ANNUAL REPORT OF PROGRAM ACTIVITIES

NATIONAL CANCER INSTITUTE

FISCAL YEAR 1973

PART III

U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE · NATIONAL INSTITUTES OF HEALTH

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July 1, 1972 through June 30, 1973

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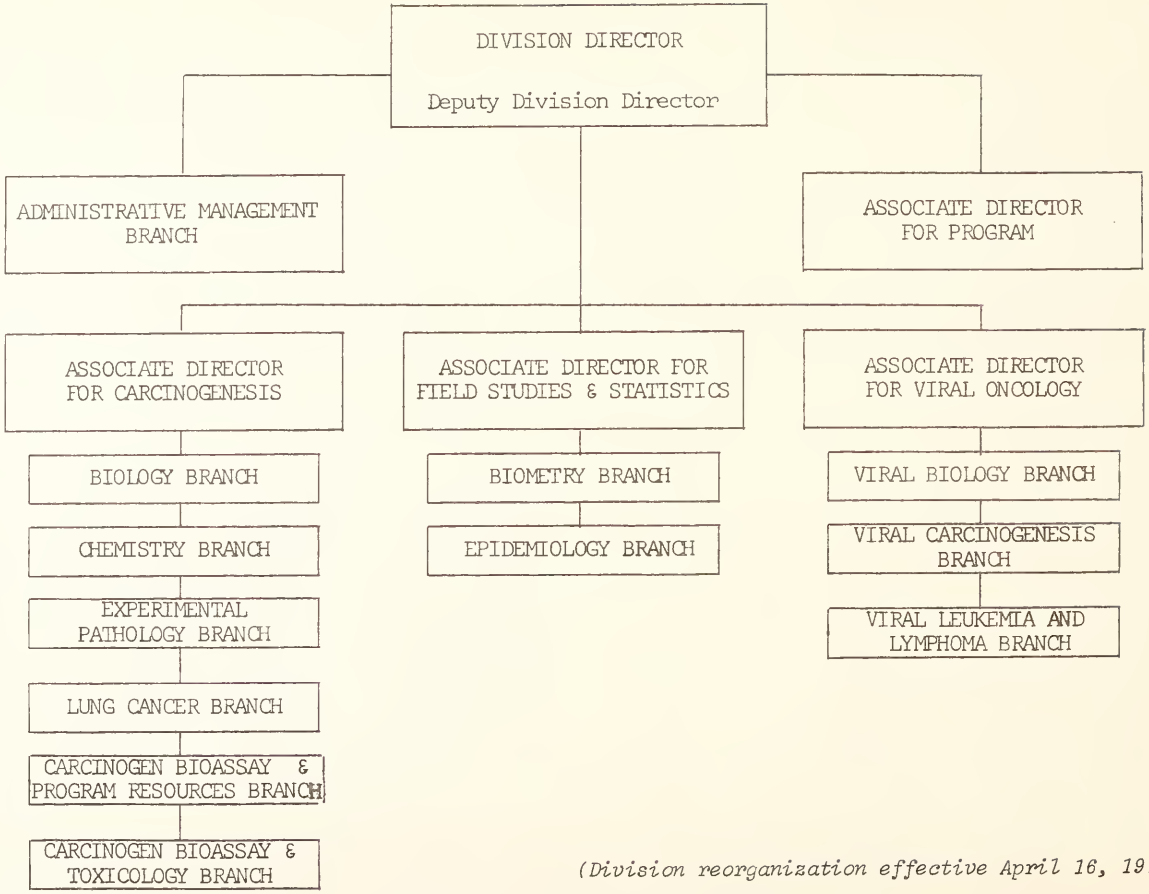

(Division reorganization effective April 16, 1973.)

SUMMARY REPORT
DIVISION OF CANCER CAUSE AND PREVENTION
NATIONAL CANCER INSTITUTE
July 1, 1972 - June 30, 1973

The summary report of the Director, the Division of Cancer Cause and Prevention, sets forth goals and objectives of the Division, a conceptual base upon which a broad attack on cancer etiology and prevention can be developed, program approaches to be used for attaining the objectives, and shows the magnitude of the task and investments required.

A simplified synopsis of major goals, and of pathways to them, is given in Figure 1. More detailed program logic networks for Demography, Carcinogenesis and Viral Oncology will be found under specific area presentations.

Activities in DCCP are specifically directed towards cancer prevention. Research output is continually utilized in resetting more advanced priorities and in formulating those activities that offer the highest promise of success. The major structures of this scientific management process are indicated in Figure 2.

Newly enacted legislation and funding for a National Cancer Program pose novel challenges and opportunities in research and for cancer control activities in man. This legislation and program recognize the promising advances of cancer research which now make it possible to formulate direct intervention in man's behalf: the definition and engineering of a less hazardous environment, and the identification of those individuals who need increased attention because of their higher risk to develop cancer.

As DCCP is increasingly committed to cancer control activities, research will be equally emphasized because the continuing success of control measures in man will depend almost exclusively on the results of past and future research.

IMPACT OF CANCER

Cancer occurs in all parts of the world, but variation in the incidence of specific forms of cancer is the rule rather than the exception. A range of ten or twenty-fold is common and for some types of cancer it is far wider.

In general, the rate of cancer mortality in the United States occupies an intermediate rank in the worldwide range, but the rank of mortality from specific types of cancer varies markedly. The United States white population has the lowest mortality from cancer of the stomach and close to the highest from cancers of the colon and female breast. As shown in Table 1, the incidence rates of various cancers in the U.S. are expected to increase from a low of 25% each for leukemias and breast cancer to a possible high of 180% for cancer of the pancreas. This table also presents leads towards identification of etiologic factors and towards prevention.

Available evidence suggests that environmental agents and social practices, rather than conventional genetic factors, are largely responsible for variation in the occurrence of cancer in different populations. It should therefore be possible to reduce the occurrence of the major types of cancer in the United States to the level of the lowest ranking country for that type. Such a reduction would cut mortality from cancer by one-third. This is the minimum goal we should aim for.

More than 52 million Americans now living will eventually have cancer (one in four persons) according to present rates. Cancer will strike over the years in approximately two of three families. In the 1970's in the United States alone, there will be an estimated 3.5 million cancer deaths, 6.5 million new cancer cases, and 10.0 million under medical care for cancer unless the present trends are moderated. Such trends mean that in 1976 there will be 346,000 cancer deaths and 747,000 new cases and in 2000, 471,000 cancer deaths and 1,085,000 new cases.

In the current year there will be near 350,000 cancer deaths in the U.S. or one cancer death nearly every one and one-half minutes. There will be 680,000 new cancer cases and 1,000,000 Americans will be under care for cancer. The Third National Cancer Survey estimates that the earning income loss due to cancer deaths alone currently deprives our economy of $18 billion annually. This does not include the costs for diagnosis and treatment of the disease. About one-half the cancer cases are below age 65; life expectancy and economic earning power are reduced over a considerable portion of the economically productive ages.

Although cure rates for cancer patients have steadily improved over the past several years, the mortality (both in actual numbers and in rates corrected for population growth and other changes) continues to rise. This is because new cancer cases are appearing at an even greater rate than the improved rate of cures. Some kinds of cancer are increasing faster than others; by the year 2000, if present trends continue, cancer of the lung will have increased 52 percent, large bowel 40 percent, leukemias 25 percent and pancreas 180 percent to an overall total of 1.2 million new cases per year.

PROGRESS, PROBLEMS AND OPPORTUNITIES

A. DEMOGRAPHY. Cancer can be prevented by identifying its causes and removing them, and/or by increasing the body's defenses against them. Thus, it is important to know not only what causes cancer, but also who is unduly susceptible or resistant. Susceptibility may be immense. For example, if an identical twin develops leukemia during the first year of life, his co-twin is virtually certain to develop leukemia within weeks or months. This increased risk fades quickly with age, and entirely disappears by 6 years of age.

Recently there has been a substantial increase in our list of characteristics of persons who are unusually susceptible to leukemia, lymphoma and other cancers. Once such a list has been created, it can be examined for a common denominator. Many items can be explained by a known or possible genetic defect, sometimes extensive enough to cause various abnormalities in the appearance of

chromosomes. It should be noted that the only environmental agent known to be leukemogenic in man, ionizing radiation, and the strongest suspect among the chemicals, benzene, produce long-lasting chromosomal abnormalities in somatic cells.

By contrast, persons at high risk of lymphoma (solid tumors of lymph tissue) have inborn immunological deficiencies (ataxia-telangiectasia, Wiskott-Aldrich syndrome, or congenital x-linked agammaglobulinemia). Consistent with these observations is the increase reported in the occurrence of lymphomas among persons given immuno-suppressive drugs following renal transplantation. A sudden upturn in one form of lymphoma, Hodgkin's disease, at about 8 years of age, also suggests a relationship to immunological development. Normally, at this age, there is a marked spontaneous involution of lymphoid tissues throughout the child's body. Lymphoid tissues are centers of immunologic activity. It is tempting to think that some failure in this normal regression accounts for the upturn in the occurrence of the neoplasm.

There are many other inborn defects that carry high risk of cancer of various types. In the aggregate, they do not account for a large proportion of cancer but they probably do constitute a large percentage of cancers among young people. In this group, prevention or early detection and treatment can provide decades of healthylife. Peaks soon after birth in the frequency of diagnosis of leukemia, neuroblastoma, Wilms' tumor, and certain forms of brain cancer indicate that many of these neoplasms are likely to have been initiated during intra-uterine life.

Knowledge of these relationships permits physicians to examine children with certain defects more closely than usual, since early detection and prompt removal of these tumors can be life-saving. Scientists, on the other hand, can explore the origins of the tumors in the light of what is known of the associated birth defects. Thus, instead of studying a tumor in terms only of itself, new avenues of approach have been opened. What is learned through these rare mistakes of nature may well apply to prevention of large categories of cancer. In particular, the epidemiologic identification of individuals and populations at increased risk of different cancer patterns should aid the investigator in virology and chemical carcinogenesis in searching for specific etiological factors.

INTERNATIONAL ACTIVITIES

A contract with NAS-NRC supports and extends cancer research at the Atomic Bomb Casualty Commission in Japan. This has paid off in a modified Japanese attitude toward ABCC, so that there is now substantial involvement of several Japanese universities, including the development of a tissue registry at Hiroshima. These resources will provide on-the-ground data resources for the development of epidemiologists. The Japanese Migrant Studies in Hawaii (in cooperation with the Heart and Lung Institute) have provided a very strong suggestion that meat - in particular beef - is associated with the great increase (4-fold) in cancer of the large bowel in Hawaiian Japanese. This work is being followed up by work in the Chemical Carcinogenesis Area in the search for bacterial flora possibly capable of producing carcinogens in the lower bowel.

With the UICC an international comparison is being made of childhood cancers, according to cell type. A working relationship has been developed with the Manchester Childhood Tumor Registry, the only population-based registry concerned with pediatric cancer. This work ties in with the concerns of the area with the etiology of childhood cancers, both in relation to problems of environmental exposure, and in their genetic components.

Work has continued on exploiting special populations in India (the Bombay cancer registry) and in Cali, Colombia (problems of cervical cancer, Hodgkin's disease). Migrant studies of Norwegian and Polish migrants to the United States are being continued - although at a relatively low financial level. Recent work has supported an association between adenomatous polyps and the incidence of colon cancer.

DOMESTIC ACTIVITIES

Two major thrusts define domestic studies: data gathering and analysis, and the testing of etiologic hypotheses. With respect to the former, data collection for the Third National Cancer Survey is near an end and activities will shift to data analysis and interpretation. A special short-duration survey of skin cancer has developed information indicating that the incidence of basal cell and squamous cell cancer of the skin may be substantially higher than previous estimates. On the basis of studies in Dallas, San Francisco, Iowa, and Minneapolis-St. Paul it would appear that there are 250,000 to 300,000 new cases of skin cancer in the United States each year. This is of the order of double to triple previous estimates. Hospital cost data have been gathered by the TNCS. (The median hospital payments made within two years of diagnosis are between $2000 to $3000). Information on treatments and survival has been reported in the 4th End Results Report. Preliminary data given in the last annual report has not been importantly changed. Improvement in diagnosis (higher proportion diagnosed while localized) have been made in sites which account for almost 30 percent of all new cases diagnosed (Breast, Prostate, Bladder, Melanoma, Brain). All of these sites have shown improvements in survival. Other sites showing improvements in survival (without much reported changes in stage at diagnosis) are all the leukemias except acute myelogenous leukemia, Hodgkin's disease, larynx, thyroid and multiple myeloma.

The Third National Cancer Survey will give way to a network of population-based cancer registries. At the moment serious problems exist with these registries (and potential registries) in Connecticut where some proposed organizational changes in the Health Department may substantially reduce the usefulness of the register, and in New Orleans, where no satisfactory administrative arrangement for operating the registry has yet been agreed upon. Useful preliminary work has gone on in New Mexico, California, Iowa, Hawaii, Detroit and Utah. The Biometry Branch is now in a position to undertake the coordination of such a national registry network. The place of the contribution of the Comprehensive Cancer Research Centers is yet to be worked out.

With respect to problems of developing and testing theories of cancer etiology, a substantial review of the epidemiologic implications of a virus etiology theory was made by Dr. Robert Miller, to supplement earlier work on problems of time-space clustering, and the collaborative work with IARC on Burkitt's lymphoma in Africa and naso-pharyngeal carcinoma in south-east Asia. Genetic and familial studies have shown several new family cancer syndromes relating to Hodgkin's disease and immunologic disorder among close relatives, and familial gastric cancer and immunologic abnormalities.

Some important declines in cancer incidence and mortality have occurred. Two major cancer registries have reported large reductions in incidence of acute leukemia in children. Mortality from Wilm's tumor has declined - apparently as a result of improved treatments. Stomach cancer incidence continues to decline. Cancer of the uterus (all forms) has continued to decline in both incidence (excluding carcinoma of the cervix in situ) and mortality. In whites mortality from carcinoma of the colon and rectum has been declining. In non-whites there has been an increase in both incidence and mortality.

Major increases in incidence have occurred in bladder (in men), lung, pancreas, esophagus (in blacks), and prostate.

Several areas of work are in an unfulfilled state. A proposed volume on high risk individuals awaits response from the American Cancer Society. Exploitation of Veteran's Administration clinical data on cancer has not yet begun - although several preliminary discussions have been held. The "Alert Practitioner" program, designed to make physicians more etiology oriented has been in operation over a year, and some possible etiologic findings were reported at the first annual conference. The program remains to be extended to physicians handling adults. The review of the epidemiologic evidence relating to BCG vaccination awaits the concurrence of the Chicago group for permission to review the hospital records.

B. CHEMICAL CARCINOGENESIS. The observed relation between exposure to certain chemicals and the development of cancer in man has been historically the first step towards knowledge of the etiology of cancer. A listing of agents presently known to produce cancers in man includes some 10 specific chemicals, 12 mixtures and crude products and, in addition, radioactive materials and radiation (including x-rays and ultraviolet light). The number of items is relatively small because studies capable of identifying an agent as directly causative of cancer in man require large-scale and time consuming epidemiologic studies and have been conducted for a very small proportion of the potential exposures of man. In fact, essentially all materials that have been demonstrated to be carcinogenic for man have also been found to be carcinogenic in animals.

Most chemical carcinogens known from animal studies appear active in a variety of animal species. It is estimated that more than 1000 chemicals have so far been found carcinogenic in animal bioassays out of about 6000 that have been tested.

The major types of cancer in man that can be directly related to known carcinogenic exposures are as follows: (a) cancer of the lung related to cigarette smoke and certain other inhalation hazards (particularly asbestos, chromates, and radioactive materials); (b) cancer of the skin related to exposure to a variety of crude tar products and combustion products as well as to ultraviolet light and other forms of radiation; (c) cancer of the bladder related to exposure to aromatic amines and their derivatives; (d) leukemias related to exposure to radioactive materials; and (e) mesotheliomas related to asbestos exposure. A number of other human cancers have been considered to be related to exposure to environmental carcinogens but the evidence is less clear.

Because of the widespread use of some of these materials, the proportion of the total population exposed and affected is extremely difficult to estimate, with the exception of such materials as cyclamates, DDT and tobacco smoke. Since it has been estimated that the majority of the present cases of lung cancer in the United States are attributable to cigarette smoking, that factor alone appears to be responsible for a death toll in the neighborhood of 40,000 deaths a year. Current projects to produce a less hazardous cigarette must therefore be expanded.

The development of our knowledge on the causative factors of cancer in man can be projected along the following lines: (a) epidemiologic studies to correlate specific exposures to certain types of cancer in man with particular emphasis on occupational exposures; (b) additional bioassays in animal models which will identify chemicals as carcinogenic in animals and therefore potentially carcinogenic in man; (c) studies of animal human correlations by comparisons of bioassay results with the results of epidemiologic studies or by bioassays on isolated human tissues by in vitro techniques; and (d) by studies of pharmacologic and metabolic correlations among species.

The major areas of opportunity and need for progress in cancer prevention through studies in Chemical Carcinogenesis are: (a) develop better means for the removal of hazardous chemicals from the environment; (b) develop means to enhance the ability of the host to detoxify environmental agents; (c) determine the extent to which chemicals act in synergistic concert with viruses or with physical carcinogens such as irradiation; and (d) develop more rapid and more sensitive means to detect and bioassay the cancer-inducing effects of chemicals for man. The latter area of activity is particularly important. For example, it is estimated that approximately 200,000 new chemicals enter our environment each year. With present assays it takes approximately 2 years and 200 mice at approximately $200 per animal to test 1 chemical. It is hoped that newer studies in animal and human tissue cultures may diminish the 2 years and nearly $40,000 now required to test a single compound. However, until this is achieved, the bioassay resources are limited to a few hundred compounds annually and it is necessary to select compounds to be tested on the basis of their distribution in the environment, intake in man and similarity to known carcinogens.

PROGRESS HIGHLIGHTS

1) Bioassay. A contract at the Stanford Research Institute, directed at establishing a ranking of suspicion of carcinogenic hazard for compounds present in man's environment, provides information for the best utilization of current limited bioassay resources and may give early guidelines to the chemical industry, as to the hazards that may be expected by the introduction of a new compound.

The significance of current bioassays for carcinogenicity derives mainly from two considerations, namely, that a) humans in general react like other animals to a variety of insults, the differences being usually quantitative rather than qualitative and b) current bioassay methods, even when performed with adequate biometrical and qualitative controls, can be expected to detect only those compounds that are strongly carcinogenic.

These two reasons prescribe that the greatest attention be given to positive results in current carcinogenicity tests.

Testing and retesting of environmental chemical pollutants at NCI continue to include agricultural chemicals such as pesticides, artificial sweeteners such as cyclamates and saccharin, hormonal compounds generally used as oral contraceptives, and natural products of various molds and fungi. Food preservatives are also under constant scrutiny.

A major expansion of the bioassay program took place in FY 1972. In the present fiscal year the emphasis is on strengthening its organization, the standardization, and the monitoring of protocols.

A computerized Bioassay Data System was designed and tried out and is now operative in a majority of the bioassay contracts. It provides, for the first time, an effective centralized monitoring resource and a basis for data analysis.

2) Smoking and Health. A major effort is underway, under the advice of the Tobacco Working Group, to reduce the risk of the smoker. Current research includes: a) Skin painting and ciliotoxic and cytotoxic bioassays of less hazardous cigarette models. These include cigarettes with various additives, papers of different porosity, reconstituted tobacco sheets of different densities and nature, reconstituted cellulose artificial tobaccos, high and low nicotine tobaccos, tobaccos grown under different fertilization conditions, "puffed" tobaccos, freeze-dried tobaccos, exane extracted tobaccos, etc.; b) Chemical characterization and ciliotoxicity bioassays of several experimental cigarette filters; c) Development of novel bioassay methods such as the wax/tar pellet implantation technique in rodent lungs; d) Development of inhalation methods in small rodents; e) Determination of nicotine effects by chronic inhalation in dogs; f) Development of experimental methods to measure smoke dose intake in man; g) Pharmacologic and behavioral approaches to demonstrate the feasibility of helping the high-risk smoker through drugs and smoke cessation clinics; h) Epidemiological studies on the health consequences of smoking associated factors, such as demographic and social variations, occupational and environmental characteristics, smoking habits, inhalation

characteristics and smoking product used (cigarettes, cigars, pipes).

Preliminary and tentative results suggest that some cigarette paper types, reconstituted sheets and artificial tobaccos may be desirable for the manufacture of less hazardous cigarettes, however, considerably more research is necessary to duplicate and confirm the initial results, and to establish that improvements related to the carcinogenicity of the condensates do not result in worsening of other characteristics affecting the cardiovascular and respiratory systems. Plans for future research include: a) In team with the National Heart and Lung Institute, develop methods to determine the cardiovascular and respiratory effects of cigarette smoke in man and experimental animals, for the evaluation of less hazardous smoking products; b) In team with the United States Department of Agriculture, develop agronomic tobacco varieties that may result in less hazardous smoking products; c) Conduct epidemiological surveys to determine multifactorial causes of diseases associated with smoking; these may include occupation, social habits, diseases, genetic and metabolic characteristics of the smoker that may increase the risks associated with smoking; d) Development of drugs that may help the high-risk smoker in counteracting some of the harmful effects of the smoke or in discontinuing the smoking habit. In this area nicotine antibodies have been successfully produced by scientists under contract with the NCI and may become a significant tool in the treatment of the high risk patient.

3) Development of Bioassay Models. Major programs have been established for the development of biological models for tumor pathogenesis closely correlated to the main types of cancers in man. Lung Cancer and colon cancer programs are well established; pancreas cancer and prostate cancer programs were initiated in FY 1972 and are now developing programs on endocrine cancers and childhood cancers. Their emphasis is on the use of animal models and human material to identify pathogenetic steps susceptible of inhibition or prevention.

In vitro models for neoplastic transformation of cells in culture by chemicals have been developed using cells from hamsters, mice, and guinea pigs. A major advance was the development of a host mediated transformation assay, responsive to carcinogens that require metabolic activation by the host. These new methods are being used to establish their value both as screening techniques and as tools for identifying key steps in carcinogen activation and interaction, susceptible to inhibition. The applicability of these techniques to transformation tests of human cells is currently being investigated. Genetic factors and growth conditioning factors required for neoplastic transformation by chemicals have been identified. Enhancement of chemical cell transformation by radiation and of viral transformation by chemicals have been demonstrated. Transformation of epithelial cells in vitro by chemicals has also been obtained in preliminary experiments. A major advance has been made with the transformation of human cells in vitro by chemical carcinogens, conditioned by corticosteroids.

4) Studies of Carcinogenesis Processes. New emphasis has been given to the study of carcinogen metabolism and toxicology, and a large collaborative effort is underway in the documentation of the conditions of formation and activation of nitrosamines. Pharmacologic inhibition of the formation of carcinogens has been obtained by blocking nitrosation of amines. Other classes of carcinogens are also under study (e.g. hydrazines, metals).

Major metabolic steps in the enzymatic activation of several classes of carcinogens have been identified with special emphasis on polynuclear hydrocarbons. Their reproducibility in human tissues has already been demonstrated in some cases. Micromethods, applicable to determinations in small samples of human cells, have been developed for measuring carcinogen activation levels. Inhibitors of carcinogen activation have been identified.

Important progress has been made in the application of immunologic methods to the study and control of carcinogenesis and tumor growth in animals and in man. These methods have also contributed valuable knowledge applicable to another program area, i.e. cancer immunotherapy in man.

C. VIRAL ONCOLOGY. A considerable number of viruses are known which either directly or indirectly cause tumors in different vertebrate species. Some of these replicate in and cause transformation of cultured human cells. The RNA viruses are responsible for many naturally occurring tumors in animals, and this group is most likely to be the cause of some human neoplasms. Certain DNA viruses may also be involved in oncogenesis either as causative agents or as necessary co-factors in cell transformation. Thus far, the only DNA viruses associated with natural cancers of animals and man are herpesviruses. Although candidate viruses have been isolated more frequently in recent years, none as yet has met the rigorous criteria to be considered a human tumor virus. Nevertheless, there is cause to be optimistic, for scientists have acquired the knowledge, techniques, experimental systems, and insight to study this problem. Even if virus particles may not be the direct cause of human cancer, virologists are pursuing the exciting possibility that cellular genes--genes containing information similar to that of RNA or DNA tumor viruses-- are expressed in human cancer.

Current knowledge suggests several methods for analysis of human tumors for virus-specific genetic information: (1) Molecular hybridization between cancer cell RNA and natural and synthetic DNA prepared by viral RNA - DNA polymerase; (2) Search for RNA - DNA polymerase activity in human cancers; (3) Search for human virus-specific antigens related to known tumor virus antigens in human cancers; (4) Application of new techniques (e.g. chemical treatment) for the induction of virus or virus genetic information in human tumor cells.

1) RNA Viruses.

Characteristics. RNA tumor viruses possess a number of similar biological and biochemical characteristics, are widely distributed in vertebrate species, and are transmitted vertically under natural circumstances. Infections are often covert, resulting in only partial expression of the viral genome. Each Type C virus, regardless of origin, possesses a major internal antigen which permits species delineation (gs-1), and shares a determinant which is interspecies specific (gs-3). There is now good evidence that internal antigens can be detected in embryonic, adult, and tumor tissues of various animal species. This finding is highly significant because the antigen can be found in situations where no virus can be demonstrated.

Of greater importance in terms of understanding the determinants of cancer is the reported linkage between RNA tumor virus and early viral gs antigen expression with the development of cancer late in life.

All RNA tumor viruses contain 60-70S RNA and an enzyme known as RNA-dependent DNA polymerase (RDDP) which permits transcription of viral RNA into DNA. As an antigen, RDDP, like the gs antigen, has both species and interspecies characteristics.

To provide a basis for further understanding information was sought to elucidate the mechanisms involved in the integration of RNA tumor virus information into host cells, and the relationship of this reaction to cell transformation. Recent results indicate the initial product of the RDDP is covalently linked RNA-DNA that is primed for DNA synthesis by a 4S RNA associated with 60-70S RNA. The yield of DNA is also increased by a stimulatory viral protein.

The protein products of virus gene expression in relation to cell transformation are also being studied.

Within the past year progress has been made toward the understanding of the biochemical (molecular) events which occur in the process of cell transformation. These studies are important because they can be applied to human normal and tumor cells to identify virus-specific sequences that have characteristics similar to those of animal RNA tumor viruses. Such studies also help to provide information on mechanisms of virus expression and regulation which could lead to new methods for controlling human cancer at the cellular level.

Analysis of human cancers for evidence of RNA tumor viruses. The powerful tools of molecular hybridization have permitted the analysis of human tumors for viral genetic information. Evidence from animal model systems indicate thus far that every cell line infected with or transformed by RNA tumor viruses possesses virus-specific RNA sequences readily detectable by molecular hybridization. Corresponding neoplasias of murine and human origin were found to exhibit remarkable similarities. For example, human breast carcinomas contained RNA possessing sequence homology to that of murine mammary tumor virus (MMTV).

Following reports of RDDP in the virions of RNA tumor viruses, it was important to determine whether the enzyme is restricted to tumor viruses and whether it is restricted to tumor cells. Attempts are now being made to determine whether the polymerase is present in human cells. RDDP obtained in the particulate fraction (1.16 g/m) of human leukemic cells was purified and characterized. The enzyme could be distinguished from major DNA polymerases of normal cells.

Considerable effort is being directed to search for RNA viruses which might be oncogenic for man. For the past several years sufficient numbers of observations reporting the finding of particles similar to Type C and Type B viruses in human malignancies have been made to conclude that viruses of these types may also infect man. Of the four candidate viruses isolated in the last year, two, ESP-1 and RD-114, have been well enough characterized to be considered

animal in origin. ESP-1 was shown to be murine; RD-114 has been classified as an endogenous feline virus.

Following published reports that endogenous animal RNA tumor viruses could be activated by treatment of cultured cells with halogenated uridine, the technique was successfully applied to a human tumor cell culture, and the agent, released in small amounts, is presently undergoing preliminary characterization, both intramurally and within the research contract program.

Attempts to confirm earlier findings showing a correlation between the presence of virus-like particulates in human milk, the RDDP activity associated with fractions separated from the milk, and the breast cancer history of the milk donor have not been successful. A specific inhibitor in milk which interferes with the assay, a ribonuclease, may be responsible for the inconsistent results.

2) DNA Viruses.

Characteristics. As recently as 1964, herpesviruses were generally considered to lack oncogenic activity. Prior to that time, the only evidence linking these viruses to animal neoplasias was the finding of herpes-like particles in cells of renal adenocarcinomas of the frog. The discovery of the Epstein-Barr virus (EBV) in cultured lymphoblastoid cells from patients with Burkitt's lymphoma in 1964 stimulated extensive studies to examine herpesviruses as possible etiological agents of cancer in several animal species, including man.

Herpesviruses interact with their host cells in a productive or non-productive manner. During the productive growth cycle, the synthesis of infectious progeny is invariably accompanied by destruction of specific target cells. The non-productive cycle is very similar to that observed with other oncogenic DNA viruses. Stimulation of cellular DNA synthesis, acquisition of virus-induced antigens, incorporation of viral nucleic acid and transformation of normal cells into established lines capable of indefinite proliferation have all been described. Activation of virus synthesis in non-productively infected cells by exposure to mutagens (BUDR, IUDR) or irradiation is usually paralleled by cell death. Herpesviruses, like the RNA tumor viruses, also establish persistent covert infections. Whether latent herpes infections are the result of low-level productive or non-productive interactions has not been determined.

The mechanisms underlying herpes-induced oncogenesis remain obscure. The close association of these viruses with several animal malignancies suggests, but does not prove, their involvement as etiological agents.

Relationship to human cancer. The most outstanding evidence for the existence of human oncogenic herpesviruses is derived from seroepidemiological, biochemical, and biological studies, suggesting an association between various herpesviruses and specific malignant diseases of man. The Epstein-Barr virus is clearly suspect as playing some role in the genesis of Burkitt's lymphoma, nasopharyngeal carcinoma and, to a lesser extent, the sarcomatous form of Hodgkin's disease and chronic lymphocytic leukemia.

The Epstein-Barr virus is closely associated with Burkitt's lymphoma (BL) but the absence of an experimental animal susceptible to infection with this agent has made it difficult to pinpoint its role in the induction of this disease. Therefore, although the evidence which suggests that EBV is the etiological agent of Burkitt's lymphoma is circumstantial at present, it is so strong that a causal rather than a casual relationship is suggested. The tumor is most prevalent in regions of Africa where conditions are extremely favorable for insect-vectored disease. Cases in certain areas have been shown to cluster in time and space. Most African BL patients were found to have relatively high titers of antibodies to EB viral capsid antigens (VCA) and cell membrane antigens (MA) when compared to control populations of similar age and sex distribution. Loss of anti-MA during chemotherapeutically induced remission has preceded recognition of a recurrent tumor by several months. In addition, BL patients frequently possess antibodies to EBV-induced early antigens (EA). Changes in the titers of antibodies to EBV-related early antigens are prognostically significant.

The establishment of continous lymphoblastoid cell lines following exposure of lymphocytes from normal donors to EBV again suggests an oncogenic potential for this virus.

A serological association has also been described between EBV and nasopharyngeal carcinoma (NPC) that is as pronounced and consistent as that observed in Burkitt's lymphoma. Virtually all NPC patients have detectable antibody to EBV VCA and MA in high titer. The serologic picture and the demonstration of EBV-DNA in biopsies and lymphoid cell lines derived from this tumor secure the position of this carcinoma as an EBV-associated tumor.

The relationship between EBV and Hodgkin's disease (HD) is not as clear as that observed between EBV and BL or NPC. Clustering studies strongly suggest a horizontally transmissible agent as one factor in the pathogenesis of the disease. Although increased antibody levels to EBV have been recorded in persons with HD, it is clear that a significant percentage of patients have no detectable titers.

Although these arguments are highly suggestive, they are not conclusive as far as the etiology of BL and NPC are concerned. While there is good reason to believe that EBV is involved in the oncogenic process, its role could be that of an accessory factor or passenger virus in the tumor. No EBV DNA has yet been found in American BL tissues by the RNA-DNA hybridization technique, even though this tumor is histopathologically similar to African BL. RNA sequences homologous to that of the Rauscher murine leukemia virus RNA have recently been described in BL biopsies suggesting the possible interaction of Type C viruses in the genesis of this disease. If EBV acts to prime the cell for neoplastic change, it is possible that one or more combinations of environmental and host factors interact to promote oncogenesis. Thus, the association of EBV infection with more than one disease becomes more plausible. Should this virus prove to be a necessary co-factor in any or all diseases with which it appears to be associated, control of infection would be of paramount importance.

An expanding body of evidence has strengthened the causal relationship between herpes simplex virus type 2 (HSV-2) and carcinoma of the human uterine cervix, the second most common malignancy in women in the United States.

The frequency of antibody to HSV-2 is generally greater in women with cervical neoplasia than in normal controls matched for promiscuity and other risk factors.

All findings strengthen the association of HSV-2 with cervical carcinoma but still do not prove a causal relationship between the virus and this malignant disease. Studies to determine if this virus is the sole etiological agent, or an essential co-carcinogen in the induction of this disease will be continued.

3) Treatment and Control. A rational approach to tumor therapy may not depend on the isolation of a bonafide human tumor virus. Indeed, existing model systems have provided sufficient information which can be applied to the control of human cancers. For example, chemical inhibitors (antienzymes, gene repressors) which act on specific stages of viral replication or stimulation or host immune mechanisms (vaccines) to virus or virus-mediated, tumor-specific antigens may prevent or control oncogenesis.

The systematic screening of rifamycin derivatives which block the RDDP activity of RNA tumor viruses may help in the development of inhibitors that possess properties for the control of specific gene expression. Some fluoranthrene di-substituted cationic derivatives and analogs are also strong inhibitors of RDDP and of cell transformation.

Many compounds have been tested for their capacity to inhibit the reproduction of murine leukemia and sarcoma viruses in vitro. A few of the most active ones also showed inhibitory activity against these viruses in vivo. Among the chemicals tested for non-specific stimulation of the RES, imidazolethiazole, pyran copolymer, and Tilaron proved effective in increasing survival of mice in induced remission.

A DNA binding protein in non-virus producing AKR mouse cells has been isolated and found to have many of the properties of known repressor proteins described in bacterial virus systems. This finding could lead to understanding the mechanisms by which mammalian cells control gene expressions and how genetic mechanisms regulate RNA tumor virus expression.

Formalin killed vaccines prepared from murine leukemia viruses have produced significant reductions in tumor incidences in mice given potent chemical carcinogens. Because of the reported immunological and biochemical relatedness between MuLV (Rauscher) and human leukemic cells, terminal cancer patients were immunized with inactivated RLV. Evidence for humoral and/or cell-mediated immunity against this virus was found in more than one-half of the immunized patients. Whether this treatment produces beneficial effects for the patients can be only determined after many years of observation.

The immunogenicity of virus-induced tumor cells has been shown to be increased by infection with influenza virus. Tumor transplantation antigens on these cells can be isolated in cell-free form. This material protected mice against challenge with tumor transplants causing either suppression or complete regression. These studies have obvious implications for immunotherapy of human tumors.

SUMMARY

Between 30% to 90% of cancer deaths in man is preventable. Mortality statistics from around the world show that if cancer mortality in the United States, site for site was comparable with that of the lowest country reporting for each site, approximately 100,000 lives would be saved each year. The major sites in which gains should be made are cancer of the colon and rectum, cancer of the lung, breast cancer, cervix and uterus (particularly among non-whites), cancer of the ovary, cancer of the prostate, and probably leukemia.

Information is already in hand to reach these goals for lung cancer and for cancer of the cervix and uterus. These 2 sites alone would account for 60,000 or more savable lives. A concerted research effort is called for in cancer of the colon, breast cancer and cancer of the prostate. There is promise of substantial pay-off in these sites. For example, migrant studies have provided very strong evidence that cancer of the colon is a disease with a very strong environmental base, possibly related, among other, to high beef meat consumption. Japanese migrants to Hawaii show a 3 to 5-fold increase in contrast to Japanese on the home islands. When we find all elements of the new environment that lead to so large increases, application of this knowledge to the present United States population could reduce the U.S. incidence by another 25-30,000 lives a year.

Thirty percent (or 100,000 lives) is a minimum estimate. If we add to the preventable cases (in terms of current knowledge, or knowledge that should be available in the next few years, earlier diagnosis and improved treatments--where prevention has broken down), this number should be easily doubled. For example, if the most recent knowledge on early diagnosis of breast cancer were applied across the country, we might anticipate 12,000 fewer deaths each year from breast cancer alone. If the finding that early pregnancy (before age 20) cuts in half the probability of developing breast cancer, could be translated into a prophylactic treatment for young women (i.e., create the hormonal milieu of pregnancy without actual pregnancy) another substantial reduction in breast cancer deaths may follow.

Current work on identifying the cancer "susceptibles", if successful, will lead to cutting down cancer deaths. First, by identifying susceptibles, we will know what people to watch closely and regularly, so that we might diagnose their disease early, and thus treat is successfully. Second, by identifying the cancer susceptibles, it should be possible to protect them from those environmental assaults which could be carcinogenic to them--while essentially innocuous to others.

Ninety percent reduction in cancer deaths could be reached within the present century, if, in addition to current knowledge on prevention, and universal application of the best diagnosis and treatment techniques, our research uncovered a) the interacting environmental elements that cause migrants' cancer rates to change toward those of their new countries; b) those environmental elements which lead to different rates in different countries in the world; and c) the susceptibles whom we must and can protect.

While there are major problems in the prevention of virus-induced cancers, not heretofore experienced in other areas of infectious disease, we should anticipate substantial inevitable success in the control (including prevention) of these diseases in man. These problems include the probable difficulties in using conventional vaccines for prevention if a major means of virus exposure is vertical rather than horizontal. Secondly, the "peaking" phenomenon of acute lymphocytic leukemia in Caucasian children at about 4 years of age and other similar phenomena suggest that it would take 5-10 years before the results of a preventative field trial were known. Even with a "clustering" disease of relative high incidence, such as Burkitt lymphoma in equatorial Africa, nearly 1,000,000 children would have to be included in the "treated" cohort observed for 5-8 years to determine whether the expected 40-50 lymphomas would be eliminated or decreased.

These difficulties notwithstanding, opportunities for cancer prevention and control are very bright through application of existing knowledge and through a prioritied commitment to seek new information with innovative application for man.

A Note on Management. The obligatory opportunity to plan and manage biomedical activities from basic or fundamental research upwards through development and application, with effectiveness and efficiency, requires skillful staff and line personnel of unabashed motivation, objectivity, and tact. Tact is of paramount importance because NCI management must persuade constituent scientists to attack specific problems of human disease. We adhere to the belief that NIH exists primarily to allow people to live better and longer and that NCI abets this goal through the conduct of research and development for prevention and treatment of cancer in man. Tact, including the courage and reason to say no, is important also because while the need for widespread program participation is high, many scientists do not understand that even successful completion of their proposed project may not necessarily contribute to the attainment of program goals. This thrust towards the timely control of disease must be tempered with appreciation for and selection of fundamental projects with some expectation of contribution to eventual application to man. This can and should be done through both grants and contracts.

We are fortunate to have this admixture of talents and motivation in the Associate Scientific Directors, Drs. Gori, Moloney, Saffiotti and Schneiderman; the Assistants to the Acting Director, Drs. Bryan and Depue, and in the Administrative Officers, Mr. John Patterson and Nicholas Olimpio. Particular recognition is entirely warranted to their associates whose motivation to program goals exceeds their desire for personal recognition.

Please refer to summary and individual reports for details of activities within DCCP programs for FY 1972.

CANCER ETIOLOGY AND PREVENTION PROGRAM

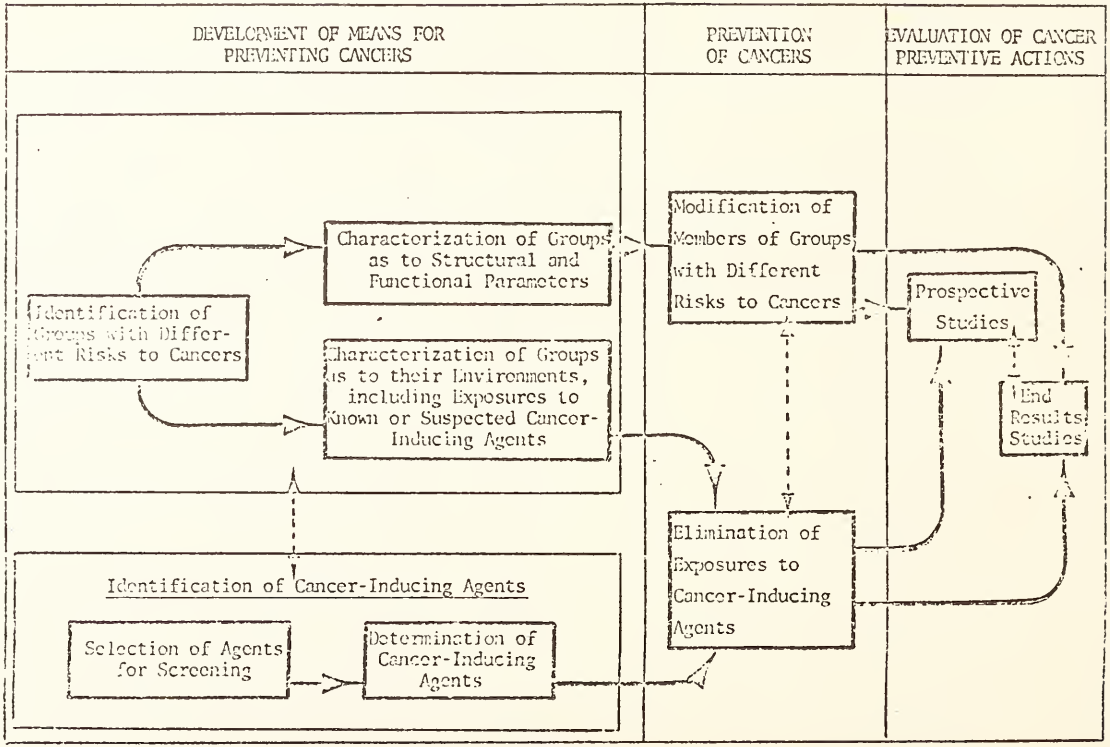

FIGURE 1

FIGURE 2

PROGRAM PLANNING
PROGRAM PRIORITIES
OPERATIONAL SEGMENTS
PROGRAM MANAGEMENT
PROPOSED SEGMENT PRIORITIES
SEGMENT MANAGEMENT
OPERATIONAL PROGRAM PRIORITIES
SEGMENT PRIORITIES
SEGMENT PROJECTS
INFORMATION RETRIEVAL
PROGRAM MONITORING & EVALUATION
PROJECT MONITORING & EVALUATION
OVERALL PROGRAM
INDIVIDUAL SEGMENTS
PLANNING
PRIORITY SETTING
PROJECTS
MONITORING

TABLE 1: CANCER INCIDENCE: EXPECTED NUMBERS, 1970-2000; CHANGE IN RATES, 1970-2000; DEATHS, 1970

SITE	NEW CASES (INCIDENCE) 1970	2000	DEATHS	INCIDENCE YEAR 2000 % CHANGE* IN RATE	MAJOR CAUSATION	MEANS OF PREVENTION
Lung	68,000	144,000	62,000	52	Tobacco Smoke Air Pollution (including on-the-job)	Stop smoking - Reduce pollution Less hazardous cigarette
Large and Small Bowel	75,000	143,000	44,000	40	Intestinal Flora? Heredity? Diet?	Viruses? Other insults. Identify susceptibles and eliminate their exposure.
Breast	69,000	121,000	30,000	25	Virus? Diet? (Hormones) Genetic?	Vaccines. Identify susceptibles.
Pancreas	19,000	101,000	18,000	180	Diet? Virus? Other Insults?	Identify etiology. Identify susceptibles.
Prostate	35,000	61,000	17,000	27	Hormones? Diet?	Identify etiology. Identify susceptibles.
Stomach	17,000	5,000	16,000	(80) decrease	Diet Poor socio-economic conditions	Diet modifications. Sociologic modifications?
Leukemias	19,000	33,000	15,000	25	Viruses. Radiation. Genetic.	Vaccines. Identify susceptibles and limit radiation.
Skin	112,000	186,000	5,000	20	Actinic rays. Genetic.	Limit radiation exposure. Identify susceptibles.
Misc.	120,000	217,000	75,000	30	Multiple	Identify extrinsic and intrinsic factors and modify them.

*Expected change in age adjusted incidence rates. Year 2000 compared to 1970.

A. SUMMARY REPORT
OFFICE OF THE ASSOCIATE SCIENTIFIC DIRECTOR FOR DEMOGRAPHY
DIVISION OF CANCER CAUSE AND PREVENTION
JULY 1, 1972 - JUNE 30, 1973

Introduction

A year's deaths from cancer deprive our economy of 18 billion dollars in unrealized earnings income. Another 10 percent needs to be added to these costs for the costs of treating a diagnosed case. These first estimates derive from the early reports from the Third National Cancer Survey - and underlie the emphasis of the Demography Area on problems of the origins of cancer - so that we might learn enough to prevent the disease.

Several approaches are being taken to develop knowledge on the etiology of cancer. We do not feel committed to any single theory of cancer causation, nor, on the other hand, do we find it possible to work on all things at all times. The areas in which we are working derive from the special strengths, knowledge, techniques and understandings that our staff members have.

Administrative Relations and Problems

Our special areas of competence lead us to be concerned that there is no one on the Cancer Board who is a specialist in any of these areas. We urge that an epidemiologist, or a biostatistician, or a person concerned with the incidence (and prevention) of cancer in humans be the next appointment to the Cancer Board. The makeup of the Board affects the decisions it makes and the advice it gives. We find it distressing that there is no one on the Board who devotes his professional life to the direct prevention of human cancer.

At present there are several "organ site" task forces operating under Cancer Institute jurisdiction. Almost every one of these has concerns that interact with the concerns of the Demography Area - epidemiology, evaluation of effectiveness of treatment, and experimental-human relationships. We have attempted to provide expertise and liaison with each of the task forces - particularly with respect to problems of epidemiology. The questions of what research a task force should support and what we should support have been informally resolved. We are supporting all the epidemiologic and biometric work in which we have special expertise - for example international studies, studies related to migrants, to childhood cancer, to familial clustering, to the relationship with diseases of domestic animals, diet studies, and to the evaluation and determination of high risk groups, particularly in breast cancer and Hodgkin's disease. The task forces should be charged with supporting the other important research that needs to be done. With respect to industrial exposures as causes of cancer, we are in a straddle-both-camps position: we have done extensive industrial epidemiology in the past and are presently using county-by-county mortality data on sites that may have occupational exposure aspects (e.g., bladder) to find leads for industrial epidemiology. We are in close contact with other government (and private)

agencies - and are prepared to assist in the development of new research and its support, but in which the major control of the research lies in the hands of the other agency. However, we feel this area is underworked. We have been attempting to add an air-pollution industrial epidemiology specialist as a consultant, but personnel limitations have been extremely frustrating.

As a result of our policy of concentrating on our own strengths we must be provided with a mechanism for learning what other people are doing, where and how. Often this poses no difficulty because of our active liaisons. However, with a limited staff universal liaison is not possible and we have proposed the establishment of an international roster of epidemiology (what's being worked on) and epidemiologists (who is doing it). Successful contract talks have been held with the IARC, and approval as pertinent to the Demography program was given by the Demography Program Review Group. Because of its international data aspects the proposal has gone to the group involved with the International Cancer Data Bank, where it now resides.

Both the Biometry Branch and the Epidemiology Branch moved from the Federal Building to the Landow Building. Aside from difficulties with computer facilities, we have found no serious problems arising out of the move. The Landow Building also houses the Chemical Carcinogenesis Area and from time to time we have found their proximity useful. When the computer facilities are set up appropriately the move could involve some net gain.

Major Activities and Accomplishments

International Activities

We were very active in international cooperative activities last year. A contract was set up with NAS-NRC to support and extend cancer research at the Atomic Bomb Casualty Commission in Japan. This has paid off in a modified Japanese attitude toward ABCC, so that there is now substantial involvement of several Japanese universities, including the development of a tissue registry at Hiroshima. These resources will provide on-the-ground data resources for the development of epidemiologists. The Japanese Migrant Studies in Hawaii (in cooperation with the Heart and Lung Institute) have provided a very strong suggestion that meat - in particular beef - is associated with the great increase (4-fold) in cancer of the large bowel in Hawaiian Japanese. This work is being followed up by work in the Chemical Carcinogenesis Area in the search for bacterial flora possibly capable of producing carcinogens in the lower bowel.

The consequences of exposure of humans to possible carcinogens, were developed in two volumes (handbooks) of chemical carcinogens prepared by the IARC.

With the UICC an international comparison is being made of childhood cancers, according to cell type. A working relationship has been developed with the Manchester Childhood Tumor Registry, the only population-based registry concerned with pediatric cancer. This work ties in with the concerns of the area with the etiology of childhood cancers, both in relation to problems of environmental exposure, and in their genetic components.

This work derives from an early interest in problems of radiation exposure (see work with ABCC, described above) that has continued through participation in the work of the International Commission on Radiation Protection. This in turn is related to studies of U.S. uranium miners, and World War II radiology technicians.

Work has continued on exploiting special populations in India (the Bombay cancer registry) and in Cali, Colombia (problems of cervical cancer, Hodgkin's disease). Migrant studies of Norwegian and Polish migrants to the United States are being continued - although at a relatively low financial level. Recent work has supported an association between adenomatous polyps and the incidence of colon cancer. Because of its usefulness with respect to migrant populations, we are supporting work on the Cancer Registry of Israel - to bring it up to a high functioning level. The potential exists for re-establishing an international network of cancer registries - as recommended in NCI Monograph 15.

Domestic Activities

Two major areas are involved in our domestic studies: data gathering and analysis, and the testing of etiologic hypotheses. With respect to the former, data collection for the Third National Cancer Survey is near an end and activities will shift to data analysis and interpretation. A special short-duration survey of skin cancer has developed information indicating that the incidence of basal cell and squamous cell cancer of the skin may be substantially higher than previous estimates. On the basis of studies in Dallas, San Francisco, Iowa, and Minneapolis-St. Paul it would appear that there are 250,000 to 300,000 new cases of skin cancer in the United States each year. This is of the order of double to triple previous estimates. Hospital cost data have been gathered by the TNCS. (The median hospital payments made within two years of diagnosis are between $2000 to $3000). Information on treatments and survival has been reported in the 4th End Results Report. Preliminary data given in the last annual report has not been importantly changed. Improvement in diagnosis (higher proportion diagnosed while localized) have been made in sites which account for almost 30 percent of all new cases diagnosed (Breast, Prostate, Bladder, Melanoma, Brain). All of these sites have shown improvements in survival. Other sites showing improvements in survival (without much reported changes in stage at diagnosis) are all the leukemias except acute myelogenous leukemia, Hodgkin's disease, larynx, thyroid and multiple myeloma.

The Third National Cancer Survey will give way to a network of population-based cancer registries. At the moment serious problems exist with these registries (and potential registries) in Connecticut where some proposed organizational changes in the Health Department may substantially reduce the usefulness of the register, and in New Orleans, where no satisfactory administrative arrangement for operating the registry has yet been agreed upon. Useful preliminary work has gone on in New Mexico, California, Iowa, Hawaii, Detroit and Utah. The Biometry Branch is now in a position to undertake the coordination of such a national registry network. The place of the contribution of the Comprehensive Cancer Research Centers is yet to be worked out.

With respect to problems of developing and testing theories of cancer etiology, a substantial review of the epidemiologic implications of a virus etiology theory was made by Dr. Robert Miller at an Interdisciplinary Communications in Cancer Conference of the Smithsonian Institution ("Belmont Conference"), and at the 7th National Cancer Conference. These reviews supplement earlier work on problems of time-space clustering, and the substantial collaborative work with IARC on Burkitt's lymphoma in Africa and naso-pharyngeal carcinoma in south-east Asia. Genetic and familial studies have shown several new family cancer syndromes relating to Hodgkin's disease and immunologic disorder among close relatives, and familial gastric cancer and immunologic abnormalities.

Some important declines in cancer incidence and mortality have occurred. Two major cancer registries have reported large reductions in incidence of acute leukemia in children. Mortality from Wilm's tumor has declined - apparently as a result of improved treatments. Stomach cancer incidence continues to decline. Cancer of the uterus (all forms) has continued to decline in both incidence (excluding carcinoma of the cervix in situ) and mortality. In whites mortality from carcinoma of the colon and rectum has been declining. In non-whites there has been an increase in both incidence and mortality.

Major increases in incidence have occurred in bladder (in men), lung, pancreas, esophagus (in blacks), and prostate.

Extensive statistical assistance has been given the group attempting to develop a safer cigarette. A new section has been organized in the Biometry Branch concerned with the design and analysis of chemical cancer data - including controlled trials. The section has strength in both medicine (2 M.D.'s) and biostatistics (2 Ph.D.'s). Support has been given to the Cancer Control Area and to the diagnostic research activities of the Division of Cancer Biology and Diagnosis.

Several areas of work are in an unfulfilled state. A proposed volume on high risk individuals awaits response from the American Cancer Society. Exploitation of Veteran's Administration clinical data on cancer has not yet begun - although several preliminary discussions have been held. The "Alert Practitioner" program, designed to make physicians more etiology oriented has been in operation over a year, and some possible etiologic findings were reported at the first annual conference. The program remains to be extended to physicians handling adults. The review of the epidemiologic evidence relating BCG vaccination awaits the concurrence of the Chicago group for permission to review the hospital records. Two staff members of Chinese ancestry are available for work with the People's Republic of China, if such needs become evident.

Summary

A substantial amount of new information has been developed on incidence, treatment, survival, costs, and mortality from cancer. Some data point to meats, most likely beef, as a major factor in the increase in colon cancer

in Japanese migrants to Hawaii. Mortality data, arranged on a county-by-county basis are providing leads for environmental epidemiology. International cooperation has proceeded at a high level. The Area has attempted to resolve the jurisdictional issue associated with organ-site task force by supporting research in which staff members have special competence. There are a substantial number of administrative problems - particularly developing from reduced or limited personnel to manage increased programs.

HONORS AND AWARDS

Marvin A. Schneiderman, Ph.D.

Adjunct Professor, Biostatistics, University of Pittsburgh.
Clinical Associate Professor, Medical Statistics, Georgetown University Medical School.
Visiting Lecturer, Visiting Lecturer Program in Statistics, Committee of Presidents of Statistical Societies (COPSS).
Chairman-Executive Secretary of the Committee of Presidents of Statistical Societies (COPSS), American Statistical Association, Biometric Society, and Institute of Mathematical Statistics.
Associate Editor, CANCER RESEARCH.
Member, Council for Analysis and Projection, American Cancer Society.
Member, Executive Committee of the Biopharmaceutical Subsection, Biometric Society.
Member, Board of Directors, American Statistical Association.
Councillor-at-Large of the Society for Occupational and Environmental Health.
Member, Cancer Center Advisory Committee at Howard University, Washington, D.C.
Member, Task Force on Aging Populations, NCI.
Member, International Council, International Biometric Society.
Member, Advisory Board, Research Support Center, Veterans Administration Hospital, Washington, D.C.

PUBLICATIONS

Marvin A. Schneiderman, Ph.D.

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CONTRACT NARRATIVE
OFFICE OF THE ASSOCIATE SCIENTIFIC DIRECTOR FOR DEMOGRAPHY
DIVISION OF CANCER CAUSE AND PREVENTION

Fiscal Year 1973

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO (NIH-E-71-2180)

Title: Group Testing for Screening Carcinogens

Contractor's Project Director: Dr. Robert M. Elashoff

Project Officer (NCI): Dr. Marvin A. Schneiderman

Objectives: To develop mathematical techniques based on the theory of group testing to permit more rapid and efficient testing of chemicals for potential carcinogenesis.

Major Findings: Analysis was done of results of pilot experiments - particularly the "8 week" experiments for determining chronic dose levels. There was extensive participation in design and analysis of pilot experiments for carcinogenesis screening -- modification of research plan (including randomization procedures), dose finding procedures and development of alternate ways to develop MTD for chronic studies.

Four manuscripts were prepared for publication on: 1) Economical first stage screening for tumorigens, 2) Further contributions to group testing, 3) Group testing with continuous variables and 4) Estimation by pooling in a Bernoulli process.

Significance to Biomedical Research and the Program of the Institute: Carcinogenesis testing is a long-term expensive process. With over 20,000 materials to test for carcinogenicity unless more efficient methods are developed testing will take a very long time. This research holds promise for more rapid testing, and for yielding information on interactions of materials.

Proposed Course: Continued close collaboration with groups doing biological testing of the materials to speed the development of mathematical models.

Development of non-sequential group testing schemes (a compromise between financial economy and time-economy).

Development of further applications of group testing.

Analysis and monitoring of short-term experiments.

Date Contract Initiated: June 24, 1971

Current Annual Level: $169,565

B. SUMMARY REPORT
BIOMETRY BRANCH
July 1, 1972 - June 30, 1973

The Biometry Branch has three main functions -- to conduct research on the etiology of cancer in man; to provide statistical consultation and support for other people conducting research; and to provide baseline data against which administrators and researchers can measure their successes (and failures) in reducing cancer incidence and mortality. These functions encompass a wide range of scientific and managerial skills in bringing people and data together.

Third National Cancer Survey

The Third National Cancer Survey conducted in seven metropolitan areas, two states, and Puerto Rico registered all new cancer cases diagnosed among residents in the years 1969-1971 and obtained demographic data and a description of the tumor for each patient. Information on the financial and social impact of the disease on the patient and family was collected for a sample of patients. The Survey field work will terminate shortly after the end of the 1973 fiscal year and the transition to the analytical phase leading to the preparation of monographs and other publications has already begun. The Demography Section of the Branch will be reorganized and given the primary responsibility for analyses of Survey data to be carried out in-house. The Special Cancer Survey Section which was responsible for management of the Survey field activities will become the focal point for field liaison with an expanded network of cancer registries, known as the SEER Program.

The Third National Cancer Survey represents such a rich source of data that the Branch will need assistance to mine it adequately. Investigators from other institutions must be recruited to take on specific publication assignments with or without research contract support. This will be particularly necessary for the Survey aspects dealing with delivery of medical care, and the direct and indirect treatment costs, topics for which the Branch possesses no expertise. To assure a well-rounded program of analysis and publications by Branch staff and outside consultants a Committee on Utilization will be appointed with representation from the National Cancer Institute, contractors participating in the Survey, chronic disease epidemiologists, and medical care analysts. The Survey director, Dr. Cutler, will chair this committee.

The findings from the first survey year, 1969, were summarized in last year's report. The data for two years have been assembled and will form the basis for the first round of publications. The two-year results are generally consistent with those reported previously, although the greater volume of experience permits some points to be more firmly established.

Information available from a preliminary review of data on the hospitalization experience of cancer patients deserves comment. The data pertain to a 10-percent sample of patients with cancers diagnosed in 1969, with information on all hospitalizations within two years of the first admission. The

average number of admissions per patient was 1.7. Seven percent were admitted 4 or more times within the two year follow-up period; 13 percent were in hospital for 50 days or longer. The average payment to hospitals was $1,520 for first admissions and $2,289 for all admissions within the two year period. For nearly 10 percent of patients, hospital payments were $5,000 or more. Of all monies paid to hospitals for the care of cancer patients, the distribution by source was:

Medicare	41%
Blue Cross	22
Other Insurance	18
Patient	7
Medicaid	5
Other Sources	7
	100%

Population-based Cancer Registries and Study Centers

Implementation of the National Cancer Program requires information for administrative purposes that must be supplied by a network of cancer registries. The national goal of research and action directed towards prevention, diagnosis, and treatment depends on current, valid information on the nature and magnitude of the total cancer problem and its components, so that progress in these areas can be measured.

An ad hoc Advisory Committee on Population-based Epidemiology Research Centers which met in November 1971 made several recommendations concerning an optimum mix of registries drawn from the following four sources:

a) individual cancer specialty hospitals;

b) comprehensive cancer research centers (loosely defined as cancer specialty hospitals augmented by special research facilities);

c) population-based cancer registries (which will provide the bulk of the descriptive incidence and end-results data);

d) population-based cancer study centers (which will conduct epidemiological and clinically oriented studies in addition to reporting descriptive incidence and end-results data).

Such a network would represent a coordinated effort directed to cancer surveillance, descriptive and analytical epidemiology and end-results evaluation. The output of this multi-purpose program would provide the basis for:

a) prompt identification of secular trends in specific cancers;

b) identification of variations in disease incidence that may suggest etiological hypotheses;

c) assessment of the efficiency and quality of the medical care system and of the efficacy of primary and secondary preventive measures.

In implementing the Committee's recommendations the contractors for areas participating in the Third National Cancer Survey are being evaluated as candidates for the expanded registry network because they have the professional and technical personnel trained in the appropriate methodology. However, recruitment of additional participating registries is not limited to this category. The Third National Cancer Survey findings are proving useful in identifying the candidate registries and populations with the greatest and most immediate study potential. Planning and/or implementation agreements have been negotiated with contractors in several key localities (Hawaii, San Francisco-Bay Area, Seattle, New Mexico, Connecticut) and other proposals are under active review (Iowa, Utah, Detroit, New Orleans). Steps are being taken to assess other candidates that can contribute to a balanced registry coverage for such population parameters as region, race, ethnic group, residential and occupational exposures.

The Biometry Branch is in a position to undertake the national coordination of such a registry network because of its past experience in collaboration with the End Results Group. The preparation of national data for incidence and end results and management of this data resource poses no technical problems. Existing procedures for data collection and processing for the End Results Group and for the Third National Cancer Survey appear adequate and will require only minor modifications and adaptation. However, coordination of work on cancer epidemiology and the more sophisticated studies of prognostic factors carried out by Population-based Cancer Study Centers and Comprehensive Cancer Research Centers present a completely new program dimension not confronted hitherto by the Branch. The existing model for data handling represented by work with the End Results Group will not be relevant, since each center will be engaged in a broad panorama of multi-disciplinary research and training activities that is not amenable to centralized direction. A new mechanism to facilitate exchange of information among the research centers and to enable them to develop programs compatible with broad national goals must be developed. A committee which includes the directors of the several research centers as members should be established. We will strive to avoid a rigid committee program and structure in favor of an evolutionary approach which could identify useful committee activities as they develop over time.

End Results Program

The End Results activities now represent one component in a larger registry network, emphasizing population-based registries, known as the SEER Program. While the End Results program as formerly constituted yielded important and significant findings on trends in cancer therapy and associated end results, sustained work with the registry materials has indicated the need for more population-based registry data.

The End Results Section, in publications describing the survival experience of cancer patients, continues to satisfy numerous national demands for this type of information. A major comprehensive summary, End Results in Cancer, Report No. 4 was distributed at the Seventh National Cancer Conference held

in Los Angeles, September 1972. This report provides the most comprehensive set of data now available on the diagnosis and treatment of cancer and the associated survival of patients.

End Results in Cancer, Report No. 4 supplemented by several publications reporting on more detailed investigations of prognostic factors, when combined with the expanded special study opportunities provided by the expanded registry network, make this an opportune time to resume collaboration with cancer registries in other countries. The present U. S. resources will permit the National Cancer Institute to play a leading role in international collaborative efforts. Numerous topics worthy of investigation had been defined in an earlier collaboration with registries in five other countries, the findings from which were summarized in NCI Mon. 15. We plan to resume our role in international comparative studies of end results and given the history of past work it is our belief that establishment of a new international network of collaborating cancer registries within the coming 2-3 years is an attainable goal.

Clinical and Diagnostic Trials

In last year's annual report we discussed our plans to organize a new section devoted to clinical biometry. During the past year the organization of this new section was completed and given the title, "Clinical and Diagnostic Trials Section." The Section was formed about an already existing nucleus of people responsible for statistical operations of the clinical trials conducted by the Veterans Administration Cooperative Urological Research Group. The new section is concerned with protocol design, data recording, collection and editing and methods of routine and special analysis of data from prospective randomized clinical trials of cancer diagnosis and therapy. The Section also acts as a statistical office providing the above services for several large scale clinical trials conducted by outside groups and provides consultation concerning operation and analysis of clinical trials, clinical decision making and related problems to clinicians and scientists of the National Cancer Institute and other institutions. Currently the Section includes four people at the professional level -- two with primary training as Doctors of Medicine and two with Ph.D.s in statistics. Emphasis is placed on developing appropriate statistical methodology for analyzing data from controlled clinical trials.

Migrant Studies

The Branch studies of migrant populations oriented to changes in site-specific cancer risks continued to be supported primarily by research contracts with investigators in the countries of origin. Present work remains focused on migrants from Japan and Norway to the United States, and on intra-country migration in Colombia. The collective evidence assembled continues to support the hypothesis of a dominant role for environmental factors in the sites under investigation. The importance of exposures early in life varies with site and this feature dominates the epidemiology of stomach cancer. On the other hand the sustained rise in colon cancer among Japanese migrants to Hawaii and among Norwegian migrants to the U.S. Mainland to approach prevailing

U.S. levels suggests that exposures in adult life after arrival in the United States have modified the risks for this site, one possibility being that the expression of a precursor state may occur at a younger age. One candidate precursor lesion has been proposed by several investigators -- adenomatous polyps -- can be considered in this connection. Comparative population studies on the congruence of colon cancer and intestinal polyps have been carried out in Colombia, New Orleans, Hawaii (Japanese) and Japan as part of the migrant study program. The distribution of intestinal polyps in populations at high and low risk for large bowel cancer strengthen the view that the two conditions are associated. The distribution of both adenomatous and hyperplastic polyps among the high-risk Hawaiian Japanese parallel closely the New Orleans experience, while the findings from Japan (low risk) portray low polyp prevalence and parallel the results from Colombia. There can be no doubt that among Japanese migrants a profound change in the number and anatomical distribution of adenomatous and hyperplastic polyps has accompanied the marked rise in large bowel cancer risks.

The point which emerges with increasing clarity is that studies of the etiology of colon cancer are unlikely to be resolved by work conducted within the confines of the single population. The broad perspective afforded by comparative population studies is needed to generate etiological hypotheses. Colon is probably not unique in this respect and clarification of the epidemiology of several cancer sites for which etiologic relationships remain obscure may come from comparative study data on sedentes and migrants.

Japan-Hawaii Cancer Study

The case-control studies of gastrointestinal cancer among Japanese migrants in Hawaii and Japan has laid the groundwork for studies on diet and related environmental and host factors that can best be pursued by prospective observations on defined cohorts. The cohort of Japanese males in Hawaii assembled by the National Heart and Lung Institute for its studies of cardiovascular diseases are now being utilized for studies of gastrointestinal cancer. The current protocols cover extensive clinical and pathological observations with emphasis on findings that may elaborate the role of precursor lesions for both stomach and colon cancers.

Problems

The biggest problem in recent years confronting the Biometry Branch has been the need to conduct the Third National Cancer Survey with a smaller staff than originally budgeted. This factor forced modifications in survey plans and retrenchment in the program originally conceived. While the field work phase of the Survey is coming to an end, the Branch continues to suffer from staff shortages in the transitional period. A substantial staff must be deployed to the analysis of data collected in the survey. Simultaneously, however, we must develop a network of population-based cancer registries to produce data needed in the management of the National Cancer Program.

Development of the network, even though the problem will be eased by transforming the Third National Survey operations in some localities to continuing

registries, will require the attention of senior Branch staff in its formative years, therefore, the manpower pressures will persist during the next 3-5 years. The ultimate solution to the Branch problems appears to be through decentralization of much of the analytic epidemiology activities to the population-based study centers. This calls for reliance on the prime contractor concept and a search must be made for prime contractors capable of operating the population-based cancer study centers. However, the use of prime contractors should not be pushed to extreme limit. Effective national coordination of the registry and study center network depends on the retention of a nucleus of investigators within the Biometry Branch capable of developing their own studies and who can simultaneously contribute scientific and management skills to the operation of the overall study program.

The pressure from the tasks just enumerated greatly restricts deployment of personnel into new program areas. We are currently unable to entertain studies of employee populations greatly desired by investigators concerned with occupational carcinogenesis. The migrant studies can pursue only a small fraction of the promising lines of investigation. One activity that can be reinaugurated without imposing too many demands on staff is the reestablishment of the international network of cancer registries for comparative studies of treatment end-results. Previous experience culminating in the symposium held in Norway in 1963, the proceedings of which were published in NCI Monograph 15, provide a sound base on which to build. This activity could form an important part of collaborative international studies for which the National Cancer Institute has a responsibility.

Despite these caveats we can repeat the optimistic assessment of prospects made in the previous annual report. Mathematical statistical developments, many of them made by Branch staff, have yielded improved techniques of experimental design in analysis now being applied to problems of carcinogenesis. The migrant studies are developing specific hypotheses on the etiology of gastrointestinal cancer, and the findings on Japanese migrants for colon cancer have played a key role in the planning of activities projected by the NCI Colon Cancer Segment and the Colon Cancer Task Force. The Third National Cancer Survey is yielding data useful for both epidemiological studies and for planning of cancer control programs. The end-results studies have identified promising leads on prognostic factors in breast cancer and similar results may be forthcoming for other sites. Finally, we take pride in the fact that younger members of the Branch staff continue to assume greater responsibilities for program direction.

Serial No. NCI-4250
1. Biometry Branch, OASDD
   Division of Cancer Cause and Prevention
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Activities during FY 1973

Previous Serial Number: Same

Principal Investigators: William Haenszel, Sidney Cutler, and Nathan Mantel

Other Investigators: David Levin, James Murray, Charles Brown

Man Years:
Total: 7
Professional: 4
Other: 3

Project Description

Objectives:

The principal objectives are to provide general planning and direction for the scientific program of the Biometry Branch, including recruitment and development of staff personnel and coordination of statistical activities.

Methods Employed:

Standard methods of program planning, management, demography, biometry and epidemiology are applied to coordinate the Branch activities and conduct the research projects.

Major Findings:

Development of the National Cancer Program has reinforced the need for a U.S. network of cancer registries for information required for planning and administration and to supply continuing data on incidence and treatment end-results. Much time has been spent in the past year on implementation of the recommendations of the Advisory Committee on Population-based Epidemiology Research Centers, whose key findings and recommendations were:

1. NCI should develop a coordinated program of cancer surveillance, descriptive epidemiology and end-results evaluation. The output of this

multi-purpose program should be the basis for:

(a) prompt identification of secular trends in specific cancers;

(b) identification of variations in disease incidence that may lead to etiological hypotheses;

(c) assessment of the efficiency and quality of the medical care system;

(d) studies by which primary and secondary preventive measures can be accurately measured.

2. Localities participating in the Third National Cancer Survey should be evaluated as potential candidates for the expanded registry network because they have professional and technical personnel trained in relevant methodology, and because of their established rapport with the medical community without which no registry can succeed. Recruitment of additional participants should not, however, be limited to this group.

3. The findings from the Third National Cancer Survey should be used to identify those registries and populations offering the greatest and most immediate study potential.

4. The descriptive incidence data from population-based registries should lay the groundwork for investigations focused on etiology, either in the form of analytic epidemiological studies or animal experiments.

5. The existing End Results Group should constitute the nucleus for an expanded data base on patient survival experience. The Group has a special role to play in evaluation of long-term results of treatment.

6. The objectives enumerated require an optimum mix of participating registries with representation from the following sources:

(a) Individual cancer specialty hospitals (example: Memorial Hospital, New York City) and other university affiliated treatment centers. Such institutions can provide: high quality data on patients with relatively rare tumors; accurate and specific diagnostic and therapeutic information; access to high-risk patients with potentially pre-cancerous conditions and/or atypical findings from routine screening tests.

(b) Comprehensive Cancer Research Centers, which may be loosely defined as cancer specialty hospitals augmented by special research facilities (examples: M.D. Anderson Hospital, Houston; Roswell Park Memorial Institute, Buffalo). These centers provide the milieu for interdisciplinary research and training which are fundamental to advances in cancer control.

(c) Population-based cancer registries. These will provide the bulk of the descriptive incidence and end results data.

(d) Population-based Cancer Study Centers. In addition to maintaining surveillance and reporting on incidence and end results, these centers will conduct epidemiological and clinically oriented studies. They will augment the conventional registry effort with diagnostic, therapeutic, epidemiologic, statistical, laboratory, and other skills on which valid data on incidence and end results depend. Some Population-based Cancer Study Centers may be part of a Comprehensive Cancer Research Center; others may be closely allied with strong departments of medicine, surgery, and pathology with special interests in cancer. The Population-based Study Centers will enhance the opportunities to conduct controlled clinical trials among truly representative samples of cases.

7. NCI research and program goals do not presently require, or even suggest an ultimate need for, the establishment of a large scale program, national in scope, of cancer registration.

Planning and implementation contracts have been written with groups in several key localities (Hawaii, San Francisco Bay Area, Seattle, New Mexico, Connecticut) and others are under active review and negotiation (Iowa, Utah, Detroit, New Orleans). Active steps are being taken to assess other possibilities that can contribute to a more balanced population coverage by region, race, and ethnic group, residential and occupational exposures.

The Biometry Branch can undertake the national coordination of the registry network, because of its previous experience in collaboration with the End Results Group. The preparation of national descriptive data for incidence and end results and management of the data resource poses no technical problems. Existing procedures for data collection and processing for the End Results group and for the Third National Cancer Survey appear adequate and will require only minor modifications and adaptations. The Biometry Branch already has a suitable organizational structure and staff.

The coordination of work in cancer epidemiology to be carried out by population-based cancer study centers and comprehensive cancer research centers does present a completely new dimension for a national program. The existing model for data handling represented by the End Results Group is not relevant, since each center will be engaged in a broad panorama of multidisciplinary research and training activities not amenable to centralized direction and control. The mechanism to facilitate exchange of information among the research centers and to enable them to develop programs compatible with broad national goals should probably be a committee which will include the directors of the several research centers as members. A rigid committee program and structure should not be frozen at the outset and an evolutionary approach which will identify useful committee activities as they develop over time would be preferable.

Mr. Haneszel has devoted substantial amounts of time during the past year to consultation and planning for an expanded NCI program for support of epidemiological research. He is serving as a member of the Epidemiology Committee of the Breast Cancer Task Force and as a member of the Committee for the Colon Cancer Segment established by the NCI Carcinogenesis Area.

Dr. Cutler has been active developing specific plans for epidemiology research centers, particularly in the San Francisco area (5 counties), the Seattle area (4 counties), and in the State of New Mexico. He is serving as a member of the Steering Committee, Breast Cancer Task Force, and was instrumental in organizing a study to assess the contribution of thermography to breast cancer screening (i.e., thermography in addition to physical examination and mammography) involving collaboration between the Health Insurance Plan in New York and Jefferson Medical College in Philadelphia.

Dr. Levin has worked on analysis of the TNCS medical supplement data and on development of the SEER Program. He has worked on problems of design of clinical trials, lung cancer in women, epidemiology of pancreatic cancer, and etiology of esophageal cancer. He is coordinating a revision of the booklet "Cancer Facts and Figures". Dr. Levin is a member of the Medical Oncology Contract Review Committee and has worked with several other areas within NIH including the Special Virus Cancer Program's Immunology-Epidemiology section and researchers from the Medicine Branch of NCI. He has served on the mathematics faculty of the NIH Federal City College Upward Mobility Program.

Mr. Mantel has continued extensive collaboration with other investigators in the National Cancer Institute, notably with those in the Epidemiology Branch (Priester, Fraumeni, Miller) and in the Chemotheraphy area (Goldin, Venditti) as well as with other staff in the Biometry Branch (Byar, Hankey, Myers). As an Associate Editor, later Editorial Advisory Board Member, of Cancer Research and as reviewer for other cancer journals he has provided indirect consultation on the work of many other cancer investigators. An area of special concern has been drug safety, particularly as related to carcinogenic hazards, and in this connection he has been consulted by staff of the Food and Drug Administration (Anello, Friedman) and of the National Center for Toxicological Research (Gaylor). Also he has acted as advisor to staff of the Environmental Protection Agency (Roessler) on the testing of disinfectants. In relation to radiation hazards he has been consulted by Atomic Energy Commission staff (Hollister) and by Bureau of Radiological Health staff (Landau). He has advised the National Institute of Mental Health staff (Levine) on psychotropic drug studies and has been a member of the Technical Advisory Committee of the Federal Advisory Board to the Higher Education Panel, American Council on Education. During the past year Mr. Mantel has been active in development of statistical methodology in various subject matter areas, with particular emphasis on methods useful in identification of factors related to disease occurrence including cancer, comparison of alternate therapies and identification of factors influencing response to therapy. In some of this methodological-development work Mr. Mantel has been joined by Dr. Brown. Dr. Brown has participated in a study on the occurrence of intestinal and stomach cancers and in another study on factors influencing

survival of patients with such cancers. He has participated in a study of infant mortality (Fujikura, National Institute of Neurological Diseases and Stroke), and has collaborated on a genetic counseling problem.

Significance to the Program of the Institute:

The development of data resources and the provision of statistical consultation services are necessary contributions to the National Cancer Program.

Proposed Course of Project:

Planning efforts will be directed toward development of a program consisting of an optimum mixture of various types of population-based cancer registries and cancer research centers. The recommendations of the Ad Hoc Advisory Committee on Population-based Epidemiology Research Centers will continue to be utilized to the fullest extent possible.

Honors and Awards

Cutler, S.:

Member, American Joint Committee on Cancer Staging and End Results Reporting

General Secretary, International Association of Cancer Registries

Adjunct Professor, Department of Biostatistics, Graduate School of Public Health, University of Pittsburgh

Haenszel, W.:

Member, American Cancer Society Advisory Committee on Intra-mural Research

Member, Editorial Advisory Board, Environ Res.

Adjunct Professor of Biostatistics, University of Pittsburgh Graduate School of Public Health

Member, American Association for Cancer Research Committee on Environmental Carcinogenesis

Member, Working Party on Neoplasms for U.S. Subcommittee on 9th Revision of International Classification of Diseases

Member, Epidemiology Committee, Breast Cancer Task Force

Levin, D.L.:

Member, Clinical Trials Contract Review Committee, Chemotherapy, NCI

Mantel, N.:

Member, International Statistical Institute

Fellow, Institute of Mathematical Statistics

Adjunct Professor of Biostatistics, University of Pittsburgh Graduate School of Public Health

Editorial Advisory Board Member, Cancer Research

Member, Technical Advisory Committee, Federal Advisory Board to the Higher Education Panel, American Council on Education

Publications

Haenszel, W.:

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Goldberg, A.L., Glynn, J.P., Kende, M., Mantel, N. and Goldin, A.: A two-stage therapeutic design in the spontaneous AKR lymphoma system. Cancer Research 32, 1321-1328, 1972.

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Goldin, A., Venditti, J.M., and Mantel, N.: Combination Chemotherapy: basic considerations. Handbook of Experimental Pharmacology - Anti-neoplastic and Immunosuppressive Agents. In press

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Kojima, R., Driscoll, J., Mantel, N., and Goldin, A.: Some structure activity relationships for mitomycin derivatives in the treatment of leukemia L1210. Cancer Chemo. Rep. In press

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Mantel, N.: Letter: Quantal bioassay with one animal at a dose level. Biometrics 29, 225-226, 1973.

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Mantel, N. and Brown, C.: Letter: Combining of independent regressor estimates is not paradoxical. The American Statistician. In press

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Serial No. NCI-4254
1. Biometry Branch, OASDD
   Division of Cancer Cause and Prevention
2. End Results Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Studies on Cancer in Defined Populations
(Descriptive and Analytic Epidemiology)

Previous Serial Number: Same

Principal Investigator: Sidney J. Cutler, Roger Connelly

Other Investigators: Joseph Scotto, Herman Heise

Cooperating Units (SEER Program Participants):

California State Department of Public Health
Connecticut State Department of Health
Massachusetts State Department of Health
Michigan Cancer Foundation
University of Iowa
University of New Mexico
Fred Hutchinson Cancer Research Center (Seattle)
University of Utah
Research Corporation of the University of Hawaii

Man Years:

Total:	6
Professional:	4
Other:	2

Project Description

Objectives:

To provide descriptive data on cancer incidence and to investigate the variation in risks for a standard range of demographic variables including region, place of residence, race, nativity, economic status and occupation; to identify promising leads and observational settings for analytical studies to test specific hypotheses on etiological relationships.

Methods Employed:

The descriptive incidence data comes from a group of population-based registries covering defined geographic areas, which record for each cancer, pertinent data on a standard list of characteristics including age, race, sex, place of

residence, cancer site and histologic type, and which periodically produce statistical reports and supply data on individual patients, in accordance with a uniform code, to the Biometry Branch. Some participants (designated as Population-based Cancer Study Centers or Comprehensive Cancer Research Centers in what is known as the SEER Program) have their own epidemiological research units that execute studies to assess specific etiological hypotheses suggested by analysis of their incidence data. Study leads uncovered through analysis of the pooled data are used to develop investigations which may involve a varying number of participants in the SEER Program.

Biometry Branch staff carry on original research utilizing the resources of the SEER Program or through contracts with other organizations having special facilities. All working arrangements emphasize multi-disciplinary approach and collaboration with pathologists is an important component in the migrant study program.

Major Findings:

The SEER Program is a recent development and available findings represent contributions from the two groups -- California State Department of Public Health, Connecticut State Department of Health -- who have had a long history of collaboration with the Biometry Branch.

1. Data from the Connecticut Tumor Registry indicate that incidence of invasive carcinoma of the cervix has decreased from 20 per 100,000 in 1945-49 to 11 per 100,000 in 1965-69. The decrease was most prominent for women 45 to 74 years of age. Over the same time period, incidence of carcinoma in situ of the cervix increased from 1/100,000 to 28/100,000. A large component of this increase was for women 25 to 54 years of age. While the observed reduction in incidence of invasive carcinoma of the cervix may be due to the introduction of cytologic screening and subsequent treatment of premalignant lesions, further study must be done in order to conclusively explain the relationship.

2. Data from the Alameda County, California reporting area were analyzed in parallel with data from other countries, in a study of the distribution of cancers among the sub-sites of the colon and rectum. The pattern of sub-site distribution of the cancers throughout the large bowel is similar in areas of high and intermediate risk; namely, there is a decreasing relative frequency from the ascending colon towards the descending colon and a sharp increase in relative frequency at the sigmoid colon. The incidence of cancer of the rectum is in turn higher than that for the sigmoid colon. In low incidence areas, the general pattern is the same, but there may be a deficit of cancers in the sigmoid colon. Variation in incidence with respect to country and sub-site suggests that the etiologic theory of the transport rate in relation to large bowel cancer risk should be pursued further, with special attention to the incidence of sigmoid colon cancers in low-risk areas.

Significance to the Program of the Institute:

Continuing information on incidence from representative groups of the U.S. population is required so that the nature and magnitude of the cancer problem including changes over time can be determined. Through close scrutiny of variations in cancer incidence with respect to geographic and demographic characteristics of the population and time trends, specific etiologic hypotheses will emerge for testing that may lead to the identification of controllable risk factors.

Proposed Course of the Project:

1. The Connecticut registry will continue to provide data on cancer incidence and mortality as it has since 1935. Negotiations are under way to establish a research unit to more completely exploit the research potential of the registry.

2. The California registry, which has been reporting incidence for Alameda County since 1960, will cover the 5-county San Francisco Bay area continuing work initiated in the Third National Cancer Survey. A research unit, Resource for Cancer Epidemiology, is being developed and will conduct special studies, one of the first underway being a case-control study of diet and colon cancer in blacks.

3. The Massachusetts registry, which reports cancer incidence for a large segment of metropolitan Boston, will develop closer working relationships with the Harvard School of Public Health to promote more research on cancer etiology.

4. Utilization of the Third National Cancer Survey as a foundation for continuing reporting systems and research programs in the State of Iowa and metropolitan Detroit will be pursued. In New Mexico, Utah and Washington, established case reporting systems are being used as a basis for developing epidemiological research programs.

Publications

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Serial No. NCI - 4257

1. Biometry Branch, OASDD, DCCP
2. End Results Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Collaborative Studies of Cancer in Human Populations (End Results)

Previous Serial: Same

Principal Investigators: Sidney J. Cutler, Max H. Myers

Other Investigators: William I. Lourie Jr., Lillian M. Axtell, Herman Heise Ardyce Asire, Evelyn Shambaugh, Benjamin F. Hankey Anne Baranovsky, Stephen M. Baylor, Philip Prorok Charles C. Brown, Debra Silverman.

Cooperating Units: California Tumor Registry; Connecticut Tumor Registry; Massachusetts Cancer Register; Charity Hospital of Louisiana; Indiana University Medical Center; State University of Iowa Hospital; University of California Medical Center; University of Chicago Clinics; University of Michigan Hospital; University of Virginia Hospital; New Mexico Tumor Registry.

Man Years:

Total:	16
Professional:	12
Other:	4

Project Description

Objectives:

To assist other Branches of the National Cancer Institute, other health agencies, and treatment centers in the development and maintenance of programs for the collection and analysis of data on cancer in human populations with special emphasis on the end results of treatment. This includes consultation and advice on methodological problems, development of improved statistical tools for the analysis of observations on human populations, and active collaboration in the collection and analysis of data in a continuing collaborative program and in specific projects.

Methods Employed:

1. The End Results Section staff provides advice on the development and application of uniform definitions and procedures to participating cancer registries, provides consultation on planning and implementing special studies, edits and analyses data submitted to the National Cancer Institute in accordance with a Uniform Code, and explores more efficient data processing and statistical techniques to abstract the maximum amount of useful information from the collected data.

2. The program draws on standard statistical and acturial techniques and accepted principles for records management, with necessary modifications required for any individual study situation.

Major Findings

1. The most recent publication on survival trends, End Results in Cancer, Report No. 4, was based on over 520,000 white patients with cancer diagnosed during the 30-year period, 1940-1969. Observed 5-year survival for all patients diagnosed during the 10-year period, 1955-64 (excluding those with non-melanotic skin cancer) was 33 percent. The corresponding 5-year relative survival rate was 40 percent, i.e. 40 percent of patients expected to survive 5 years (based on normal life expectancy) in fact survived 5 years. Survival for females (median of 2 years) was more favorable than that for males (1 year). This differential was due in part to the fact that relatively poorer prognosis is associated with the most frequently occurring cancer sites in men. Median survival times ranged from over ten years for cancers of the uterine corpus, thyroid gland, salivary gland, testis and eye (these sites represent only 7 percent of all cancers) to less than 5 months for esophagus, stomach, lung, acute leukemia, gallbladder and pancreas. The latter group of sites represents over 20 percent of all cancers. Observed median survival times for 20 selected forms of cancer are given in the following table. An asterisk beside the median indicates those forms for which survival has been improving over time.

	Median survival time
thyroid	greater than 10 years
corpus	greater than 10 years
cervix	7.3 years
melanoma	7.0* years
female breast	6.0 years
bladder	3.2* years
prostate	3.0* years
Hodgkin's disease	2.8* years
colon	2.2 years
rectum	2.0 years
chronic leukemia	19 months
kidney	18 months
ovary	17 months
pharynx	16 months
brain	8 months*
lung and bronchus	5 months
esophagus	5 months
stomach	5 months
acute leukemia	4 months
children (0-14)	9 months*
adults (15 and over)	3 months
pancreas	3 months

Note: These results are for patients diagnosed during 1955-64.

* Continued improvement in survival from early to late 1960's.

2. Prognosis for patients with Hodgkin's disease is a function of a number of patient and disease characteristics such as histologic type, extent of disease (stage), presence of constitutional symptoms prior to diagnosis, age and sex. Utilizing a survival curve adjustment procedure developed by members of the End Results Section staff, each of the specific factors mentioned had a statistically significant relationship to prognosis, even after adjustment for differences in distribution with respect to the remaining factors. For example, for 270 Hodgkin's disease patients seen at the NIH Clinical Center between July 1953 and July 1968, the survival advantage of female to male patients could not be explained away on the basis of a more favorable histologic type, stage, age and symptom distribution. Multivariate analysis of these data was used to estimate median survival as a joint function of sex, symptoms and stage for patients under 50 years of age and for 2 specific histologic types. For patients with the nodular sclerosis form, estimated median survival ranged from a low of 3.4 years for males with symptoms and stage III or IV disease to a high of 13.5 years for females with no symptoms and stage I or II disease. The estimating equation based on 93 Nodular Sclerosis patients was:

$$\text{Estimated Median Years of Survival} = 8.47 + \underset{\text{Sex}}{\begin{matrix} F \\ M \end{matrix}}\begin{bmatrix} +2.31 \\ -2.31 \end{bmatrix} + \underset{\text{Symptoms}}{\begin{matrix} \text{absent} \\ \text{present} \end{matrix}}\begin{bmatrix} +2.15 \\ -2.15 \end{bmatrix} + \underset{\text{Stage}}{\begin{matrix} \text{I or II} \\ \text{III or IV} \end{matrix}}\begin{bmatrix} +0.58 \\ -0.58 \end{bmatrix}$$

A larger group of patients representing the total spectrum of types of Hodgkin's disease must be assembled in order to develop survival estimates with an acceptable level of precision. Such an effort is being undertaken in collaboration with the American Joint Committee for Cancer Staging and End Results Reporting.

3. Prior studies suggested that patients with in-situ breast cancer are characterized by particularly high levels of prognostically favorable immunologic responses. If second breast cancers arising in such patients are antigenically similar, one would expect such cancers to exhibit particularly favorable stage and survival characteristics. Review of patient records and tissue slides available at the Cancer Clinics of the Saskatchewan Cancer Commission indicated that breast cancers arising in patients having a history of precancerous mastopathy were found to have more favorable stage and survival characteristics than breast cancers preceded by normotypic benign lesions, prior invasive cancers, or unselected breast cancers. The findings are consistent with the existence of cross-reacting immunologic responses in patients with in-situ breast cancer. Negative findings in this natural experiment would raise serious questions regarding the ultimate development of effective immunoprophylaxia or immunotherapy of breast cancer.

Significance to the Program of the Institute:

Many of the benefits of the consultation program are long range in nature and consist of setting up record systems and study situations which enlarge the potential study resources of NCI and related organizations. The research objectives of the Institute are promoted by continued work on the integration of various study techniques, e.g., retrospective and prospective studies, analyses of mortality data and morbidity surveys, epidemiologic investigations and cancer registries. An example of the interaction between epidemiologic and end-results investigations are the developing studies of prognostic factors and the role of these factors in cancer etiology.

Proposed course of the project:

1. This project is part of the Collaborative Program for Cancer Surveillance, Epidemiology and End Results Reporting (SEER Program) being developed within the Biometry Branch. Emphasis will continue to be placed on obtaining and reporting data on cancer therapy and associated survival patterns. The base for reporting of end results is being expanded by the addition to the SEER program of registries covering the incidence of cancer in defined populations. The New Mexico Tumor Registry is the participant most recently added to the program. Negotiations for population-based reporting of cancer incidence and end results are under way in New Orleans, Detroit, Utah and Seattle.

2. Activities in the development of special studies on prognostic factors will continue. In some cases these studies will be in collaboration with the American Joint Committee for Cancer Staging and End Results Reporting, in others with cancer specialty centers such as Memorial Hospital (N.Y.C.). With the development of the Resource for Cancer Epidemiology associated with the California Tumor Registry and the proposed Research Unit in Connecticut, the potential for an enlarged special study program will be greatly increased.

Publications

Black, M.M., Cutler, S.J., and Barclay, T.H.: Post-Biopsy Breast Carcinoma: A Natural Experiment in Cancer Immunology. Cancer 29: 61-65, 1972.

Axtell, L.M., Myers, M.H., Berard, C.W., Thomas, L.B., Kagan, R. and Newell, G.: Prognostic Indicators in Hodgkin's Disease. Cancer 29: 1481-1498, 1972.

Black, M.M., Barclay, T.H., Cutler, S.J., and Hankey, B.F.: The Association of Atypical Characteristics of Benign Breast Lesions with Subsequent Risk of Breast Cancer. Cancer 29: 338-343, 1972.

Hyman, B., Myers, M.H., and Schottenfeld, D.: The Relationship of Menstrual Status and other Risk Factors to Recurrence of Carcinoma of the Breast. Amer. J. of Epid. 96: 173-182, 1972.

Axtell, L.M., Cutler, S.J., Myers, M.H., and staff. End Results in Cancer, Report No. 4, DHEW Publication No. (NIH) 73-272.

Myers, M.H.: Breast Cancer Survival Over Three Decades, Recent Results in Cancer Research, Vol. 42, Breast Cancer: A Challenging Problem. Publication Pending.

Myers, M.H., Hankey, B.F., and Mantel, N.: A Logistic-Exponential Model for Use with Response-Time Data Involving Regressor Variables. Publication Pending in Biometrics, June, 1973.

Myers, M.H. and Axtell, L.M.: Statistical Procedures for Evaluating Survival in Hodgkin's Disease. Publication pending in NCI Monograph #36 - International Symposium on Hodgkin's Disease.

Serial No. NCI-4258
1. Biometry Branch, OASDD
   Division of Cancer Cause and Prevention
2. Demography Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Staff Studies of Cancer in Human Populations

Previous Serial Number: Same

Principal Investigator: William Haenszel and Haitung King

Other Investigators: Gary Glober, Frances Locke,
Joseph Moore, John Young

Cooperating Units: University of California Medical School
New England Deaconess Hospital
Lahey Clinic
Connecticut Tumor Registry
Aichi Cancer Institute, Nagoya, Japan
Kuakini Hospital, Honolulu
Cancer Registry of Norway
Universidad del Valle, Cali, Colombia

Man Years:
Total: 7
Professional: 4
Other: 3

Project Description

Objectives:

To describe cancer morbidity and mortality in human populations; to seek out and examine significant differences between persons who develop cancer and those who do not; to search for clues to causative or promoting factors of cancer by comparisions between high- and low-risk populations; an example of the latter being certain migrant groups in the U.S. useful for the study of host and environmental effects in specific cancer sites; to relate epidemiologic features of cancer to survival rates; to identify personal and environmental factors of potential significance in the prevention, control, early diagnosis, or successful treatment of cancer.

Method Employed:

These studies require an extremely wide variety of methods of data collection and analysis, and in many cases new methods must be developed. Where appropriate, these are described in the following sections.

Major Findings:

Ad hoc studies being actively pursued now are limited because of the demands of the Third National Cancer Survey. Extensive analyses are being completed of cancer morbidity among the Chinese in the People's Republic and other areas.

The study of cancer morbidity is based on microscopically examined and/or registered cases during the past 50 years as specified below:

Microscopically Examined Cases

Peking - about 20,000 cases
Shanghai - about 30,000 cases
Taiwan - about 2,000 cases
Hongkong - about 4,600 cases
Singapore - about 8,000 cases

Registered Cases

Shanghai - about 20,000 cases
Hawaii - about 400 cases

Review and analysis of statistical data are nearly completed. Some interesting associations include a possible link of high prevalence of esophageal cancer among Northern Chinese and consumption of ksoliang (a strong drink), the relevance of schistosomiasis in the development of colonic cancer, the occurrence of liver cancer during the intra-uterine life of the fetus, clonorchis sirensis as a liver carcinogen, a noticeably higher rate for female breast cancer among Mainlanders than among Taiwanese, a relatively high frequency of choriocarcinoma and of bone cancer, and an elevated prevalence of skin cancer in North China in relation to the customary use of Kang (oven-bed). Selected findings are reported in two papers.

The study of cancer mortality dealt mainly with U.S. Chinese, based on 1,254 deaths occurring in 1959-1962. Supplementary data for Taiwan, 1959-1960 and 1963-1964, Hongkong, 1961-1964, Singapore, 1959-1962, and British Colombia, 1960-1962 were incorporated into the analysis. Particular emphasis was placed on nativity differentials in mortality. There was a lower risk for nasopharyngeal cancer among U.S.-born than among China-born Chinese. Furthermore, the standardized mortality ratios for U.S. Chinese were intermediate between the high ratio in Hongkong and the low figure reported in Singapore and, particularly, Taiwan. The ranking of these areas seems consistent with what is known of the high risks for nasopharyngeal cancer within China. Specifically, the majority of the Chinese in Hongkong originated in nearby Kwangtung Province, whose capital provides the popular term for nasopharyngeal malignancy, i.e., "Canton Cancer". Although the migrants from Canton predominate in the U.S. Chinese, the intermediate U.S. position may also reflect representation of non-Cantonese populations with lower risks.

The Migrant Studies Program currently consists of four components: Norwegian, Japanese, Colombian and Polish (supported by PL-480 funds in Poland). Observations have established the nature of the changes in cancer-risks in these migrant populations. In all populations efforts are directed toward obtaining comparative epidemiologic and supporting pathological data while exploiting the special capabilities of each collaborator and to take advantage of the experiments of nature represented by migration. The major efforts to date have been directed to stomach, large bowel and prostate.

Stomach. The relation between cancer and premalignant conditions of the stomach has received special attention in Colombia, Hawaii and Japan and the distinction between intestinal and diffuse types of stomach cancer has helped clarify the role of intestinal metaplasia of the gastric mucosa as a possible precursor lesion. Recent review of stomach cancers among Japanese in Hawaii and Miyagi prefecture, Japan revealed that age slopes for log incidence were different for intestinal and diffuse type cancers. For the intestinal type the native and migrant Japanese had the same age slopes, but the curve for Hawaiian-Japanese was displaced to the right. Such observations are helpful in development of hypothesis on pathogenesis.

Case-control studies of stomach cancer and diet have been undertaken for Japanese and Norwegian migrants to the United States. Hawaiian-Japanese cases reported higher consumption of salted and dried fish and pickled "salted" vegetables and given the distribution of secondary amines in these foods a role for nitrosamines can be considered. Some parallels between the Japanese and the Norwegian findings (to be published) exist with respect to the use of processed, salted foods.

A cohort of Japanese males in Hawaii assembled by the National Heart and Lung Institute is being studied for gastrointestinal cancer. One major objective of the cohort studies is an attempt to transform the epidemiology of stomach cancer into the epidemiology of a potential precursor -- intestinal metaplasia. Observations in Colombia, Japan and Poland all agree on strong associations in the distribution of gastritis, gastric ulcers, metaplasia and gastric cancer, much different from the prevailing U.S. picture, where there is no evidence for any association between gastric ulcers and cancers.

Colon. Additional studies of the distribution of intestinal polyps in high- and low-risk populations for large bowel cancer in New Orleans, Honolulu (Japanese) and Japan expanded on the findings for cancer of the colon and rectum and adenomatous polyps published by Haenszel and Correa. Stemmerman and Yatani (Cancer, in press) have reported both the distribution of both the adenomatous and hyperplastic polyps among Hawaiian Japanese to parallel closely the New Orleans experience, while unpublished data from Japan portray a low prevalence of both adenomatous and hyperplastic polpys, which parallel the previous results from Colombia. There can be no doubt that among Japanese migrants a profound change in the number and the anatomical distribution of adenomatous and hyperplastic polyps has accompanied the marked rise in colon cancer risks in the migrant Japanese.

Prostate. The work of Akazaki (JNCI, in press) offers the possibility of resolving anomalous observations on the prevalence of occult prostatic carcinoma and clinically active disease for Japanese populations at home and abroad. Akazaki distinguished between two forms of occult disease -- proliferative, non-proliferative -- and was able to demonstrate a rise in prevalence of the proliferative type among Hawaiian-Japanese consistent with their increased incidence of clinically active disease. An effect limited to a subset of more aggressive appearing occult lesions suggests a role for some unidentified promoting factor.

Significance to the Program of the Institute:

This program helps establish baselines for cancer mortality and morbidity in various populations from which changes can be measured, ascertains trends in risks which may reflect the action of various etiologic agents, identified high- and low-risk population groups which can be studied and compared, and provides observations on human populations useful for correlation with laboratory studies. Ad hoc investigations are undertaken because of unusual or unexpected findings from the routine monitoring of incidence and mortality rates.

The migrant population studies represent a major biometric effort of the Institute. If these studies elucidate the environmental factors involved in the incidence of certain forms of cancer and indicate those which are predominantly environmental and those which are predominantly genetic, it may be possible to apply these findings to the United States population for purposes of cancer prevention and cancer control.

Proposed Course of the Project:

The migrant studies will emphasize analysis of data bearing on the pathogenesis of the major sites under study, particularly large bowel. The findings from the case-control studies of gastrointestinal cancers and diet will be used in developing the protocols for the ongoing cohort study of Hawaii Japanese males and the extension to cohorts in Japan. Field studies using the gastrocamera and other screening techniques of the population at very high risk to stomach cancer in one department of Colombia will receive high priority. Greater efforts in the synthesis of data in hand and being collected are required and this will require closer collaboration with the principal investigators for the several related programs being supported by research contracts.

The immediate course of the other projects is not predictable, since many are undertaken as the need becomes apparent and as staff time is available. A number of studies are in various phases of completion, and the items listed below represent long-term efforts and shortage of staff resources will delay completion until at least the following year.

1. Data have been collected on non-cancer deaths among the Chinese in the same areas described under Major Findings, and will be useful for checking the validity of findings on the relationship between stomach malignancy and duodenal ulcer in Chinese populations. Plans are being made for updating information on various aspects of cancer in Mainland China and for investigating consumption of herbs among the Chinese in selected areas.

2. Ten years of follow-up data are available for 3,500 polyp-free patients, and fifteen years of follow-up data have been collected on an additional 500 patients, all examined in the mid-1950s at the Lahey Clinic, Boston. The Lahey Clinic has collected fifteen years of follow-up data on 300 patients examined during the same time period found to have rectal or colonic polyps at examination. Both the polyp-bearing and polyp-free groups experienced fewer than expected colon and rectal cancers during the first ten years post examination. However, the polyp-bearing group showed a far greater than expected number of these cancers during years eleven to fifteen post examination, so that it is of extreme importance to complete the fifteen-year follow-up for the entire polyp-free series to permit accurate comparisons between the two groups.

3. Approximately 1,000 cases of cancer of the prostate and urinary bladder and matched controls have been interviewed and the records coded, and analysis is well underway. Analysis of data will cover demographic, medical, occupational and diet history. Work has been delayed because of staff commitments to the ongoing Third National Cancer Survey.

4. Investigation of mortality among defined cohorts of clergymen will be continued. Analysis to date demonstrates a reduced risk of death in all age groups and for most major causes of death, including many cancer sites. While the reasons for these reduced risks are not yet known, further accumulation of massive data at both national and international levels would provide useful clues for testing specific hypotheses.

Honors and Awards

Haenszel, W.:

Clinical Associate Professor, Georgetown University School of Medicine

Member, Board of Directors, Washington Statistical Society

Editor for Invited Papers, Biometrics

Reviewer for: Biometrics; Blood; Cancer Chemotherapy; Cancer Research; J.A.M.A.; Technometrics; and Transaction Biomedical Engineering

King, H.:

Advisor-Consultant, D. C. Interagency Council on Smoking

Clinical Professor of Community Medicine and International Health, Professorial Lecturer and Research Associate in Demography, Georgetown University

Reviewer for Journal of Chronic Diseases

Organizer, Symposium on Health Revolution in the People's Republic of China at the 1972 annual meeting of the American Public Health Association

Publications

Haenszel, W.:

Correa, P., Duque, E., Cuello, C. and Haenszel, W.: Polyps of the colon and rectum in Cali, Colombia. International Journal of Cancer 9: 86-96, 1972

Haenszel, W., Kurihara, M., Segi, M. and Lee, R.K.C.: Stomach cancer among Japanese in Hawaii. Journal of the National Cancer Institute 49: 969-988, 1972.

Haenszel, W. and Hougen, A.: Prevalence of respiratory symptoms in Norway. Journal of Chronic Diseases 25: 519-544, 1972.

Haenszel, W. and Correa, P.: A review of epidemiological findings on cancer of the large bowel. Presented at the 71st Annual Meeting of the American Proctologic Society in New York City, June, 1972. Accepted for publication in Diseases of the Colon & Rectum.

King, H.:

King, H.: Cancer Research Organization and Preventive Programs. In Joseph Quinn (ed.): Medicine and Public Health in the People's Republic of China, Fogarty International Center. DHEW Publication No. (NIH) 72-67, 1972.

King, H.: Cancer Research. In Topics of Study Interest in Chinese Medicine and Public Health, Fogarty International Center. DHEW Publication No. (NIH) 72-395, 1972.

Serial No. NCI 4260
1. Biometry Branch, OASDD,
   Division of Cancer Cause and Prevention
2. Clinical and Diagnostic Trials Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Statistical Consultation for the Veterans Administration Urological Research Group

Previous Serial Number: Same

Principal Investigator: David P. Byar

Other Investigators: Mitchell Gail, Larry Muenz, Nell Sedransk

Cooperating Units: V. A. Urological Research Group (26 hospitals)

Man Years:
Total: 7
Professional: 4
Other: 3

Project Description

Objectives:

To provide consultative services in statistical and epidemiological methodology necessary for the various programs sponsored by the Veterans Administration Urological Research Group. This includes assistance in the design, interpretation and evaluation of the clinical trials conducted by the Veterans Administration Urological Research Group, and provision for studies operating concurrently with the clinical trials such as epidemiological studies.

Increased workload and processing data from these clinical trials plus additional responsibilities, particularly in the area of consultation with other outside groups and the arrival of three new staff members has resulted in the creation of a new section, the Clinical and Diagnostic Trials Section. Plans for this Section were noted in last year's report in the Branch Chief's comments under the heading Clinical Biometry.

Methods Employed:

Standard methods of biometry, statistics, probability, and epidemiology with necessary modification as required by the particular problem. New

techniques are developed by the personnel working with the Section to handle specific problems.

Major Findings:

This Section assists the investigators in developing and evaluating methods of treatment for cancers of the male genitourinary tract. Such assistance is generally acknowledged in the publication of findings of the medical investigators. New statistical techniques are published independently.

The Section is more deeply involved in six separate clinical trials:

(a) Main Prostate I, which compares the effect of two treatments (prostatectomy alone, and prostatectomy with estrogen) for Stages I-II, and of four treatments (estrogen, orchiectomy, estrogen plus orchiectomy, and placebo control) for Stages III-IV. The intake of new patients into this study was terminated on March 31, 1967. A total of 2,315 patients had been entered by that date.

(b) Main Prostate II, which also compares the effect of two treatments (prostatectomy alone and placebo control) for Stages I-II and of four treatments (three levels of estrogen and placebo control) for Stages III-IV. This study is designed as a sequel to Main Prostate I. Intake of new patients was terminated in June 1969 after 561 patients had been entered in the study.

(c) Main Prostate III, which compares placebo versus prostatectomy for operable Stage I-II patients. Inoperable Stage I-II patients are randomly assigned to placebo or 1.0 mg. Diethylstilbestrol. All eligible Stage III-IV patients are randomized to one of four treatments: 1.0 mg. Diethylstilbestrol, 30 mg. Provera, 2.5 mg. Premarin, or 30 mg. Provera plus 2.5 mg. Premarin. As of September 1, 1972, 802 patients had been entered in this study.

(d) Focal Carcinoma, which compares several treatments for very early prostatic lesions of the type commonly found in TUR or autopsy specimens from old men. There are now 175 patients in this study.

(e) Urinary Bladder I, which compares surgery alone, radiation alone, and combined therapy for bladder cancer. There have been 72 patients admitted.

(f) Urinary Bladder II, which compares transurethral surgery followed by oral placebo, oral pyridoxine, or topical installation of thio-TEPA in treating Stage I tumors of the bladder. Intake of new patients into this study was begun in November 1971.

In addition to these six clinical trials, the VA group has several other studies for which the National Cancer Institute provides consultation as requested.

The data from two trials -- Main Prostate III, and Urinary Bladder II -- are not yet sufficient for detailed study.

The results of Main Prostate I have already caused widespread changes in the general treatment of prostatic cancer, because of the finding that the standard estrogen treatment for prostatic cancer carries a very great risk of death from cardiovascular diseases. In addition, the clinical benefits with respect to the cancer are smaller than was generally believed. Overall mortality rates in Stages I, II, and III, and in "early" Stage IV are substantially higher in estrogen-treated patients than in those not receiving estrogens. This hazard was not recognized before the results of this study were available.

During the past year, efforts in this project have been concentrated on three objectives: elucidation of the mechanisms by which estrogen exerts its harmful effects, classification of patients with respect to the probable benefits or losses associated with each treatment tested, and studies (Prostate II) on the effects of various doses of diethylstilbestrol.

Preliminary results from Main Prostate II indicate (1) that there is no evidence thus far to show that 5.0 mg. diethylstilbestrol is superior to 1.0 mg. in controlling the cancer in either Stage III or IV, (2) that in Stage III there are substantially fewer heart and cardiovascular deaths on 1.0 mg. diethylstilbestrol compared to the 5.0 mg. dose, (3) that in Stage IV both the 1.0 mg. and 5.0 mg. doses are more effective than the 0.2 mg. dose in controlling the cancer, and (4) that in Stage III the superiority of placebo over the 5.0 mg. dose (and to a lesser extent over the other two doses of diethylstilbestrol) is accounted for by the excess deaths from non-cancer causes in the estrogen treated patients. Taking all these rather complex relationships into account we see no justification for the continued use of 5.0 mg. diethylstilbestrol in either Stage III or IV.

At the time that Main Prostate I was organized there was no reason to suspect an adverse effect of estrogen on the cardiovascular system, so much important information was not collected and submitted in a uniform way. We are now attempting to gather some items retrospectively (pre-treatment EKG, history of heart disease, admission blood pressure, etc.) but the analysis will be difficult. Main Prostate II includes very careful study of the cardiovascular system before treatment and at each follow-up, and may eventually provide answers to this problem. We are currently studying this problem by means of a multivariate Bayesian analysis using ten pre-treatment variables including a number of laboratory values (e.g. cholesterol, fibrinogen, plasminogen) and clinical measurements (e.g. weight and blood pressure). Completion of this work will require extension of existing statistical methodology and testing of the results by half-replication techniques.

It must be noted that cardiologists have not accepted the estrogen findings as readily as urologists. Results of the VA study have been of concern to the National Heart and Lung Institute because of its involvement in the

Coronary Drug Project in which several drugs (including estrogen) are actually being used in the hope of reducing mortality among men with a recent myocardial infarct. NHLI assigned a staff member to make a detailed review of our original records and other data on Stages I-II, where the adverse effect of estrogen is most marked. His review completely supported all of our published conclusions. In November 1970 the first report of the Coronary Drug Project appeared (JAMA 214: 1303, 1970). In this report, data were presented which indicated that 5.0 mg. conjugated estrogen daily led to a significant increase in non-fatal events of myocardial infarction, pulmonary emboli, and thrombophlebitis compared to the placebo group. This treatment has since been dropped from their study.

During the last year we completed and published an analysis of the Focal Carcinoma study and found that almost none of the patients had died of cancer of the prostate. Adjustment procedures revealed that survival of older patients who did not have radical mastectomy was as good as that of the comparable group of younger patients from Study I who did, when age was taken into account.

An analysis was also completed and published of the Urinary Bladder I Study revealing no difference in the three treatment groups being compared. However, there were many flaws in the study, and the number of patients entered was small. Our paper reported the negative findings, and pointed out some of the difficulties of conducting a successful clinical trial in a rapidly progressing disease such as this.

We have also used the data collected from Prostate Study I to examine the general methodological question of analysis of data from clinical trials when not all patients remain on the assigned treatments and how to compare the secondary treatments when the second phase treatment was not part of the original design. A detailed analysis of the data from Study I was completed and published in which we addressed ourself to these problems. Another study completed and published last year was an investigation of the effects of treatments in Prostate Study II on serum testosterone levels in patients with cancer of the prostate. Random samples of 30 patients from each of the stage treatment groups in stages III and IV were selected from the computer records and serum samples of these patients were sent to a special laboratory for testosterone determinations. Statistical analysis of the data carried on in the Section revealed that 1.0 mg. daily of diethylstilbestrol produced a drop in serum testosterone almost equivalent to that of the 5.0 mg. dose although the drop was not quite so prompt. This work is continuing and being extended to the treatments in Prostate Study III.

Significance to the Program of the Institute:

This Section serves the members of the Veterans Administration Urological Research Group by assisting in preparing and revising protocols, forms, and records; giving statistical guidance in the design and execution of clinical

trials; processing and analyzing data that is received daily on patients in the various studies; and assisting the members of the Group with reports on the progress of the various studies.

In return for these services, the Clinical and Diagnostic Trials Section has access to the largest complete set of data available anywhere on the course of prostatic cancer. Over 2,300 patients have been entered in Main Prostate I with follow-up as long as ten years. These data are proving extremely useful and interesting not only in the comparison of treatment effects, but also in defining the general behavior of prostatic cancer in relation to a host of personal, clinical, and laboratory characteristics.

Proposed Course of the Project:

The cooperative studies undertaken jointly by the Veterans Administration and this Section are planned to be a self-generating sequential series to continue as long as fifteen years. Consultative services of this Section have expanded considerably over the past year as the data from Main Prostate I and II have ripened and as patients have accumulated in Main Prostate III.

In the coming year, we will continue to give most of our attention to the analysis of Main Prostate I and II, and especially of the patients in Stages III-IV, since the other stages do not yet include large numbers of patients with long-term follow-up.

Studies of specific cardiovascular effects of estrogen on patients in Main Prostate I have been undertaken, based on data obtained retrospectively from the four largest hospitals in the study. A great deal of effort has been put into preparing and editing these data and they are now ready for a detailed analysis using sophisticated biometric methods. Using data from Prostate Study II we examined the effect of the four doses of estrogen on blood pressure. Our analysis revealed a dose related change after six months of treatment in the patients' pulse pressures. This analysis is now complete and the results are being prepared for publication.

Because the amount of useful data collected in these studies is more than our present staff can adequately analyze, contracts for assistance in the analysis were offered to the Yale University Department of Epidemiology and Public Health. During the past two and a half years extensive work has been done extending work originally begun by us on the use of a multivariate exponential survival model. Specifically, we have studied a step-wise procedure for eliminating variables from the analysis in order of their importance in predicting survival based on the likelihood function. A goodness-of-fit test has been devised for checking the agreement of the model with the observed data, and the model has been tested by means of half-sample pseudoreplication. These results are currently being prepared for publication. We have also investigated the effects of including first order interactions in the model, and have found that with these data they do not add appreciably to the information. We have compared estimates of the variance of the

parameters of the model based on the asymptotic variances with those obtained in half-sample pseudoreplication and found that when estimating three such variances on samples of only 100 observations, the two estimates were in good agreement.

An additional aspect of this work has been the investigation of the concepts of optimal treatment based on a patients presenting signs and symptoms. The application of the exponential model suggests that no one treatment is best for all patients at a given stage but rather that the treatment is best determined by taking into account additional covariate information. During the past year considerable attention has been paid to the theoretical aspects of this problem. As expected the work on this contract will be completed in this its third year, ending in July 1973.

The addition of three new professional staff members to the newly formed Clinical and Diagnostic Trials Section has provided an opportunity for pursuing several aspects of general epidemiological interest in assessing the results of clinical trials. Dr. Larry Muenz, a Ph.D. in statistics, has been studying the properties of the Mantel-Haenszel chi-square statistic when used to compare survival distributions. Dr. Mitchell Gail, an M.D. with considerable sophistication in statistical methodology, has investigated such topics as tests for the ratio of two Poisson parameters, allocation of patients in clinical trials, and subjective value systems for comparing morbidity and making early predictions of survival in clinical trials. Dr. Nell Sedransk, a Ph.D. in statistics who is spending a year with the Section as a Senior Staff Fellow, has completed an investigation of the allocation of experimental units in sequential trials.

Members of the Section have been involved in extensive activities as site visitors and consultants to various programs outside the branch. The Section Head is a member of the Breast Cancer Task Force Treatment Subcommittee and Chairman of the Etiology and Prevention Subcommittee of the National Prostatic Cancer Project Working Cadre. Other members of the Section have been involved in assisting in the design of clinical trial of treatment for testicular tumors and for immunotherapy in malignant melanoma.

During the last year we have continued our contract with the Wolf Research and Development Corporation who assist us in analysis of the large amounts of data at our disposal by providing programming assistance. We are currently using a full time systems analyst and a full time programmer. This contract has been extended for another year. This relationship has been surprisingly convenient and productive.

Serial No. NCI 4260

Honors

David P. Byar, M.D.

Invited Address, First National Conference on Urological Cancer, sponsored by the American Cancer Society, March 30, 1973.

Invited Address, Stanford-Berkeley Joint Statistical Colloquium, April 10, 1973. "Selecting Optimal Treatment Using Concomitant Information"

Invited Address, Case Control and Matching in Epidemiology Studies, Winnipeg, Canada, June 22, 1973.

Mitchell Gail, M.D.

Invited Address, Ciba Symposium, London, England, August, 1972. "Time-lapse Studies on the Motility of Fibroblasts in Tissue Culture"

Nell Sedransk, Ph.D.

Invited Address, Sixth Annual Symposium on Interface Between Statistics and Computer Science, October, 1972. "Guidelines for Evaluating Data Management for Cooperative Clinical Trials"

Publications

Byar, D. P., and The Veterans Administration Cooperative Urological Research Group: The treatment of prostatic cancer: Studies by the Veterans Administration Cooperative Urological Research Group. Bull. New York Acad. of Med. 48 (5): 751-766, June 1972.

Byar, D. P., Mostofi, F. K., and The Veterans Administration Cooperative Urological Research Group: Carcinoma of the prostate: Prognostic evaluation of certain pathological features in 208 radical prostatectomies. Cancer 30 (1): 5-13, July 1972.

Bailar, J. C., III, and Byar, D. P.: Expectant treatment of early inoperable (stage III) carcinoma of the prostate. In Current Controversies in Urologic Management. (R. Scott, Ed.) Philadelphia, W.B. Saunders Company, 1972.

Blackard, C. E., Byar, D. P. and the Veterans Administration Cooperative Urological Research Group: Results of a clinical trial of surgery and irradiation in stages II and III carcinoma of the bladder. J Urol 108 (6): 875-878, December 1972.

Byar, D. P. and the Veterans Administration Cooperative Urological Research Group: Survival of patients with incidentally found microscopic cancer of the prostate: Results of a clinical trial of conservative treatment. J Urol 108 (6): 908-913, December 1972.

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Serial No. NCI-4265

1. Biometry Branch, OASDD, Division of Cancer Cause and Prevention
2. Mathematical Statistics and Applied Mathematics Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Consulting in Statistics and Applied Mathematics

Previous Serial Number: Same

Principal Investigators: John J. Gart, Hugh M. Pettigrew, Robert J. Connor, Donald G. Thomas, M.W.J. Layard

Other Investigators: Jun-mo Nam, Alroy M. Smith

Cooperating Units: None

Man Years:

Total:	3.5
Professional:	3.0
Other:	0.5

Project Description

To provide consulting assistance and collaboration to NCI scientists on the applications of mathematical statistics and applied mathematics to the interpretation and analysis of scientific experiments.

Objectives:

The principal objectives are (1) to collaborate with NCI scientists on mathematical problems related to cancer research; (2) to provide consulting assistance in statistics and applied mathematics to NCI investigator; and (3) to accelerate the use of quantitative methodology in various aspects of the NCI intramural program and extramural program.

Methods Employed

The methodology of applied mathematics, mathematical statistics and probability are applied to biomedical problems. Often variations of existing techniques are developed to suit the special requirements of a particular problem. The members of this section consult with a great many investigators in all areas of intramural research in the NCI.

Major Findings:

During this year the staff consulted with many different branches and laboratories of the NCI,sometimes with several investigators in an individual branch or laboratory. In addition the staff has consulted with other researchers at other Institutes of N.I.H.

In conjunction with The Tobacco Working Group, Dr. Gart has been directing the analysis of experiments directed toward producing a less hazardous cigarette. This involves the extensive and complex analysis of mouse painting experiments at Hazleton Laboratories. One such large analysis involving 7,000 mice has been completed; the second of similar size is in progress. The results of the first skin painting experiment are being correlated with the tobacco characteristics, physical characteristics of the cigarettes, and the chemical characteristics of the condensate and tobacco. The latter data were produced by the U.S. Department of Agriculture, Meloy Laboratories, and the Oak Ridge National Laboratory, respectively.

In relation to the painting studies Mr. Thomas has improved the performance of Breslow' s program for the censored rank test with the addition of a sorting routine, a more reasonable input format that facilitates the combining of samples, and the addition of plots of survival curves. The Weibull curve fitting program was improved with the addition of plots of observed and fitted survival curves.

In relation to the correlation analyses, Mrs. Smith has developed step-down multiple regression programs, both weighted and unweighted. Mr. Thomas has developed a step-up multiple regression program for such data.

Dr. Pettigrew, in conjunction with Dr. Jane Taylor of the Endocrine Evaluation Branch and Dr. Norbert Page of the Carcinogenesis Area, has been providing statistical consultation on the analysis of data gathered under contract by Dr. Albert Segeloff of the Ochsner Clinic, New Orleans, La., to investigate the possible synergism between radiation and estrogen administration in the induction of mammary tumors in A x C rats.

Dr. Connor was invited to serve as statistical consultant for the Program and Data Analysis Unit' s Carcinogenesis Bioassay Data System. He will advise on statistical methods for the data system. Dr. John Cooper, Special Assistant to the Associate Scientific Director Carcinogenesis, and Dr. Norbert Page, of the carcinogenesis area are directing this effort.

Mr. Thomas served as the section' s expert on high speed computing and during the year consulted with every member of the section on many projects which depend on computers. Several useful new computer routines were added to the sections library this year. We began using IBM' s new Time Sharing Option this year and found it very useful in speeding program development. Our computer usage expanded and we acquired a second IBM 2741 remote terminal.

Mr. Nam consulted with Dr. Rogentine of the Immunology Branch on a large scale second study of HL-A blood frequencies, and analyzed the data of 201 controls to determine the distribution of HL-A antigen in normals.

Dr. Gart and Mr. Nam have been consulting with Dr. Ulland of Litton Bionetics and Dr. Elizabeth Weisburger of Biology Branch on a large scale experimental study done on contract at Bionetics and are proceeding with the statistical analysis to determine the potential carcinogenicity of several chemicals.

Mrs. Alroy M. Smith continues to play an important supportive role in the data analysis of this section particularly in writing and implementing statistical analyses on the high speed computers.

For Dr. Gerald Clamon of the Lung Cancer Unit, Dr. Pettigrew is currently analyzing data obtained by performing gel electrophoresis on tissue homogenates prepared from normal and cancerous tissues, in order to see if the tissues differ with respect to isozyme distributions.

For Dr. Pietro Gullino, Laboratory of Biochemistry, NCI, Dr. Pettigrew is analyzing data to study the effect of castration on rats bearing carcinogen-induced mammary tumors.

With Dr. M.A. Chirigos and Dr. John W. Pearson of the Virus and Disease Modification Section, Viral Biology Branch, Dr. Pettigrew has completed the analysis of two experiments to study the effect of immunotherapy on survival of AKR Mice. He is now providing statistical

consultation on another series of experiments combining chemotherapy and immunotherapy, attempting to correlate survival with various measures of the animal's immune response, such as time to rejection of skin grafts.

Dr. Pettigrew has analyzed data of Dr. Gary Pearson of an experiment demonstrating enhanced cytotoxicity of normal spleen cells against MSB and MSC cells in the presence of an immune serum with high antibody titer.

Dr. Pettigrew has consulted with Dr. Samuel Wells, Dr. Roger C. Millar, Dr. James F. Burdick, and Ms. Hilda Wexler of the Surgery Branch on a variety of topics, ranging from a proposed clinical trial for sacroma patients to studies of metastatic behavior in murine tumor systems.

Dr. Connor is continuing as statistical collaborator on a study of immunosuppressive agents and their relation to carcinogens at the Department of Pathology, University of Utah. This project is being done as a contract with Dr. Curtis Harris, Carcinogenesis Area, as the project officer. Dr. Connor has made site visits and expects to assist in the statistical analysis for the study.

Dr. Connor completed his analysis and assisted in the writing of a paper with Dr. D. Lavrin of Bionetics and Drs. S. Rosenberg and W. Terry of the Immunology Branch, on a study of BCG and MER effects on the induction of tumors in mice. The paper is to be published in Cancer Research.

With Dr. Yamamoto, Biology Branch, Mr. Nam analyzed an animal experiment to find the immunosuppression by griseofulvin, an antibiotic substance, and performed a statistical analysis.

Mr. Nam consulted with Dr. Hansen of the Pathology Laboratory on the infectivity of Leukemia patient by Cytomegalovirus (CMV) Hepatitis and analyzed an autopsy data of Leukemia patients withCMV Hepatitis and those without CMV Hepatitis.

Mr. Nam consulted with Dr. Schwartz of the Immunology Branch on the statistical problems in evaluation of the cellular immune responses, and advised on the statistical methods and presentation of data.

Dr. Gart has continued his consultations with Dr. J.H. Robbins of the Dermatology Branch. This research centers on the quantitative typing of patients with xeroderma pigmentosum. He has also advised Dr. Boone of the Cell Biology Section on problems in statistical methodology.

Dr. Connor has advised several scientists for the Biology Branch (e.g. Dr. T. Borsos, Dr. B. Zbar, Dr. R. Bast, Dr. E. Leonard) on problems in the design and analysis of their experiments.

He also has consulted with other NCI researchers on the analysis of their results. They include: Dr. R. Herberman, Dr. J. Cohen, and Dr. R. Oldham of the Laboratory of Cell Biology; Dr. J. Neefe of the Immunology Branch, Dr. D. Key of the Dermatology Branch, Dr. D. Char of General Laboratories and Clinics, Dr. S. Bennett and Dr. R. Hoye of Surgery Branch, and Mr. E. Barkley of Viral Oncology.

Dr. Connor has provided and continues to provide statistical advice on the development of data analysis systems for NCI contract work done at Bionetics.

In connection with the planning of several contracts, Dr. Gart has advised Dr. Owen of the Division of Cancer Cause and Prevention.

Dr. Connor has served as a statistical consultant to a variety of NIH scientists, recommending experimental designs and/or specific statistical methods for the analysis of their research results. These scientists include, Dr. S. Rosen of NIAMDD, Dr. R. Wood of NIAMDD, Dr. J. Niswander of NIDR and Dr. K. Brown of NIDR. Also he completed his analysis of data on a study of Down's syndrome for Dr. M. Coleman and reviewed the statistical analysis for her book reporting this work.

Dr. Gart has advised several investigators outside of NIH on the design and analysis of studies relating to research on tobacco. These include Dr. Bock of the Roswell Park Memorial Institute, Dr. Colburn and Dr. Petrasovits of Canadian National Health and Welfare, Dr. Tso of the U.S. Department of Agriculture, and Lirio Covey and Dr. Ernest Wynder of the American Health Foundation.

Significance of the Problem to the Institute:

Members of this section are assuming an essential role in much research within the NCI. Their activities include not only statistical analysis but also in planning valid experiments.

Proposed Course of the Projects:

Several of the projects mentioned in the Major Findings section will continue. In particular the consultation with the chemical carcinogenesis and the association with the tobacco painting research is expected to continue.

Honors and Awards

Dr. Gart continued to serve as a member of the Biometric and Epidemiological Methodology Advisory Committee of FDA.

Dr. Gart was appointed a member of the Biometry Panel of the Scientific Advisory Board of the National Center for Toxicological Research.

Dr. Pettigrew was an invited participant of the workshop on uniform procedures in cancer research held in Buffalo, New York in November 1972 under the auspices of the American Association of Cancer Institutes.

Dr. Connor served as a referee for The Journal of the National Cancer Institute. Mr. Nam and Dr. Gart served as referees for Cancer Research.

Publications

Gargus, J.L., Gart, J.J., Kwapien, R.P., and Gori, G.B.: Replicate control values in large-scale mouse dermal bioassays of tobacco smoke condensates. Abstract of talk presented to The Society of Toxicology, March 21, 1973.

Kagan, A.R., Hafermann, M., Hamilton, M., Pierce, R., Morton, D. and Johnson, R.: Etiology, diagnosis and management of pericardial effusion after irradiation. Radiol. Clin. Biol. 41: 171-182, 1971.

Lavrin, D.H. Rosenberg, S.A., Connor, R.J. and Terry, W.D.: Immunoprophylaxis of Methylcholanthrene induced tumors in mice using Bacillus Calmette-Guerin (BCG) and Methanol Extracted Residue (MER), Cancer Research, in press, 1973.

Robbins, J.H., Gart, J.J., Levis, W.R. and Burk, P.G.: The millipore filter assay technique for measuring tritiated thymidine incorporation into DNA in leucocyte cultures. Clin. Exp. Immun. 11:629-640, 1972.

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Serial No. NCI-4267

1. Biometry Branch, OASDD, Division of Cancer Cause and Prevention
2. Mathematical Statistics and Applied Mathematics Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Research in Mathematical Statistics and Applied Mathematics

Previous Serial Number: Same

Principal Investigators: John J. Gart, Robert J. Connor, M.W. J. Layard, Hugh M. Pettigrew, Donald G. Thomas

Other Investigators: Jun-mo Nam, Alroy M. Smith

Cooperating Units: None

Man Years:

Total:	3.5
Professional:	3.0
Other:	0.5

Project Description

Objectives:

To conduct basic research in mathematical statistics, probability and applied mathematics; to develop new statistical methodology which is especially appropriate to bio-medical sciences.

Methods Employed:

The methods employed are the modern theories of mathematical statistics, probability and applied mathematics. High speed electronic computers are often used to compute appropriate mathematical tables and test approximations by simulation techniques.

Major Findings:

The Research of the members of this section covers a wide spectrum of topics in mathematical statistics, probability and applied mathematics. These are summarized below.

1. The "variance" test for the Truncated Poisson Distributions.

John J. Gart has been investigating the "variance" test of the Poisson distribution in the case of truncated samples. The results are expressed in terms of ratios of generalized Stirling numbers. The methods are useful for testing the fit of data (radioactivity or other counting data) when the zero reading, for instance, is not observable or relevant. A paper based on these results is being prepared for submission.

2. Grouping and Testing for Trend in Categorical Data.

Robert J. Connor has continued his research efforts as reported last year. In particular, he is nearing completion of his study on grouping for testing trends in categorical data for small sample sizes. This work investigates the effects of number of groups and of the method of grouping.

3. Models and Methods in Gel Electrophoresis.

Hugh M. Pettigrew is engaged in developing models to account for various phenomena associated with the use of gel electrophoresis as a technique for estimating the relative amounts of various isomers of enzymes in tissue homogenates. The models are being tested by experimentation in collaboration with Dr. Gerald Clamon of the Lung Cancer Unit, NCI, and may lead to improved design of gel experiments. Appropriate statistical methodology for the analysis of the resulting data will be developed. If the isozyme patterns in various normal and cancerous tissues can be characterized sufficiently well, then it will be possible to develop methods to test a given tissue homogenate, e.g. from organ culture, for presence of tumor cells.

4. Exact and Asymptotic Methods for the Combination of 2 x 2 Tables.

Donald G. Thomas completed work on a computing program which gives, for a set of combined 2 x 2 tables, the maximum likelihood estimate of the common odds ratio, an exact test for the main effect assuming no interaction, an exact test for interaction and exact confidence limits for the odds ratio. An option is provided for the computation of these results by asymptotic methods for use with large samples. This work has been submitted for publication in Applied Statistics.

5. Detection of Cyclic Trend.

Jun-mo Nam is investigating the proper statistical methods to estimate and test a cyclic trend. These methods, for example, may prove very useful to identify a seasonal trend in disease.

6. Models for cytotoxicity.

Hugh M. Pettigrew is studying (with Dr. Gary Pearson) the properties of experiments to determine the cytotoxicity of spleen cells against target cells in vitro in order to assess the sources of variability in order to increase the precision and reproducibility of results. Experiments will be devised to test any models that are proposed to explain the phenomena involved.

7. Exact Tests in Bioassay.

Donald G. Thomas is developing efficient computer programs to perform an exact test for trend in an m-strata bioassay. Also, an exact test for equality of slopes and equality of intercept in two sample bioassay problems.

8. Validity of Normal Confidence Limits in Bioassay with Small Samples.

Jun-mo Nam is continuing his investigation of the adequacy of nominal confidence limits calculated from the minimum logit χ^2 method and an improvement of those limits for small samples.

9. Sample Sizes in Comparison of Proportions.

John J. Gart has collaborated with Dr. Mitchell Gail on the preparation of a more precise table for determining sample size in experiments comparing proportions. This work will appear shortly in Biometrics.

10. Models for Mammary Tumors in Rodents.

Hugh M. Pettigrew is engaged in developing models for mammary tumor systems in rodents, and considering extensions of existing statistical theory that may be necessary to evaluate data from such systems.

11. Restricted N-Tuple Partitions of M.

Donald G. Thomas developed an efficient computer algorithm which gives all partitions, in lexicographical order, of the number M which are N-tuples with each I-th element of the N-tuple $\geq$ L1(I) and $\leq$ L2(I) where L1(I) and L2(I) are any integers such that L1(I) $\leq$ L2(I) for all I=1 to N $\geq$ 2. This algorithm has been found useful in enumerating complex sample spaces in order to compute exact probabilities.

Significance to the Program of the Institute

The interplay between mathematical theory, data analysis and experimental research is an important element in biomedical research. Many of the "Major Findings" reported above are new statistical techniques which have or may be directly applied to data collected by the medical researchers at NCI or other workers on problems in cancer research. Others are mathematical models which may elucidate the biological phenomena involved in cancer research and which may also aid in the planning of subsequent experiments or epidemiologic studies. The opportunity for initiating fundamental research on mathematics and mathematical statistics is essential for enabling members of the section to achieve professional recognition among their peers in their own scientific disciplines. More importantly, the possibility of doing such unconstrained research is a prerequisite for the consulting work of the section to be carried out at the highest professional level.

Proposed Course of the Project:

Many of the projects described in major findings will be continuing, e.g. such as models and methods for various bioassay systems and exact and approximate analyses of counting data. Other problems, not referred to above, shall also be attacked in the upcoming year; they will include the development of new statistical methods and mathematical models in various biomedical problems, particularly in the area of models for the analysis of survival curves in carcinogenesis experiments.

Honors and Awards

John J. Gart was elected an honorary fellow of the Institute of Mathematical Statistics.

John J. Gart served as an external referee for the Pure and Applied Mathematics Grants Selection Committee of the National Research Council of Canada.

John J. Gart served as program chairman of the Biometric Society for its 1972 Annual meeting in Montreal.

John J. Gart continued to serve as member of the Regional Committee of the Biometric Society.

John J. Gart continued to serve as an Associate Editor of The American Statistician.

Robert J. Connor was an invited lecturer at the University of Illinois at Chicago Circle in March 1973.

John J. Gart was an invited lecturer at The Johns Hopkins University in April 1973.

Hugh M. Pettigrew and Jun-mo Nam served as referees for The American Statistician. John J. Gart served as a referee for The Journal of the American Statistical Association. Donald G. Thomas served as referee for The American Statistician and Applied Statistics.

Publications

Connor, R.J.: Grouping for testing trends in categorical data. J. Am. Stat. Assoc. 67: 601-608, 1972.

Gail, M.H. and Gart, J.J.: The determination of sample sizes for use with the exact conditional test in 2 x 2 comparative trials. Biometrics 29: in press, 1973.

Gart, J.J.: Interaction tests for 2 x s x t contingency tables. Biometrika 59: 309-316, 1972.

Gart, J.J.: The statistical analysis of chain-binomial epidemic models with several kinds of susceptibles. Biometrics 28: 921-930, 1972.

Gart, J.J.: Discussion of paper by D.R. Cox. J. Roy. Stat. Soc. B, 34: 212-213, 1972.

Gart, J.J.: A review of the theory and application of methods for the comparison of proportions. Proc. 17th Conference on the Design of Experiments in Army Research, U.S. Army Research Office, in press, 1973.

Gart, J.J. and Thomas, D.G.: Numerical results on approximate confidence limits on the odds ratio. J. Roy. Stat. Soc. B 34: 441-448, 1972.

Hamilton, M.A.: The stochastic approximation to a discrimination problem. Ann. Math. Stat. 43: 1096-1109, 1972.

Nam, J.: Optimum sample sizes for the comparison of the control and treatment. Biometrics 29: 101-108 , 1973.

Thomas, D.G.: Tests of fit for a one-hit vs. two-hit curve. Appl. Stat. 21: 103-112, 1972.

Serial No. NCI-4268

1. Biometry Branch, OASDD
   Division of Cancer Cause and Prevention
2. Special Cancer Survey Section
3. Bethesda, Maryland

NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Third National Cancer Survey

Previous Serial Number: Same

Principal Investigators: Harvey Geller and Sidney J. Cutler

Other Investigators: Paula Baylis, Roger Connelly, Daniel Cramer, Susan Devesa, J. David Godwin, James Larson, Constance Percy, Joseph Scotto, Theodore Weiss

Cooperating Units: University of Alabama School of Medicine
Georgia Regional Medical Program
State of Colorado Department of Health
University of Texas Southwestern Medical School
Michigan Cancer Foundation
University of Iowa
Mayo Clinic
University of Minnesota, Department of Epidemiology, School of Public Health
University of Pittsburgh, School of Public Health
Puerto Rico Commonwealth Health Department
State of California, Department of Public Health
Iowa State University
Epidemiologic Pathology Unit, NCI
Electronic Data Processing Staff, BB, NCI

Man Years:

Total:	9
Professional:	5
Other:	4

Project Description

Objectives:

To prepare a complete register of all new cancer cases diagnosed in the resident population of seven standard metropolitan areas, two states, and the Commonwealth of Puerto Rico during the period 1969-71, plus all other cancer cases seen by doctors or hospitals in those areas; to obtain demographic data and a description of the tumor for each patient; and to study certain economic social, and other aspects of the disease in a sample of patients.

Extensive use is being made of computer capabilities in the processing of the survey documents. Each document is processed separately, with the computer tagging and combining all the records for an individual patient. The computer is conducting a large number of edit checks--consistency among documents on the same patient, as well as among items on the same document. The sample selection is also a computer operation with the printing of the appropriate labels included in the operation. The data processing time has been reduced to a minimum. Within five weeks after a record is received at NCI, the data are converted on to computer tapes, edit runs are completed, and printouts of error reports and updates of survey control reports are mailed back to the field offices.

Methods Employed:

In each survey area, NCI has contracted with local organizations to hire field staff and gather data. Data are being requested from doctors and obtained from hospital records and death certificates. With approval of their doctors, a sample of cancer patients is being interviewed. Local staffs do the preliminary editing and coding of data, and are responsible for disposition of the original records at the close of the survey. Institute staff in Bethesda, Maryland, complete the editing and coding of data and convert it to magnetic tape for use in preparing detailed reports through electronic computers.

Basic information is being collected on every active cancer case, except certain skin cancers, during the three-year period. This includes race, age, place and date of birth, sex, and marital status. Medical information includes the primary site and histologic type of tumor, date and method of diagnosis. Information available from any existing cancer register or continuing survey in a survey area is being utilized to avoid duplication of effort. All reports are treated as strictly confidential.

Death certificates mentioning cancer are obtained from the appropriate vital statistics office. Local survey staff or hospital personnel abstract information about cancer patients from hospital records.

Trained interviewers, after receiving written permission from attending doctors, obtain from a selected sample of patients information on the amount of disability incurred, time lost from productive activity, cost of treatment, and sources of payment. Questions are also asked regarding religion, number of children, socioeconomic status, and personal habits such as smoking and drinking. Hospital records on this selected population are reviewed for more detailed information on cost of hospitalization, methods of meeting this cost, treatment for the cancer, and the extent of the disease at time of diagnosis.

The main products of this survey will be a detailed analysis of cancer incidence and prevalence rates, the extent of the disease at time of diagnosis, the medical treatment, and the hospital costs. There will also be a large number of special reports either by the survey staff or others with an interest in more detailed analysis of the data being collected. Reports for each individual survey area will also be prepared.

Workshops are conducted periodically to resolve problems which the supervisors have encountered. The format of round table discussions on specific questions and informal discussions among the supervisors have proved very effective in carrying out the instructional and communicative objectives of these workshops.

Editing the data within a document and among documents on the same patient is accomplished by extensive use of the computer. Data are accessed on a weekly cycle and collated into the master file. Each item is reviewed to insure that it is within set limits and that the coded information is a valid code. Other edits include determination of consistency or validity between items on the same abstract form. The computer also checks for consistency in data among the various forms on the same patient. There is a feedback to the survey areas of any invalid, inconsistent, or questionable data. The survey field offices research the problems and correct the data stored in the computer tapes. The data are reviewed periodically to insure that corrective action has been taken by the survey field offices.

As the survey approaches the completion of the data-gathering operation, a variety of reports are being produced to assist in assurance of complete reporting and accuracy of the information. These include 1) tabulations of cases reported by each hospital in each field office area by year so that non-uniformity in numbers can be investigated; 2) listing of cases that require follow-back, such as death certificate abstracts, to insure accuracy of the data; 3) listing of cases that require supplemental data on medical care and hospital costs to insure complete reporting of these cases; and 4) special listing of cases with erroneous or questionable data to insure that the cases are researched and corrections made.

Because of limited staff available, some of the quality control procedures have been modified. The reinterview program was eliminated because of the problems in obtaining the reinterviews, and the lack of feedback either to the survey field office staff or the NCI. The reabstract program has also been modified by eliminating the reabstracting of a random sample. At the present time, reabstracting is accomplished at the same time as the initial abstract, and comparisons are made at the hospital with the records available.

A special skin cancer survey was started September 1, 1971, which collected data on cases diagnosed through February 29, 1972. Only four of the survey areas--Iowa, Dallas, Minneapolis, and San Francisco--are participating in this special study. Special effort was made to contact all physicians who would see or treat skin cancer patients to obtain their cooperation. Pathology laboratory files will supply data on cases that were histologically diagnosed. In addition, abstractors will complete separate forms for skin cancer patients found during their normal visits to participating hospitals.

This special study is being handled as a separate operation. A separate report will be published presenting the skin cancer data as soon as the data are tabulated and analyzed.

Major Findings:

A preliminary report based on the 1969 incidence data was distributed to the National Cancer Advisory Council in October, 1971. The report showed that 61,410 cancers were diagnosed in the survey areas (excluding the non-Denver portion of Colorado, and Puerto Rico) during 1969. Since the survey areas include ten percent of the total United States population, it is estimated that approximately 610,000 new cancers were diagnosed in that year. The report further showed that when the rates for sex and race are adjusted to the age distribution of the United States in 1970, the incidence rate for the black population (338 per 100,000) is substantially higher than for the white population (311 per 100,000). The sites with markedly higher rates among black males are the prostate and the esophagus. Among females, the black females have higher rates for both invasive and in situ carcinoma of the cervix uteri, while the white females had higher rates for cancer of the breast and corpus uteri.

While the areas covered by the present survey are not exactly the same as the areas covered in the survey of 1947, a number of trends are worthy of note. Adjusting the rates to the United States 1950 population, the incidence rates among males increased from 280 in 1947 to 304 in 1969, while the rates among females decreased from 294 to 256 (excluding carcinoma of the cervix uteri) for the same time periods. The increase in the male rate is due largely to substantial increase in the incidence of cancers of the lung and prostate, and a somewhat lesser increase in incidence of colon cancer. These increases more than counterbalanced the drop in incidence of gastric and rectal cancers. The decrease in female rate is the result of a drop in the incidence of cancers of the cervix uteri (invasive), stomach, and rectum.

The data also showed that when the survey areas were divided into three geographic regions--North, South, and West--cancer of the lung, prostate, digestive tract, breast, and uterus demonstrated geographic differences.

Preliminary findings from the special skin cancer survey indicate that the estimated incidence of the site is grossly understated. Even in the survey area with the lowest rate, non-final tabulations indicate more than twice the number of cases than expected. Because of this large increase of cases over expected, the data are being reviewed with a number of consultants. It is anticipated that final publication of the findings will be completed by the end of this fiscal year.

A preliminary report on the hospital costs over a two-year period for cases with a single primary, diagnosed in 1969, was produced in December, 1972. This report showed that the average length of stay for the first admission to a hospital was 17 days. The average payment to the hospital by the patient, insurance companies, Medicare or Medicaid was $1,520. The length of stay and payments to hospital varied according to the site of the cancer, the lowest, both in length of stay and payments, being for patients with carcinoma in situ of the cervix (7 days and $642 in payments), and the highest, for length of stay, was for patients with cancer of the brain and nervous system (27 days). The highest payments occurred for patients with cancer of the stomach ($2,249).

During the two-year period, the average number of admissions per patient was 1.7. During this two-year period, $7.2 million was paid for 5,235 admissions by 3,151 sample patients, for an average payment of $2,289. Applying these figures to the estimated 1.3 million cancer patients under medical care in 1969, there was $1.8 billion paid to hospitals for hospitalization of cancer patients. Due to the fact that almost half of the number of cancer patients are over age 65, it was not surprising that the major source of hospital payments was Medicare, which accounted for 41.1 percent of the payments. Blue Cross with 22.0 percent of the payments, and private insurance companies with 18.1 percent of the payments, were the other major sources of hospital payments. On the average, the patient himself had to pay only 6.7 percent of the hospital bill out of his own funds.

Significance of the Program to the Institute:

Registration of all cancer cases in a defined population is the only way to acquire reliable data on cancer incidence rates. The rates themselves are indicators of the significance of cancers in the various sites or of different types. This data bank would also provide a valuable source for epidemiologic investigations by identifying population groups that have a high or a low incidence of cancers of specific sites. Some of the factors that would be studied include residence, occupation, place of birth, use of contraceptive devices, and the use of tobacco and alcohol.

Other products of the survey that will be important to the mission of the National Cancer Institute will be studies on the delivery of medical care to a large and representative sample of cancer patients, treatment provided the cancer patient, extent of disease at time of diagnosis, direct and indirect costs of cancer, and the extent and duration of disability from cancer.

The survey will produce unique data which can be used by other investigators as a starting point for more intensive investigations. One area of special interest is rare tumors. Because of the low numbers of these tumors, epidemiologists feel that investigations will yield significant results and possible etiologic leads.

The data contained in the preliminary report, and the additional tabulations generated from the 1969 incidence data have been a valuable source of information not available anywhere else in the country. As a result, a large number of queries have been received on cancer sites and histology to which we are now able to respond with data. The information has also been useful in program planning and in preparing reports for NCI, NIH and HEW use. The survey data have also indicated there were errors in estimates of incidence by site used by various cancer agencies. The extent of the data being collected will enable us to provide a more sensitive method for determining estimates of the incidence of cancer in the future.

Proposed Course of the Project:

This project is in the final phases of the data-gathering operation. A timetable has been set so that all abstracting, follow-back on missing documents or data, collecting sample data, and correcting errors will be completed by

the end of August, 1973. It is planned to continue to produce, at frequent intervals, computer listings and tabulations that will assist in this final data-gathering and correcting effort. These listings and tabulations will include such information as cases that require follow-back for initial information as a result of a death certificate listing cancer, but with no hospital or doctor report for that case; or a pathology report of cancer, but with no hospital report for that case; sample cases with supplemental data missing, indicated by a medical or financial supplement with no corresponding document on file; or a hospital abstract, but with no supplement forms on file for that hospitalization; comparisons of reporting by year for each hospital to indicate where there may be cases missing.

The usual error and inconsistency reports will continue to be produced with renotification to field offices of errors not yet corrected or inconsistencies not yet resolved.

The special skin cancer survey is in the final phases of data-gathering and data correction. As soon as these operations have been completed, tabulations will be run using previously planned programs. A number of meetings have been held with various consultants to review the preliminary findings and to further plan the analysis and presentation of the data. Because of requests from such agencies as the Department of Transportation and NASA, the findings will be presented as soon as possible. However, because the survey areas were not randomly selected and the findings indicate significantly higher incidence rates than previous estimates, the data should be reviewed and methods of estimating national figures evaluated by consultants.

A good deal of effort was spent obtaining data on medical costs and medical care on the sample cases. The procedures developed as a result of this effort have been evaluated and are now part of the standard operations. The main change is that we are requiring this supplemental data for a two-year period after diagnosis for 1969 and 1970 incidence cases, and for one year for 1971 incidence cases. For cases diagnosed prior to 1969, the two- and one-year periods start from the date the patient was seen for the cancer after January 1, 1969. This means that hospital records for activity in 1972 had to be examined, which presented some problems in some of the hospitals.

The preliminary report on the hospital costs was used to evaluate the method of handling the data. Some problems still exist which have to be investigated. Some deal with the question of presenting cost data for patients who die during this follow-up period. Others deal with how the hospital bills are paid. In some private insurance cases, the patient was paid by the insurance company directly, and he then paid the hospital. This is not reflected in the information received from the hospital financial reports.

The interview data will require attention. Some preliminary hand tabulations demonstrated there were inconsistencies between the data reported in the interview and data reported on the financial supplement. Other portions of the interview appear to be complete, and the data seem valid. The procedure for data conversion is being investigated by the systems analysts and should be on computer tapes in the near future. This has a low priority and will be undertaken when time is available.

It was decided at the meeting of the Advisory committees and principal investigators that some core staff remain at each of the survey areas. This staff would be used to complete any phases of operation that remain, to collect special data if required for additional studies, and to complete tabulations and analysis of the local field office data for feedback to the community. It was also decided that a utilization committee be established to assist in the analysis and presentation of the basic information, and to effectuate ultimate utilization of the survey data by identifying areas which could yield significant information by a more detailed analysis, and by indicating special studies using the survey data as a data source.

It has been proposed that a report be developed as soon as possible presenting the survey data on as much detail as the preliminary report. This would get wide distribution and acquaint the medical community of the availability of the data. Special site-specific studies will also be undertaken with findings published as they are completed. These special studies could be accumulated in a monograph at some future date so that all of the survey findings and analyses will be together.

Honors and Awards:

None

Publications:

Preliminary Report, Third National Cancer Survey, 1969 Incidence, September 9, 1971.

Preliminary Report, Third National Cancer Survey, Payments to Hospitals, December 4, 1972.

Serial No. NCI-4269
1. Biometry Branch, OASDD, DCCP
2. Automatic Data Processing Management Section (ADPMS)
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Data and Information Processing Consultation and Assistance

Previous Serial Number : Same

Principal Investigator: Theodore Weiss

Other Investigators: Guy D. Brunetto, Altamease A. Gales, Terence D. C. Kuch, James E. Larson, Frank W. McKay, Norman Oslik, James M. Stump

Cooperating Units: None

Man Years:

Total: 8
Professional: 6
Others: 2

Project Description

The Automatic Data Processing Management Section provides consultation and assistance to the scientific and administrative staff of the Division of Cancer Cause and Prevention. It has expertise in applying data and information processing technologies to individual as well as cooperative epidemiologic, laboratory, and clinical research projects, program planning and appraisal activities, and administrative management operations.

Objective:

To further the research effort through the appropriate application of computer science and information technology; and through minimizing the mechanical and logistical constraints upon investigators arising from scientific investigations which involve data and information collection, conversion to machine-readable form, purification, computation, storage, retrieval, analysis, and dissemination.

Methods Employed:

Consultation and assistance is provided to research projects in three disciplinary areas:

Planning and Appraisal: participation in the planning and evaluation of research projects which have data and information processing activities; assisting investigators and their research contractors develop sources of computer-related services; conducting studies and disseminating information on the feasibility of using computer-related techniques, such as optical scanning versus key-to-disk or keypunching for data capture, computer-related generalized proprietary software packages versus custom-designed programs and systems, and computer-related equipment, such as on-line communications terminals; and developing standards of quality and performance of data and information processing products and services provided by research contractors, the ADPMS, and commercial ADP contractors;

Information Systems Development and Statistical Computing: providing technical assistance through forms design, systems analysis, systems design, the preparation of reference and procedure manuals, and computer programming services using digital computers, scientific computers with analog-digital converters, and related electronic instrumentation; providing generalized software systems and programs for investigator's own use; and conducting workshops and individual instruction in the use of these programs and systems; and

Operational Monitoring and Control: coordinating and monitoring the conversion of raw data into forms appropriate for computer processing and the day-to-day operation of data processing systems and activities; and maintaining and operating a Remote Job Entry Terminal Facility with such equipment as the IBM 360/20.

Major Findings:

Demography Area

There have been a number of accomplishments this fiscal year toward improving the library of data processing and statistical computing programs and systems available as generalized investigative instruments; toward improving the accessibility and capability of data processing equipment and services; and toward improving the content and delivery of information to Program Directors for their planning, budgeting, and evaluation of their computer services.

- A conversational mode (i.e., on-line time-sharing, interactive between the user and the computer) generalized file management, statistical computing, and analytical graphics system, the Conversational Computing Statistical System (CCSS) was installed at NIH by the ADPMS, Mr. James M. Stump, principal data processing consultant, and the Epidemiology

Branch, NICHHD, and DCRT. This system, developed by the Department of Biostatistics at the University of Washington allows an investigator with little or no technical knowledge of computers to input, edit, calculate, and output his data through a "conversation" with the computer, it prompting the user much as the "Wylbur" system does for text editing functions. The selection of the CCSS system was based on a review of software in use at a number of biomedical computer centers.

- The Generalized Reporting Program (GRP), installed by Mr. Stump, was modified to further reduce the amount of programming required to use this program on several research projects. GRP allows an investigator to write a computer program using a few simple instructions (in a non-conversational mode) to generate formatted listings and tables from a single computerized data file. The program and instructions to execute the program may be entered via the remote on-line typewriter terminal system (WYLBUR).

- A statistical computing and tablemaking system (Tablemaker) was designed by Mr. Stump and implemented under his direction. Tablemaker allows an investigator to write a computer program using a few simple instructions (in a non-conversational mode) to calculate frequency counts and age-adjusted and age-specific incidence rates and produce formatted tables containing these data.

- Two contracts were implemented through the efforts of Mr. Stump to provide systems design and computer programming services to meet specific data handling and analytical workloads in the Demography Area.

- A Remote Job Entry Terminal Facility became operational in February 1973 in the Landow Building. It was constructed according to specifications developed by Mr. Weiss. The Facility contains an IBM 360/20 (Remote 17) with card reader and high speed printer, a number of IBM 2741 communication terminals, a keypunch/card interpreter, and a sorter-counter. A production desk type service is operated by the ADPMS. In addition, a central Landow-DCRT pickup and delivery service operates from the Facility. Remote 17 is funded jointly by the National Heart and Lung Institute and the National Cancer Institute.

The ADP Management Section provided consultation and assistance to eight Demography Area research projects during the year:

The Third National Cancer Survey

Consultation, as well as extensive systems analysis, computer programming and operational services were provided under the direction of Mr. James E. Larson, principal data processing consultant, to the Special Cancer Survey Section in support of the data processing and preliminary data analysis phases of the Third National Cancer Survey. This included directing all program maintenance of the Survey's data processing system, and monitoring and coordinating the day-to-day data processing operations involving the conversion of data from approximately one million documents to machine-readable form, and the processing of these data by computer. In addition, a considerable number of new programs and sub systems were developed to support special operational and analytical requirements. Sub systems developed this year were used to produce and analyze 1970/71 incidence, payments to hospitals, treatment modalities, the incidence of skin cancer, and to make comparisons with the 1947 Ten City Survey.

The Japan-Hawaii Cancer Study

A comprehensive and flexible data processing system, encompassing clinical, laboratory, and epidemiologic activities and data has been developed by the ADPMS for the Japan-Hawaii Cancer Study. (See Japan-Hawaii Cancer Study Project Report, Biometry Branch, NCI, for a description of the research project.) This system is now operational in Hawaii. The principal data processing consultant and systems designer is Mr. Norman Oslik. He is presently on assignment in Hawaii for approximately one year to get all aspects of the system operational on a routine basis, and to recruit and train a systems analyst/programmer to maintain the system in the future. Appropriate portions of the system have been installed at the Kuakini Clinic, various hospitals and specimen processing laboratories, a specimen repository, the University of Hawaii Computer Center, and at a commercial keypunching facility.

At the inception of the project in 1971, when the scope of the data processing effort was recognized, two essential decisions were made. The first was to obtain a general software contract for computer programming services. This contract was terminated in February 1973 as these services were no longer required. The second decision, following a study on the subject, was to use an existing generalized data management computer system to eliminate some of the routine computer programming that otherwise would have been required to implement a system for the Japan-Hawaii Cancer Study. The system selected is the National Information Processing System (NIPS), originally developed by IBM for the Department of Defense.

Several diverse objectives were identified for the design, implementation, and operation of this system. The automated system had to be developed

and implemented in a relatively short time period to handle approximately thirty separate data collection forms, many of them multipage forms. A record linkage system had to be devised that would facilitate access to, and retrieval of patient data from a large number of data sources. The system had to be constructed to easily incorporate new and modified data collection forms, and their corresponding edits and consistency checks for new sub-studies and clinical and laboratory procedures that were not definable at the inception of the main study.

Presently the system has the capability of adding to and retrieving from the computer file, data from any of twenty-seven data collection forms, including patient history questionnaires, physical examination reports, laboratory reports, pathology reports, and hospital surveillance abstracts. The addition of new data collection forms into the Study in the future can be accomplished with a minimum of impact on the existing system.

In addition, because of the experimental nature of some of the laboratory tests, it was considered essential to have a method of verifying the accuracy of the test procedures. A unique specimen and laboratory number will be assigned to each specimen, that is separate and distinct from the identification number of the patient, which will not accompany the specimen to the laboratory. Occasionally, a duplicate specimen will be sent to the laboratory and the results compared to the first test to determine if proper techniques are being employed.

Clinic procedural and reference manuals have been developed and implemented for collecting data, for establishing control over documents to prevent loss during keypunching operations or afterwards, for sampling data to detect coding and keypunching errors beyond the capability of the computer edit, and for all other clerical and professional activities.

The confidentiality of the data collected has been of utmost concern. Various procedures have been implemented to protect the data. All persons working on the Study are required to sign confidentiality pledges indicating that they will not divulge the identity of any subject to persons outside the Study staff. All data are stored in record rooms that are kept locked when Clinic personnel are not present. Computer printouts which contain name and address data and which are no longer used are incinerated with other Kuakini Hospital confidential records. Data in the computer are stored in two separate files. One file contains all of the data collected for a subject, but not his name and address. The other file contains only name and address data. In the future, an additional safeguard will be implemented to encode the stored name and address data and decode it only when used by the JHCS system. The actual computer tape would be unintelligible to anyone who did not know the decoding algorithm.

Output for preliminary and continuing analyses, as well as for reference purposes are being produced to aid in the operation of the Study. All of the data collected are added to the computer files. After each monthly

update of the file, computer-generated letters containing appropriate test results are produced and sent to the subjects and their physicians. Also monthly, edit errors detected by the computer are researched and the data files corrected using a relatively simple computer file change document that can be coded to correct any item of data on file.

One of the critical aspects of data analysis involves the use of the data collected in the Honolulu Heart Study from 1965-1972. That data, previously available in twelve different computer files, have been reorganized into a single file; and along with data currently available from the Third Examination are currently the subject of various preliminary analyses using existing statistical programs (e.g., UCLA BMD), and others specifically written for this Study.

Data processing work is continuing in several areas. Maintenance of the JHCS computer system will be an ever present task. This includes the design of new data collection forms and their addition to the computer system, writing edit routines for the new data, correcting programming errors, making refinements to existing programs, if appropriate, and making adjustments required by changes in software or hardware at the University of Hawaii computer center.

Clerical and automated procedures for family linkage will need to be developed. These procedures will establish links between data records to allow the retrieval of data of blood relatives for genetic studies. The population data base created by Dr. M. P. Mi of the University of Hawaii, Department of Genetics is being considered a source of information for this aspect of the Study.

Finally, a package of computer programs for analysis will have to be developed using existing programs wherever possible.

Continuing Demography Studies

The Japanese Migrant Studies, Norwegian Migrant Study and the Study of Cancer Among Retired Railroad Employees received systems analysis and computer programming support from the ADPMS. Mr. Stump served as the principal data processing consultant on these projects. Miss Altamease Gales wrote several file management and statistical programs for each project. (For a description of the research see Project Reports, Biometry Branch, NCI.) Pathology data was added to the Japanese Migrant Studies Stomach Cancer File using an update program written for this project. The Generalized Report Program (GRP) was adapted to process the Colon/Rectum File and a variety of reports were produced for analytical purposes. New death information was added to the migrant/sibling records of the Norwegian Migrant Study File, and a considerable recoding of data was required in order to facilitate analysis. Also, data files containing the control group of British migrants and U. S. native born citizens were reformatted and standardized for use in this study. Subsets of the computer files for the Study of Cancer Among Retired Railroad Employees, which consists of 1.5 million records, were produced to allow for a more economical analysis of the data. In addition, the Generalized Report Program (GRP) was adapted for use in this study.

U. S. Cancer Mortality Study

Mr. Frank W. McKay developed and implemented two computer systems for the Epidemiology Branch's U. S. Mortality Study. One system produces race, sex, site, and age analyses of cancer mortality for the total United States. The other produces similar tables for individual counties.

An extensive ADP technical effort, directed by Mr. McKay, reformatted and recoded the NCHS U. S. Mortality and U. S. Census Population files for use by these systems.

Study of Cancer in Renal Transplant Patients

The American College of Surgeons-NIH Organ Transplant Registry in Chicago, Illinois, collects demographic and clinical data on human renal transplant patients from clinical centers throughout the world. Data on all renal transplants performed during the period 1952-71 have been extracted from the Registry's files and are the subject of a study of cancer in these patients. Mr. Stump is assisting the Epidemiology Branch on this study. Using incidence rates obtained from the End Results Program/Connecticut Cancer Registry data, Mr. Stump modified and expanded an existing computer system to calculate expected rates of cancer incidence (by site) for this patient population, and to produce a variety of reports on selected demographic and clinical characteristics.

Veterinary Medical Data Program

The Epidemiology Branch, Epizoology Section has been collecting and analyzing clinical data submitted by participating veterinary institutions throughout the country since 1964. An existing data processing system, comprised of ten computer programs, was modified to accommodate an expanded data collection form, to incorporate more extensive data edits, to produce additional reports, and to expedite processing and reduce operational costs.

Mr. Stump developed the detailed specifications for these modifications, prepared a Request for Proposal for the required programming services, and participated in the contractor selection process. He and Mr. Guy D. Brunetto coordinated the activities of the data conversion contractor, the programming contractor, and the NIH computer center to assure the timely completion of the work.

Carcinogenesis Area

The Program and Data Analysis Unit in the Carcinogenesis Area received assistance from the ADPMS during the fiscal year. Mr. Terence D. C. Kuch served as the Project Officer on a contract to operate a (sub) system for document storage and retrieval services in support of the Carcinogenesis Bioassay Data System (CBDS). This (sub) system was developed in the preceding reporting year by Mr. Kuch and involved conducting a comprehensive study of current document storage and retrieval techniques and equipment, the development of detailed (sub) system specifications, the recommendation of special

microfilming and retrieval equipment, the preparation of a comprehensive RFP, and participation in the selection of a contractor to operate the (sub) system.

Other consultations in the Carcinogenesis Program included advising a research contractor on the preparation, organization, analysis, and submission of bioassay data to the CBDS; to the Experimental Pathology Branch on the computerization of statistical analyses of experimental data from vitamin B12 studies; to the Biology Branch on obtaining a contractor to develop a method for, and to computerize the calculation of molecular orbits of known and suspected carcinogens; to the Information and Resources Segment Advisory Group as a participating member of the Advisory Group; and to various investigators on an ad hoc basis.

Viral Oncology Area

Consultation and assistance have been provided the Office of Program Analysis and Communications by Mr. Theodore Weiss to assure continuity of expertise and services in the systems planning and further development and implementation of the Special Virus Cancer Program Biological Resources Information System. A comprehensive staffing plan, workplan, and Request for Proposal including task statements, was prepared for this office. This effort was coordinated with the Contracting Office and the General Services Administration, whose Basic Ordering Agreement with commercial suppliers of software was used to obtain the expertise and services required.

In addition, the Immunology and Epidemiology Segment, Special Virus Cancer Program was advised by Mr. Weiss on automating the data storage and analysis aspects of various research projects.

Administrative Management Section, Office of the Scientific Director

Each month the ADP Management Section updates its computer files containing contract summary data for the Division of Cancer Cause and Prevention and produces the Contract Commitment Report. This is a fairly extensive operation because of the amount of contract activity.

The system developed by the ADPMS for budgeting and reporting on expenditures for computer services produces and distributes to the Division's Program and Project Directors the monthly report, "Summary of Division of Computer Research and Technology Costs". It is being expanded to furnish information to individual users of computer services, and to report on the utilization of ADP on-line equipment, such as the "Wylbur" terminals and IBM 360/20 Remote Job Entry Terminal.

Significance to Biomedical Research and the Program of the Institute:

The systematic capture, organization, and display of complex and diverse data are of considerable importance in the planning, conduct, and management of the research efforts. As multidisciplinary and collaborative activities increase in scope and complexity, the problems of linking, manipulating, analyzing, and communicating large quantities of data and information become unmanageable without the assistance of computer-related technology.

Proposed Course of the Project:

Consultation and technical support will continue to be provided to the Division's research activities. The level of support is expected to increase as the National Cancer Plan is implemented.

Honors and Awards

Weiss, T.: Member, Contractor Selection Committee, Office of the Associate Director for Program Planning and Analysis, Office of the Director, NCI, National Cancer Program Management Information System; Member, Contractor Selection Committee, Division of Cancer Control, NCI, Breast Cancer Demonstration Project Central Coordinating and Statistical Center; Member, Coordinating Committee on Data Processing, Association of American Cancer Institutes; Member, Grant Application Review Committee, Division of Cancer Grants, NCI, Cancer Institute of Philadelphia, Department of Biostatistics and Computing; Member, Contract Review Committee, NHLI, Central Coordinating Center for Lipid Research Studies, University of North Carolina; Member, Contractor Selection Committee, BHME, Division of Nursing, Computer Services; Speaker and Panel Member, 1973 Regional Computer Users Forum and Exposition, Washington, D. C., sponsored by Computerworld, subject Contracting for Software Services.

Kuch, T.D.C.: Consultant to Secretary's Advisory Committee on Automated Personal Data Systems, DHEW; Member, Advisory Panel, Humanization of Information Systems (Computing Science Programme, Simon Fraser University); Member, Thesaurus Committee, Engineers' Joint Council; Board of Directors, Association of Computer Programmers and Analysts; Board of Directors, The Population Society (American University); Member, American National Standards Institute X3K5, Information Processing Vocabulary; Reviewer for Installation Management Reviews (Association for Computing Machinery); Chairman, Committee for Intersociety coordination, Special Interest Group on Computers and Society (Association for Computing Machinery); Consultant, Federal Trade Commission, Record-Linkage Abuses in Commercial Credit Systems.

Publications

Kuch,T.D.C.: Professionalism. National Conference of the Association of Computer Progrvmmers and Analysts, 1971. (Invited Paper).

Kuch,T.D.C.: A Study of Position Titles in the Computer Systems Field. Springfield, Va.: National Technical Information Service, 1972.

CONTRACT NARRATIVE
BIOMETRY BRANCH
DIVISION OF CANCER CAUSE AND PREVENTION
Fiscal Year 1973

Office of the Chief

CALIFORNIA DEPARTMENT OF PUBLIC HEALTH (64-873)
KUAKINI HOSPITAL AND HOME (71-2170)
AICHI CANCER CENTER RESEARCH INSTITUTE (72-3213)

Title: Study of Cancer Among Japanese Migrants

Contractor's Project Director: Dr. John E. Dunn, Jr. (64-873)
Dr. Grant N. Stemmermann (71-2170)
Professor Mitsuo Segi (72-3213)

Project Officer (NCI): Mr. William Haenszel

Objectives: The purpose of these contracts is to collect data bearing on the reasons for the differences in cancer incidence between Japanese on the home islands and the Japanese migrant populations in Hawaii and California. The objective is to sort out those aspects of common cancers which may be genetically involved and those which may derive from aspects of the environment or some mixture of the two.

Methods Employed: Standard demographic techniques are employed, basically using a system of diagnosed cases and matched controls. In addition, data have been collected from samples of Japanese households. The matched case-control technique is particularly useful in this kind of study. Pathology protocols are being completed for cases in the study series for several sites (stomach, colon, rectum) and considerable emphasis is being placed on the correlation of the epidemiological and pathology findings. In preparation for later case-control studies of prostate cancer, latent prostate cancer is being studied in 5 groups of patients.

Major Findings: The cancer risks for Japanese migrants differ from those observed in Japan; the migrant risks are higher for lung, colon, rectum, prostate and lower for stomach while little change has been observed for breast among post-menopausal women. The case-control studies show an excess consumption of salted and dried fish and pickled "salted" vegetables some of which are rich in secondary amines, by stomach cancer patients. Since nitrites and secondary amines can be synthesized *in vivo* into nitrosamines, a potent carcinogen, a possible etiology is suggested for further study. Latent prostate cancer is almost as common in Japanese as in Americans though Japanese have little clinical cancer of this site. A possible resolution of this anomly has been contributed by Dr. Akazaki, who has described and defined two types of latent carcinoma - proliferative and non-proliferative. The gradient in prevalence of the proliferative type lesion in his autopsy studies of specimens from Japan and Hawaii Japanese

corresponds closely to the gradient in incidence of clinical prostatic carcinoma in the two populations. The important feature of the Hawaii Japanese males is the greater amount of proliferation and not their unremarkable overall prevalence of occult lesions, which suggests the presence of some promoting factor in the migrant male population.

Significance to NCI Program and Biomedical Research: If the studies in these migrant populations elucidate the environmental factors involved in the incidence of certain forms of cancer, and indicate those that are predominantly environmental and those which are predominantly genetic, then it may be possible to apply these findings to the United States population for purposes of cancer prevention and cancer control.

Proposed Course of the Project: The contract with Aichi Cancer Center Research Institute has been initiated to continue Dr. Segi's work in Japan after his move from Tohoku University. The contract with Tohoku University has been terminated. A new NCI contract has been started with Kuakini Hospital and Home, separate from the NHLI contract, to assure proper support and direction of the important clinical and pathological cancer research being performed at Kuakini.

Date Contract Initiated:	May	1964 (64-873)
	June	1971 (71-2170)
	February	1972 (72-3213)
Current Annual Level:	$ 100,000	(64-873)
	$ 638,813	(71-2170)
	$ 127,329	(72-3213)

CONTRACT NARRATIVE
BIOMETRY BRANCH
DIVISION OF CANCER CAUSE AND PREVENTION
Fiscal Year 1973

Office of the Chief

UNIVERSITY OF CALIFORNIA AT LOS ANGELES (NIH-NCI-E-72-3209)

Title: Fecal Flora Studies

Contractor's Project Director: Dr. Sydney M. Finegold

Project Officer (NCI): Dr. Sidney J. Silverman

Objectives: Determine differences that may exist in the intestinal microflora of individuals on a traditional Japanese diet; on a Western-type diet; and when possible, individuals with intestinal polyps or early proven carcinoma of the bowel.

Major Findings: Studies of the fecal flora has been made to date on 23 subjects, 13 of whom ate one or more traditionally Japanese meals daily. Differences in the bacterial population were noted from that reported for persons on a Western-type diet: Escherichia coli and Clostridia were found in greater numbers in the feces of the Japanese. The species of Fusobacterium were different and changes in the numbers of these organisms was related to changes in the diet of the individual.

Significance to Biomedical Research and the Program of the Institute: This study is one facet in the program of the Colon Cancer Segment that seeks to establish the etiology of colonic cancer. It is an attempt to establish in the laboratory the bases for the epidemiological findings that correlate diet with intestinal carcinomas. It is a source of bacterial cultures for the in-house study (at FCRC) to determine whether intestinal organisms are capable of producing carcinogenic substances from endogenous or exongenous substances present in the gut.

Proposed Course: The work under this contract will be discontinued because of insufficient funds.

Date Contract Inititated: March 1, 1972

Current Annual Level: $71,930

CONTRACT NARRATIVE
BIOMETRY BRANCH, DCCP
Fiscal Year 1973

END RESULTS SECTION

CALIFORNIA DEPARTMENT OF PUBLIC HEALTH (69-5)
UNIVERSITY OF CALIFORNIA (73-2001)
CHARITY HOSPITAL OF LOUISIANA (64-83)
UNIVERSITY OF CHICAGO (73-3203)
CONNECTICUT STATE DEPARTMENT OF PUBLIC HEALTH (63-1148)
INDIANA UNIVERSITY HOSPITAL (70-2000)
STATE UNIVERSITY OF IOWA (64-15)
MASSACHUSETTS HEALTH RESEARCH INSTITUTE (72-2077)
UNIVERSITY OF MICHIGAN (73-3206)
UNIVERSITY OF VIRGINIA (67-13)
NEW YORK MEDICAL COLLEGE (71-2424)
UNIVERSITY OF NEW MEXICO (72-3235)
SASKATCHEWAN, CANADA (REGINA) (72-3276)
CHARITY HOSPITAL OF LOUISIANA (72-3301)

Title: End Results Evaluation

Project Officer (NCI): Dr. Sidney J. Cutler

Objectives: To obtain and analyze data on cancer incidence, survival and mortality in order to study:

1. variation in incidence and/or mortality with respect to geographic and demographic characteristics,

2. changes over time in the use of different methods of therapy and diagnostic criteria,

3. the relative effectiveness of various treatment modalities as judged by patient survival, and any changes over time in survival.

To organize special studies to investigate in more detail findings revealed by analysis of the routinely collected data.

Methods Employed: The National Cancer Institute is sponsoring a Collaborative Program for Cancer Surveillance, Epidemiology and End Results Reporting (SEER Program). Participants in this program include both population-based and medical center registries so that both incidence data and survival data can be based on as broad a cross-section of the U.S. population as possible. The Biometry Branch has professional and technical responsibility for supervising these contracts. Information on individual patients is submitted to the Biometry Branch, according to a prescribed schedule, and in accordance with a uniform code. This program makes available to the National Cancer Institute information on a very large series of patients in a form which permits

maximum flexibility in handling and analyzing the data, and also provides an extensive resource for special studies on selected series of cases. Partial support for the population-based registries and for registries in medical center hospitals is provided through the contract mechanism to make it possible for the participating registries to meet specified criteria regarding completeness, currency, and accuracy of information as well as to promote epidemiological investigation.

Significance to NCI Program and Bio-Medical Research: Information on incidence, survival, and mortality from a representative cross-section of the U.S. population is required on a continuing basis so that the nature and magnitude of the cancer problem including changes over time can be determined. Through close scrutiny of variation in cancer incidence and survival with respect to geographic and demographic characteristics of the population and differential changes over time, specific etiologic hypotheses will emerge which when tested via special study mechanism should then lead to the identification of controllable risk factors. Such research is an integral part of the ultimate goal of the National Program: the reduction in the occurrence of and mortality due to cancer.

Major Findings: Our most recent publication on survival trends, End Results in Cancer, Report No. 4, was based on over 520,000 white patients with cancer diagnosed 1940-69. Observed 5-year survival for all patients diagnosed 1955-64 (excluding those with non-melanotic skin cancer) was 33 percent. The corresponding 5-year relative survival rate was 40 percent, i.e. 40 percent of patients expected to survive 5 years (based on normal life expectancy) in fact survived 5 years. Survival for females (median of 2 years) was more favorable than that for males (1 year). This differential was due in part to the fact that relatively poorer prognosis is associated with the most frequently occurring cancer sites in men. Median survival times ranged from over ten years for cancers of the uterine corpus, thyroid gland, salivary gland, testis and eye (these sites represent only 7 percent of all cancers) to less than 5 months for esophagus, stomach, lung, acute leukemia, gallbladder and pancreas. The latter group of sites represents over 20 percent of all cancers. Observed median survival times for 20 selected forms of cancer are given in the following table. An asterisk beside the median indicates those forms for which survival has been improving over time.

	Median survival time
thyroid	greater than 10 years
corpus	greater than 10 years
cervix	7.3 years
malanoma	7.0* years
female breast	6.0 years
bladder	3.2* years
prostate	3.0* years
Hodgkin's disease	2.8* years
colon	2.2 years
rectum	2.0 years
chronic leukemia	19 months
kidney	18 months
ovary	17 months
pharynx	16 months
brain	8 months*
lung and bronchus	5 months
esophagus	5 months
stomach	5 months
acute leukemia	4 months
children (0-14)	9 months*
adults (15 and over)	3 months
pancreas	3 months

Note: These results are for patients diagnosed during 1955-64.

* Continued improvement in survival from early to late 1960's.

Proposed Course of Project: Continued attention will be given to reducing the time lag in reporting incidence data and in reporting survival data for disease entities for which therapeutic practice is changing rapidly, e.g., leukemia and Hodgkin's disease. Greater emphasis will be placed on developing additional population-based registries to provide a resource for a wide variety of epidemiologic and clinical studies.

	Date Initiated	FY-73 Negotiated
CALIFORNIA DEPARTMENT OF PUBLIC HEALTH (69-5)	7-1-62	$482,600
UNIVERSITY OF CALIFORNIA (73-2001)	11-1-72	14,480
CHARITY HOSPITAL OF LOUISIANA (64-83)	10-1-63	52,230
UNIVERSITY OF CHICAGO (73-3203)	7-1-72	42,120
CONNECTICUT STATE DEPT. OF PUBLIC HEALTH (63-1148)	6-1-63	262,056
INDIANA UNIVERSITY HOSPITAL (70-2000)	7-1-63	75,367
STATE UNIVERSITY OF IOWA (64-15)	7-1-63	33,656
MASSACHUSETTS HEALTH RESEARCH INSTITUTE (72-2077)	1-1-72	142,237
UNIVERSITY OF MICHIGAN (73-3206)	10-1-72	36,790
UNIVERSITY OF VIRGINIA (67-13)	7-1-66	41,572
NEW YORK MEDICAL COLLEGE (71-2424)	6-24-71	30,000*
UNIVERSITY OF NEW MEXICO (72-3235)	4-14-72	169,000*
SASKATCHEWAN, CANADA (REGINA) (72-3276)	6-21-72	10,000*
CHARITY HOSPITAL OF LOUISIANA (72-3301)	6-29-72	75,000*

* FY-73 Estimate

CONTRACT NARRATIVE
BIOMETRY BRANCH
DIVISION OF CANCER CAUSE AND PREVENTION
FISCAL YEAR 1973

Office of the Chief

HEALTH INSURANCE PLAN OF GREATER NEW YORK (69-88)

Title: Evaluation of Periodic Breast Cancer Screening by Mammography

Contractor's Project Director: Mr. Sam Shapiro

Project Officer (NCI): Dr. Sidney Cutler

Objectives: A. To determine whether a simple screening technique (x-ray mammography) can diagnose breast cancer early.

B. To determine if the very early diagnosis of breast cancer is really useful in improving long-term survival.

C. To investigate through a prospective study of the women screened, the relationship of a wide range of parameters to the development of breast cancer.

Methods Employed: This is a combination statistical-epidemiological-medical study in which a study population receiving mammography at annual intervals and a control group of 30,000 women each are being routinely followed for breast cancer experience through internal Health Insurance Plan files and through review of death certificates. Details of the techniques are given in a paper describing the methodology and initial findings of this study entitled, "Evaluation of Periodic Breast Cancer Screening with Mammography" by Sam Shapiro, Philip Strax, and Louis Venet, JAMA 195: 731-738, 1966.

During one reexamination cycle 40 cc of blood was taken from women in the study population and stored.

Major Findings: Findings to date strongly suggest the usefulness of annual screening which includes mammography. Over the short run period of 5 years of follow-up, the study group of women have about a one-third mortality from breast cancer than those in the control group. This holds whether the comparison is based on deaths due to breast cancer during the 5 years following the entry to the investigation, 63, control vs. 40, study; or on case fatality rates for the 5 years of follow-up after diagnosis, 42%, control vs. 28% study (1 year's lead time in diagnosis of breast cancer due to screening taken into account). The differential between control and study cases in their fatality rates is almost entirely due to the exceptionally low rate (17%) among the cases detected through screening. Both the clinical examination and mammography contributed to this favorable situation but mammography, with a fatality rate of only 2% among cases it detected in the absence of a positive clinical finding

during screening, was especially important. In examining various risk factors for the women who appeared for an initial screening the following women were found to have a 20-30% increase in risk of breast cancer: higher educated women, Jewish women, and unmarried women. Increasing age at first pregnancy, low gravidity, and menarche at ages under 15 are associated with a 50-100% increase in risk.

Significance to NCI Program & Bio-Medical Research: Early diagnostic procedures may be of use in the early treatment and potential cure of cancer patients. If it develops that mammography is such a technique, it then could be extended to other populations and would then have the effect of reducing the mortality from breast cancer within the United States.

Proposed Course of Project: The screening part of the study is complete and the emphasis from now on will be on follow-up. The women will be followed for as long a time period as necessary to demonstrate whether the technique has any usefulness as any early diagnostic device or whether its usefulness is limited. The duration of the study is contingent upon the answers to the questions implied by the objectives.

Supplemental lines of inquiry may be suggested by the Breast Cancer Task Force. Proposals for use of the stored blood plasma are being developed.

Date Current Contract Initiated: 11-1-62

Current Annual Level: $158,244

CONTRACT NARRATIVE
BIOMETRY BRANCH
DIVISION OF CANCER CAUSE AND PREVENTION
Fiscal Year 1973

Office of the Chief

ISRAEL CENTER FOR REGISTRATION OF CANCER AND ALLIED DISEASES (NIH-NCI-E-72-3272

Title: Cancer Incidence Study and Registry Assessment

Contractor's Project Director: Dr. Ruth Steinitz

Project Officer (NCI): Dr. Sidney J. Cutler

Objectives: Approved as part of a three year project, the first two years are devoted to collection of cancer incidence data so that a report in the third year may analyze such cancer incidence for the 12 years 1960-71 with complete coverage of the population of Israel. As the study progresses, additional reports are produced starting with assessment of the registry's collecting system and in the third year assessment of the data processing system. Therefore, part of the objectives are to indicate what changes are required to produce useful epidemiologic data more quickly.

Major Findings: During the first year changes in personnel practice have been instituted and some changes in technology. The collection and processing of data are on schedule.

Significance to Biomedical Research and the Program of the Institute: Because of the large number of ethnic and immigrant groups, Israeli cancer data have unique epidemiologic potential and fit well into the Branch's collaborative study program involving changes in incidence and mortality after migration. Of major importance is the fact that specific differences in cancer incidence between population subgroups are reported from Israel which far exceed those found between different population groups in the U. S. The population-based cancer registry can also be integrated in the long run with the "SEERR" Program (Collaborative Program for Cancer Surveillance, Epidemiology, and End Results Reporting) based upon domestic cancer data.

Proposed Course: This contract is expected to be renewed annually until the report due at the end of three years is received, after which a critical review will be made to see whether further support is justified in establishing specific epidemiologic studies.

Date Contract Initiated: June 28, 1972

Current Contract Level: $28,271

CONTRACT NARRATIVE
BIOMETRY BRANCH
DIVISION OF CANCER CAUSE AND PREVENTION
Fiscal Year 1973

Office of the Chief

JOHNS HOPKINS UNIVERSITY (71-2422)

Title: Epidemiological Study of Rare Sites of Cancer

Contractor's Project Director: Dr. Abraham M. Lilienfeld

Project Officer (NCI): Mr. William Haenszel

Objectives: To elucidate the causal factors in the development of selected rare cancers in patient groups which are large enought to make the findings valid and reliable. The rare sites to be studied will be liver, gallbladder, islet cell tumors of the pancreas, renal pelvis, nasopharynx, adrenal gland, testis, penis, scrotum, vagina, vulva, and male breast.

Methods Employed: The investigation is in the form of a case-control study. The appropriate cases and matched controls are identified, interviews are obtained and medical records checked. A total of five areas are included, two of which are also part of the Third National Cancer Survey: Buffalo, Detroit, Miami, Minneapolis-St. Paul, and New York City.

Major Findings: The major finding of this study so far is that it takes a great deal of time and effort to locate new cases with these rare cancers as well as appropriate controls. It is also difficult to find the new cases soon enough after diagnosis to interview them while still in the hospital. The original plan to work through the case finding systems established by the field offices of the Third National Cancer Survey did not prove feasible, and therefore, other arrangements were made in other metropolitan areas. Less than 100 cases of any one rare cancer site have been found since the inception of the study in June 1971 and adequate numbers of controls have not been obtained.

Significance to NCI Program & Bio-Medical Research: This study was designed to fill the information gap regarding the causative and related factors associated with the rare types of cancer. New knowledge from such a study could be useful in the control of the more common types of cancer as well.

Proposed Course of Project: Because of both technical and administrative problems, the contract is being phased out during a terminal year period which provides for orderly conclusion of the field work and adequate time for analysis of the data in hand.

Date Contract Initiated: 6-28-71

Current Annual Level: $100,000

CONTRACT NARRATIVE
BIOMETRY BRANCH
DIVISION OF CANCER CAUSE AND PREVENTION
Fiscal Year 1973

Office of the Chief
(With Colon Cancer Segment, Carcinogenesis)

LOUISIANA STATE UNIVERSITY MEDICAL CENTER (NIH-71-2324)

Title: Premalignant Lesions in the Large Intestine

Contractor's Project Director: Professor Jack P. Strong

Project Officer (NCI): Dr. Pelayo Correa

Objective: To obtain data on the prevalence of various kinds of epithelial intestinal tumors in white and black Americans corresponding to data obtained or being obtained from other populations of epidemiologic interest.

Methods Employed: Tumors are identified by a careful protocol study of bowel specimens obtained at autopsy.

Major Findings: A preliminary study indicated that the prevalence of small tumors is quite different in populations of low and high risk for colon cancer. A workshop was held to evaluate inter-observer variation in the interpretation of lesions. The number of cases accumulated so far should be sufficient to estimate the prevalence in population subgroups and for specific anatomic regions of the colon.

Significance to NCI Program and Biomedical Research: It is hoped to identify those epithelial lesions which are epidemiologically related to colon cancer. This will not only provide clues on the pathogenesis but identify individuals at high risk for colon cancer and provide a signal of exposure closer in time to the contact of patient and environmental factors.

Proposed Course: It is expected that the data will be analyzed this year and this will indicate the future course of the project. More information is needed on the progress made to date and the outcome of other exploratory work like the one on DNA content.

Date Contract Initiated: June 1, 1971

Current Annual Level: $33,000

CONTRACT NARRATIVE
BIOMETRY BRANCH
DIVISION OF CANCER CAUSE AND PREVENTION
Fiscal Year 1973

ADP Management Section

MARYLAND NATIONAL OPTIMATION SERVICE, INC. (NIH-71-2050)

Title: Data Conversion for the Third National Cancer Survey

Contractor's Project Director: Daniel Wallingsford

Project Officer (NCI): Theodore Weiss

Objectives: Convert the data on approximately seven hundred and fifty thousand Third National Cancer Survey documents to magnetic tape.

Major Findings: Data is being converted by the optical character recognition method (OCR). The contractor is using a system of procedures and computer programs specifically designed for him by the Institute. The system includes extensive security control over documents and information, a pre-edit of completed Survey documents, and manual and computer edit checks of the data transcription process.

Significance to Biomedical Research and the Program of the Institute: See Third National Cancer Survey Project Report, Special Cancer Survey Section, Biometry Branch,NCI.

Proposed Course: Contract is expected to terminate at the completion of the data collection phase of the Survey (approximately September 30, 1973).

Date Contract Initiated: October 19, 1970

Current Contract Level: $214,857

CONTRACT NARRATIVE
BIOMETRY BRANCH
DIVISION OF CANCER CAUSE AND PREVENTION
Fiscal Year 1973

Office of the Chief

NORWEGIAN PUBLIC HEALTH SERVICE (64-499)
UNIVERSITY OF MINNESOTA (66-919)

Title: Incidence, Prevalence and Mortality from Cancer in Selected Migrant Populations (Norwegian)

Contractor's Project Director: Dr. Einar Pedersen (64-499)
Dr. Leonard Schuman (66-919)

Project Officer (NCI): Mr. William Haenszel

Objectives: To determine the reason for difference in cancer incidence and mortality in sedente populations and migrant populations of the same ethnic and genetic composition.

Methods Employed: Standard epidemiologic-demographic methods including prospective observations on defined cohorts and case-control studies.

Major Findings: Magnetic tapes of all data collected during the initial phase of the migrant study have been sent to NCI for analysis. Personal identification numbers have been obtained for 99% of the sibling and general population cohorts and the groups are now considered ready for follow-up study of mortality and cancer incidence. A paper on "Prevalence of Respiratory Symptoms in Norway" by Haenszel and Hougen has been published by the Journal of Chronic Diseases (Vol. 25, pp. 519-544, 1972).

Data from the dietary surveys in Norway and the United States have been analyzed by Dr. Bjelke and will be included in his thesis to the University of Minnesota. Dietary data for men in the general population cohort and sibling cohort have been compared with similar data for the Norwegian-born, and Minnesota residents in the American samples. The comparisons reflect the dietary changes brought about by migration and prolonged stay in the United States. Marked regional variation in current dietary habits in Norway is reflected in variations according to region of birth among the Norwegian-born in the United States.

Description and analysis of the case-control studies in Norway and Minnesota have continued and will be included in Dr. Bjelke's thesis. Results from the histopathologic typing of stomach carcinomas for cases in both studies, carried out by Dr. Stalsberg, were entered on the magnetic data files in September 1971 and, when pertinent, have been included in the analyses. Selected findings for stomach cancer were presented in papers read at a Nordic Cancer Union Symposium on stomach cancer held in Helsinki.

Significance to NCI Program & Bio-Medical Research: This is one of a group of migrant studies to determine those elements in the incidence and mortality from cancer which may be related to environment and those which may be related to genetic factors. Isolation of environmental elements may be useful in cancer prevention within the United States.

Proposed Course of Project: This project in Norway and Minnesota will continue for several more years. The basic migrant study will continue and analysis of the data on respiratory cancers and gastrointestinal cancers will be accelerated. Plans are being made to extend the survival analysis of end results data collected by the Registry during the period 1953-67. New studies may also be started on breast cancer, thyroid cancer and Hodgkin's disease.

The University of Minnesota has defined a cohort of approximately 20,000 policyholders in an insurance company that has a high proportion of persons of Norwegian descent and will monitor the group prospectively for mortality data. The contractor has also carried out case-control interviews for gastrointestinal cancer among persons of Norwegian descent in Minnesota and is prepared to extend field work to other sites at a later date. All phases of the cohort and case-control studies in Minnesota are closely coordinated with the ongoing work in Norway. One investigator from the Cancer Registry of Norway is in residence almost continuously at the University of Minnesota to assure close liaison.

Date Contract Initiated: March 1964 (64-499)
June 1966 (66-919)

Current Annual Level: $64,854
$13,278

CONTRACT NARRATIVE
BIOMETRY BRANCH
DIVISION OF CANCER CAUSE AND PREVENTION
Fiscal Year 1973

ADP Management Section

PRICE, WILLIAMS & ASSOCIATES (NIH-NCI-E-73-3227)

Title: Computer Programming Support for the Veterinary Medical Data Program

Contractor's Project Director: Paul Williams

Project Officer (NCI): Michael Stump

Objectives: To modify the Veterinary Medical Data Program computer system.

Major Findings: The contractor modified a system of ten computer programs necessitated by the adoption of a new input form, and additional coding and output requirements. The system flow was also improved to expedite processing and reduce operational costs. A complete documentation package reflecting the modifications was prepared.

Significance to Biomedical Research and the Program of the Institute: See Veterinary Medical Data Program Project Report, Epizoology Section, Epidemiology Branch, NCI.

Proposed Course: The support represented by this contract is expected to continue through April 30, 1973.

Date Contract Initiated: December 13, 1972

Current Contract Level: $11,664

CONTRACT NARRATIVE
BIOMETRY BRANCH
DIVISION OF CANCER CAUSE AND PREVENTION
Fiscal Year 1973

Office of the Chief

RESEARCH CORPORATION OF UNIVERSITY OF HAWAII (71-2208)

Title: Demographic Cancer Research and Training Program in Hawaii

Contractor's Project Director: Dr. Richard K. C. Lee

Project Officer (NCI): Mr. William Haenszel

Objectives: The objective of this project is to plan and develop a Demographic Cancer Research and Training Program in Hawaii. This Program will form a basis for a future comprehensive cancer research center.

Methods Employed: Standard methods of planning, programming and budgeting, as well as personnel recruitment and research management are employed.

Major Findings: The work is based on current plans as described in the progress report prepared under this contract which outlines the goals of the Demographic Cancer Research and Training Program and the specific objectives for both the research and training aspects of the program. Several people are being recruited to fill positions in the developing program. The Hawaii Tumor Registry is being supported to improve its operation and data base. It will eventually have a rapid incidence reporting system, and will become a vital and integral part of the new cancer research center program. The Data and Computation Program at the University has developed a computerized population-based family file for all citizens of Hawaii. It contains demographic data and vital statistics on the population for the past 30 years, and these records are being linked via computer with new vital statistics data and Tumor Registry data, and may also be linked with data from the Hawaii Cooperative Chemotherapy Program. The Training Program included M.P.H., Ph.D. and residency programs which are available to research associates working on other parts of the Demography Program in Hawaii. The case-control study of Japanese migrants conducted under contract Ph 43-63-558 is now included in this contract and is still under the direction of Dr. Lee. The cooperative arrangements with Kuakini Hospital are continuing to facilitate the interviewing and pathology work on both studies.

Significance to NCI Program and Biomedical Research: The development of a Demographic Cancer Research and Training Program in Hawaii is directly related to the Demography Program plans for development of population-based cancer registries as recommended by the Ad Hoc Advisory Committee on Population-based Epidemiology Research Centers. It is also directly related to development of comprehensive clinical cancer research centers as defined by Congress in The National Cancer Act of 1971. The new program will serve as a cancer incidence

reporting system, a data resource, an epidemiological research effort and as a much needed training base. Eventual inclusion of the Demographic Program into a comprehensive clinical cancer research center will further increase the usefulness and productivity of the demographic work and will strengthen the total training program.

Proposed Course of the Project: This project will continue for two more years in its present form and then may be reorganized in relation to the new Cancer Research Center in Hawaii.

Date Contract Initiated: 6/15/71

Current Annual Level: $360,419

CONTRACT NARRATIVE
BIOMETRY BRANCH, OASDD
DIVISION OF CANCER CAUSE AND PREVENTION
Fiscal Year 1973

Special Cancer Survey Section

UNIVERSITY OF ALABAMA MEDICAL CENTER (Birmingham) (69-49)
CALIFORNIA STATE DEPARTMENT OF PUBLIC HEALTH (Berkeley) (69-51)
COLORADO DEPARTMENT OF PUBLIC HEALTH (Denver) (69-50)
COMMONWEALTH OF PUERTO RICO, DEPARTMENT OF PUBLIC HEALTH (San Juan) (69-72)
IOWA, UNIVERSITY OF (Iowa City) (69-42)
MEDICAL ASSOCIATION OF GEORGIA (Atlanta) (69-47)
MICHIGAN CANCER FOUNDATION (Detroit) (69-41)
UNIVERSITY OF MINNESOTA, SCHOOL OF PUBLIC HEALTH (Minneapolis) (69-45)
UNIVERSITY OF PITTSBURGH (69-43)
UNIVERSITY OF TEXAS, SOUTHWESTERN MEDICAL (69-40)
MAYO FOUNDATION (Minnesota) (70-2057)

Title: Third National Cancer Survey

Contractor's Project Director: See table on following page

Project Officer (NCI): Sidney J. Cutler, Sc.D.

Objectives: Procurement and initial processing of data for the Third National Cancer Survey

Major Findings: A preliminary report presenting the payments to hospitals during the first two years after diagnosis for cases diagnosed in 1969 was presented to the Survey Advisory Committees, as well as some initial data on therapy. The report on the special skin cancer survey is in preparation and will be released in the near future.

Significance to Biomedical Research and the Program of the Institute: See Individual Project Report NCI-4268.

Proposed Course: A schedule for completion of the various phases of operations of the survey has been developed. Most field offices will have few problems in meeting the deadline except for Dallas-Ft. Worth and Colorado, where personnel problems have hindered the survey operation. Steps have been taken to correct the situation. Close attention will be paid to these areas to see that data-gathering and error correction will be completed by the end of the summer, 1973. All effort will be made to complete all case-finding and abstracting of hospital records and death certificates as soon as possible. Computer printouts will be generated at frequent intervals to assist the field offices in this completion operation.

A workshop was held in December, 1972, during which the operations necessary to complete all phases of the survey were discussed. One of the major problems presented was that of retaining the staff as the end point approaches. This will be eased, somewhat, by a decision of the Advisory Committees, and Principal Investigators, at a meeting in February, 1973,

that a core staff be retained in each field office. This core staff will be available for any final activity that remains to be completed, for any special data-gathering operation needed for a more in-depth study of the survey data, and for analysis and presentation of the local survey area data. It was also decided at the February meeting that the two advisory committees be terminated, since their purpose of advising on the operation of the survey will no longer be applicable. In their stead, a utilization committee will be formed to assist in the determination of methods of analysis and presentation of data, and in the development of special studies using the survey data as a starting point.

A preliminary report on costs of hospitalization for cancer patients was presented to the advisory committees. There was much discussion of the data presented, and problem areas were identified. The committees were impressed with the data being collected and felt this would be a major contribution to the area of economic costs of cancer.

Almost all of the data for the special skin cancer study have been processed and preliminary reports generated. There is a large demand for these data, especially from groups studying the effects of environmental change on disease. We will be working with these groups, providing data useful in their investigations, as well as preparing the total data for analysis and presentation.

Time will be spent comparing the data for the individual years as the data collection phase approaches completion. This investigation may point out areas that need study to insure complete and accurate reporting, while full staffs are available at the field offices.

Date Contract Initiated: See table on following page.

Current Contract Level: See table on following page.

Contractor	Director	Initiated	Period	Level
University of Alabama	Dr. Peter Peacock	10/ 1/68	9/ 1/72- 8/31/73	$ 63,980
California Dept. of Pub. Health	Mr. George Linden	10/ 1/68	9/ 1/72- 8/31/73	245,000
Colorado Dept. of Pub. Health	Dr. Vernon Wohlauer	10/ 1/68	9/ 1/72- 8/31/73	207,158
Puerto Rico Dept. of Pub. Health	Dr. I. Martinez	12/ 9/68	12/ 9/72-12/ 8/73	8,525
University of Iowa	Dr. Edward Mason	10/ 1/68	9/ 1/72- 8/31/73	174,631
Medical Association of Georgia	Dr. James Cooney	10/ 1/68	9/ 1/72- 8/31/73	91,442
Michigan Cancer Foundation	Dr. Michael Brennan	10/ 1/68	9/ 1/72- 8/31/73	223,780
University of Minnesota	Dr. Leonard Schuman	10/ 1/68	9/ 1/72- 8/31/73	141,500
University of Pittsburgh	Dr. Carol Redmond	10/ 9/68	9/ 1/72- 8/31/73	136,650
University of Texas	Dr. Eugene Frenkel	10/ 1/68	9/ 1/72- 8/31/73	168,721
Mayo Foundation	Dr. Leonard Kurland	12/22/69	12/22/72-12/31/73	20,000

CONTRACT NARRATIVE
BIOMETRY BRANCH
DIVISION OF CANCER CAUSE AND PREVENTION
Fiscal Year 1973

Office of the Chief

UNIVERSIDAD DEL VALLE -- FUNDACION PARA LA EDUCACION SUPERIOR (PH43-66-907)

Title: Epidemiology-pathology studies of cancer in Colombia

Contractor's Project Director: Dr. Carlos Cuello

Project Officers (NCI): Mr. William Haenszel, Dr. John Berg

Objectives: To determine site-specific cancer incidence in Cali and other cities in Colombia and to correlate the morbidity data with pathology findings.

Methods Employed: The cancer morbidity surveys in Cali will be continued with emphasis on variation by socioeconomic class and place of birth. Internal migration within Colombia is of such magnitude that two-thirds of the residents of Cali were born elsewhere. Cancer morbidity surveys on a limited scale are being undertaken in Cartegena and Medellin, cities thought to have greatly different site-specific incidence, and in the Department of Nariño.

Major Findings: Studies of autopsy materials and surgical specimens are being conducted in Cali to define criteria which differentiate high-risk and low-risk populations. In Cali an organ bank of tissues from persons dying from accidents and acute diseases is being utilized for study of occult tumors and malignant precursors with emphasis on stomach, colon and cervix. The organ bank findings also emphasize the distribution by place of birth and are used to complement the incidence data from the morbidity surveys. Similar pathologic material is being collected in New Orleans and Hawaii.

The Cancer Registry in Cali is analyzing its data from the past ten years and preparing them for publication in the next edition of Cancer Incidence in Five Continents. The work on colon cancer is being conducted primarily in Cali and Medellin. Two papers have been published on this work during the past two years in conjunction with the Project Officer. Special studies are continuing on the precise localization of the colon cancer lesions and their histologic characteristics. A comprehensive epidemiologic study of stomach cancer is being conducted, primarily in the Department of Nariño. The problem may be related to nitrates in the water supplies. Incidence data will continue to be collected and analyzed to further identify high risk areas and subgroups of people. Studies of lymphoreticular tumors are continuing and are directed toward elucidation of environmental and host factors. Some cooperative efforts with the International Union Against Cancer (UICC) may be developed in the future. The collaborative study of thyroid cancer is continuing with tissues being sent to investigators in Hawaii for comparative

histopathologic examination. A similar collaborative study on prostate cancer is being conducted with investigators in Japan to assess the frequency of occult cancerous lesions in the male population. A case-control study of cervical cancer is underway in Cali and is related to a cervical cancer control campaign in that city.

Significance to NCI Program & Bio-Medical Research: This contract is producing significant data for comparative studies in the United States and elsewhere, with emphasis on those areas providing the greatest contrasts in risks for specific cancer sites.

Proposed Course of Project: The various aspects of this project will continue for at least three years. Emphasis will be placed on clinical and experimental studies of the genesis of intestinal metaplasia and carcinoma of the stomach. New collaborative research efforts may begin with investigators in Brazil, Costa Rica and Venezuela.

Date Contract Initiated: 6/29/66

Current Annual Level: $88,570

CONTRACT NARRATIVE
BIOMETRY BRANCH
DIVISION OF CANCER CAUSE AND PREVENTION
Fiscal Year 1973

Office of the Chief

VIRGINIA POLYTECHNIC INSTITUTE AND STATE UNIVERSITY (NIH-71-2427)

Title: Comparative Fecal Flora Studies

Contractor's Project Director: Dr. W. E. C. Moore

Project Officer (NCI): Dr. Sidney J. Silverman

Objectives: To compare the intestinal microflora of Japanese migrants to Hawaii who have intestinal polyps with those without such lesions. To determine the effect of diet on the intestinal flora and to study the neutral and acid steroids in the feces of the individuals under study.

Major Findings: Fecal specimens were obtained from members of a cohort of Japanese-Hawaiians who are participating in the Japan-Hawaii Cancer Study. To avoid changes observed in frozen and shipped specimens, the samples were cultured in Hawaii and the cultures sent to VPI for processing and identification of the microorganisms. Results of studies on 20 normal subjects have been received. One hundred forty-seven different species were observed among the 1136 isolates studied. This represents (statistically) about 94% of the cultivatable flora. Analysis of the data is in progress. Various changes in diet and environment were observed to be accompanied by changes in the fecal flora. Although individuals maintain their own specific flora when placed on the same diet, changes occur in the relative proportions of the different species.

No obvious differences have been observed between the neutral steroids in the feces of North Americans when compared to the Japanese Americans in Hawaii. Variations between individuals within the same groups are considerable. Some individuals among both the Japanese-Hawaiians (9/21) and among the Caucasian North Americans (8/28) failed to degrade cholesterol completely, as indicated by the higher amounts of cholesterol than coprostanol in their feces. Repeated testing indicated that this is a stable characteristic. The amounts and kinds of bile acids found in the feces of North Americans and Japanese-Hawaiians were similar. This may be correlated with a similarity of diet between the two groups.

Significance to Biomedical Research and the Program of the Institute: This project is part of the greater epidemiological study that will correlate laboratory, clinical, and demographic data to elucidate the factors causing intestinal carcinomas. It will help to answer the questions concerning the interrelationships between diet, intestinal microflora, bile acid components, and tumor formation. The project provides cultures for metabolic studies at the Frederick Cancer Research Center.

Proposed Course: Studies will continue on the fecal flora of polyp patients and of subjects under study in Japan. The contractor is investigating methods to enable him to study more specimens than the current two specimens per week.

Date Contract Initiated: June 30, 1971.

Current Annual Level: $179,965

CONTRACT NARRATIVE
BIOMETRY BRANCH
DIVISION OF CANCER CAUSE AND PREVENTION
Fiscal Year 1973

ADP Management Section

WOLF RESEARCH AND DEVELOPMENT CORP. (NIH-71-2270)

Title: Data Processing Support for Biomedical Research

Contractor's Project Director: William Lake, Ph. D.

Project Officer (NCI): Theodore Weiss

Objectives: This contract provides systems design and computer programming support to six epidemiological research projects.

Major Findings:

The Third National Cancer Survey Project - Technical ADP support was provided to operate and maintain the ADP aspects of the Third National Cancer Survey data processing system and produce computer-generated reports for analysis and publication of Survey data. This included the technical direction of all activities involved in the conversion of data from some 750,000 source documents to magnetic tape, the operation and maintenance of the Third National Cancer Survey data processing system, and the design and implementation of new computer programs and systems to meet specific operational and analytical needs. Specific accomplishments included:

- the operation and maintenance of the Survey's data processing system;
- the analysis, design, and production of a comprehensive extract file for the analysis of cost and treatment information from the medical and financial supplement documents;
- the production of numerous tables which were published in the Preliminary Report, Third National Cancer Survey, Payments to Hospitals;
- the development of a sub system to facilitate the comparative analysis between the 1947 Ten City Survey and the Third National Cancer Survey.

The Japan-Hawaii Cancer Study - Computer programming support and systems analysis consultation were provided to develop, install, and test the JHCS data processing system at NIH in Bethesda, Maryland, and to transfer the system to the University of Hawaii's computer center. This involved coding numerous computer programs, debugging two versions of the NIPS compiler,

conducting a detailed checkout of the entire JHCS computer system, the system at the NIH computer center, and developing a complete system and program documentation package.

The U. S. Cancer Mortality Study - Computer programs were developed to convert the NCHS U. S. mortality files into a standard format and code structure so that they can be processed by the general purpose analysis systems used by the Epidemiology Branch. Programs were also developed to produce tables for the analysis of the U. S. mortality data.

Study of Cancer in Renal Transplant Patients - Modifications were coded and tested to the Epidemiology Branch's Incidence of Second Cancers System to calculate from Connecticut Cancer Registry data expected rates of cancer incidence, by site, for the patient population included in this study.

The End Results Group Project - A general purpose computer system, Tablemaker, which calculates site specific frequencies and incidence rates was programmed and implemented. The system is file-independent and parameter controlled, and should have applications throughout the Demography Area.

Clinical and Diagnostic Trials - The contractor implemented a computer system at the VA Hospital in Minneapolis to allow laboratory data to be entered directly from remote job entry terminals into permanent computer files, and to produce a patient hospital report and a laboratory summary report from these files. In addition, Wolf provided computer programming maintenance of the Veterans Administration Cooperative Urological Research Group (VACURG) computer system, which is used to collect and analyze data on the treatment of prostatic cancer. A data base containing information on approximately 3,500 patients is updated with semi-annual submissions of new data. The system, consisting of approximately forty computer programs, was used by the contractor to edit these data, update the master files, and produce semi-annual reports containing numerous tables and other data displays. A number of computer programs were also written to produce reports for ad hoc studies.

Significance to Biomedical Research and the Program of the Institute:
See Project Reports, Biometry Branch, NCI.

Proposed Course: The support represented by this contract continued through February 28, 1973.

Date Contract Initiated: 5-03-71

Current Contract Level: $651,600

CONTRACT NARRATIVE
BIOMETRY BRANCH
DIVISION OF CANCER CAUSE AND PREVENTION
Fiscal Year 1973

ADP Management Section

WOLF RESEARCH AND DEVELOPMENT CORP. (N01 CP 33254)

Title: Computer Programming Services for the Third National Cancer Survey

Contractor's Project Director: William Lake, Ph.D.

Project Officer (NCI) : James E. Larson

Objectives: This contract provides biomedical computing software support to the Third National Cancer Survey project.

Major Findings:

Wolf is providing systems analysis and computer programming support to the Special Cancer Survey Section for the data processing and preliminary analysis phases of the Third National Cancer Survey. Specific activities included:

- the operation, maintenance and orderly close down of the Survey's data processing system;
- the modification of an existing sub system to produce incidence data for all three survey years (1969, 1970, 1971) along with a final detailed edit of all cases on file to facilitate the resolution of data discrepancies prior to the termination of field office activities;
- the development of numerous computer programs to support the data purification and analysis phases of the Special Skin Cancer Study;
- the development of a sub system to capture, edit, and analyze Patient Interview data; and
- the production of reports for the extended analysis of the Survey's "payments to hospitals" data.

Significance to Biomedical Research and the Program of the Institute: See Project Reports, Biometry Branch, NCI.

Proposed Course: The support represented by this contract is expected to continue through January 31, 1974.

Date Contract Initiated: February 11, 1973

Current Contract Level: $145,259

CONTRACT NARRATIVE
BIOMETRY BRANCH, DIVISION OF CANCER CAUSE AND PREVENTION
Fiscal Year 1973

Clinical and Diagnostic Trials Section

WOLF RESEARCH AND DEVELOPMENT CORPORATION (NO1CP-33259)

Title: Computer Services

Contractor's Project Director: Dr. William Lake

Project Officer (NCI): Dr. David P. Byar

Objectives: This program provides systems design and computer programming support for projects in the Clinical and Diagnostic Trials Section.

Major Findings: During the past year the contractor has maintained a Veterans Administration Cooperative Urological Research Group (VACURG) computer system comprised of approximately 40 computer programs which edits new data, updates master files, and produces a semi-annual report containing numerous tables and other data displays. This document consists of some 120-150 pages of extensive tabulations of data. The results are derived from complex statistical analyses, sophisticated data handling and analytical techniques, and data graphically plotted by digital computer. Since important clinical decisions are based on this report, up-to-date data must be presented as quickly as possible. Much of the computer output, therefore, must be in a form suitable for direct photographic copying. Before the report can be prepared, the data bases of five major clinical trials are updated with follow-up information received since the last report, and then submitted for a thorough computer editing.

Wolf personnel have written numerous computer programs for special studies related to the VACURG study and for other selected studies in which the Section participates. This often involves developing computer programs to facilitate the study of properties of medical and demographic data utilizing complex analytical techniques and decision rules.

In addition Wolf personnel have designed a system for recording the results of some twelve laboratory tests which are done on all patients in Prostate Studies II and III every six months. The system provides for entering this large volume of data directly into the computer system at our reference biochemistry laboratory in Minneapolis on a daily basis as the work is completed. Computer programs have been written to perform the many repetitious and complex calculations needed to convert the raw laboratory data into meaningful lab values. The complete system of editing and updating for the main file has also been designed and the results of the tests are sent periodically to the Section office for a statistical analysis in relationship to the patient's clinical records.

Proposed Course: During fiscal year 1972 this contract was extended for a further year and is currently scheduled to be in operation through the end of February 1974.

Date Contract Initiated: February 28, 1972

Current Contract Level: $52,000

CONTRACT NARRATIVE
BIOMETRY BRANCH
DIVISION OF CANCER CAUSE AND PREVENTION
Fiscal Year 1973

Office of the Chief

YALE UNIVERSITY SCHOOL OF MEDICINE (N01 CP 33235)

Title: Establishment and Development of a Connecticut Cancer Epidemiology Program

Contractor's Project Director: Dr. Robert McCollum

Project Officer (NCI): Mr. William Haenszel

Objectives: To establish a cancer epidemiology program with the primary purpose of studying and utilizing the data in the Connecticut Tumor Registry (CTR). Because of personnel restrictions imposed on the CTR by the State of Connecticut, the CTR staff is not in a position to analyze the incidence and mortality data nor to use the information in epidemiologic studies of cancer in Connecticut.

Methods Employed: Epidemiologists and statisticians will be recruited to develop a staff for the project which will have direct access to the data in the registry. The unit will not only conduct its own research and educational activities utilizing the CTR but will also actively encourage others to use and expand the resources of the registry.

Major Findings: This is a new contract.

Significance to NCI Program & Bio-Medical Research: The Connecticut Tumor Registry has been for years the cornerstone of the information reporting system which supports the major part of the Demography Program. It provides incidence data, End Results data, and related information for epidemiologic studies, both national and international. It is important that all this information be used to the maximum possible extent in order to advance our knowledge of the natural history of cancers and to capitalize on our long term investment in the CTR. With the CTR not in a position to do this work, this project presents an opportunity to fill an important need within the Demography Program. This proposal also represents a chance to further the cancer epidemiology training efforts at Yale. The total cancer program at Yale will be considerably enhanced and strengthened by having a functional Cancer Epidemiology Unit within the Oncology Division.

Proposed Course of Project: This project is expected to gradually develop into an important epidemiologic research center in cooperation with the Connecticut Tumor Registry.

Date Contract Initiated: 2/1/73

Current Annual Level: $54,299

C. SUMMARY REPORT
EPIDEMIOLOGY BRANCH
JULY 1, 1972 through JUNE 30, 1973

The objective of the Epidemiology Branch is to test ideas concerning the origins of cancer through epidemiologic studies based on medical knowledge and experience and on an awareness of resources best available at the national or international level. An idea is developed from existing knowledge, which may come from the laboratory, the clinic, or other epidemiologic studies. Emphasis is placed on epidemiologic research based on important new information from laboratory investigations at the National Cancer Institute and elsewhere.

Staff Changes: John J. Chabalko, M.D. and Robert N. Hoover, M.D. joined the staff for two years as Staff Associates.

Outside Training: John J. Mulvihill, M.D. began a two-year residency in Pediatrics at the Johns Hopkins Hospital.

Research Program:

International Activities:

During the year the Branch was especially active in conducting and planning international research. Emphasis was placed on initiating or perpetuating resources abroad that do not exist in the United States.

Atomic Bomb Casualty Commission (ABCC):

A contract was made with the National Academy of Sciences-National Research Council to support and extend cancer research at the ABCC in Hiroshima and Nagasaki. The contract provides for continuation of current studies to determine if cancer mortality rates will continue to increase as the cohort ages, especially among persons exposed before they were 10 years old (as early results suggest), and to initiate programs to increase appreciation in use of ABCC as a resource by university-based Japanese physicians and statisticians. The program provides for several Japanese faculty members to spend one year at ABCC conducting their own research under the guidance of the regular staff and the Epidemiology Branch, NCI. The first appointee has several studies in progress, and has spoken well of the program, as is perhaps reflected by the recruitment this year of five visiting scientists: 2 in epidemiology and 1 each in statistics, medicine and pathology. The contract also calls for the establishment of tissue registries for cancer surveillance. Progress has been good in Hiroshima, but Nagasaki authorities may decide that their existing tumor registry is sufficient.

International Union Against Cancer (UICC):

Under the aegis of the UICC's Committee on Cancer in Children, the Epidemiology Branch of NCI is collecting by mail data from pediatric centers throughout the world for an international comparison of

childhood cancer according to cell type. The purpose is to determine if the relative frequencies of certain childhood cancers are inordinately high or low in specific locales. The response has been most generous. More than 60 centers have contributed abstracts on series of patients, giving the diagnoses by cell type. More than 30,000 abstracts have been accessioned. Each contributor receives within a week a tabulation of his data according to a standard format, with comments as to how the relative frequencies of cancers that he has seen differ from the experience elsewhere. In this way he can identify areas of special research interest within his own locale and publish his findings as he sees fit. When unusual occurrences of tumors of specific types are noted in a particular center, histologic specimens are requested for review under an NCI contract with the Albany Medical College. A summary report of the findings from all centers will be presented to the UICC in 1974 (37).

World Health Organization (WHO):

As was hoped, the UICC study generated new interest in childhood cancer internationally. In December 1972, WHO convened a small group of consultants in pediatric oncology to review the status of cancer research and care pertaining to children and to make recommendations for local, national or international implementation. Twenty-one recommendations were made concerning etiology, prevention, pathology, therapy, education and communication. Implementation of the recommendations will be sought through a meeting of the UICC Committee on Cancer in Children in October, in conjunction with the annual meeting of the International Society of Pediatric Oncology (ISOP). By knitting together the interests of WHO, UICC and ISOP, benefits to pediatric oncology may accrue throughout the world.

Manchester Childhood Tumor Registry:

An exchange of visits among personnel in the Branch and from the Manchester Childhood Tumor Registry brought about a new and potentially valuable interaction between the two. The Manchester Registry is the only one in the world concerned with pediatric cancer, that is population-based and has very careful pathological review. It serves as a standard against which findings elsewhere in the world can be measured. The Registry is maintained with minimal financial support, and the clinical personnel have insufficient time to evaluate the data collected since 1954. By collaboration with our Branch it is expected that the Registry can be continued, with more frequent publication of its long-term findings concerning both etiology and therapy.

International Commission on Radiological Protection (ICRP):

One member of the Branch has served as Co-Chairman of the Task Group on Epidemiological Surveys of Human Populations, and prepared a statement for possible publication by ICRP on Epidemiology of Late Radiation Effects: Status and Needs. This analytical review indicated that gaps in our knowledge seem unlikely to be filled except through preserving and making the fullest use of data sources that have proved most informative in the past. Those in which the Branch has played a role are the ABCC, U.S. uranium miners and U.S. veterans who served in World War II as radiology technicians.

Other Activities:

Two members of the Branch contributed substantially to the writing of chapters concerning human effects of chemical carcinogens, in volumes concerning polycyclic aromatic hydrocarbons and heavy metals to be published by the International Agency for Research on Cancer. Members of the Branch also participated in the U.S.-Japan Panel on Environmental Mutagenesis and Carcinogenesis (U.S.-Japan Cooperative Medical Science Program), the Third Annual Princess Takamatsu Cancer Symposium in Tokyo (32), the Symposium on Multiple Primary Cancers held in Perugia, Italy (12), and the Conference on Host-Environment Interactions in the Etiology of Cancer in Man -- Implementation in Research held in August in Primosten, Yugoslavia (9).

Genetic and Family Studies:

The Branch has increasingly become known for its interest in genetic determinants of cancer. The subject is important because it can identify persons at high risk of cancer who can be screened more frequently than usual for early detection of neoplasia and can be advised to avoid even small exposures to environmental agents known to induce cancer in man. During the year two comprehensive clarifications of current knowledge were published (9, 10).

The aggregation in families of tumors of the same cell type or of certain dissimilar cell types are often referred by hospitals and practitioners to the Branch for evaluation and special study when indicated. In these studies the family history of neoplasia is carefully obtained, documentation for the diagnoses are secured when possible, physical examinations are performed, and batteries of laboratory tests are made using the most recently developed procedures at NCI and elsewhere. If feasible, physicians in the Branch conduct their examinations and obtain specimens in the field (i.e., at the local hospital), or if the procedures involved are beyond the capacity of facilities there, the family members are brought to NIH for study by our staff. These efforts have led to the delineation of several new family cancer syndromes, of value not only in studying the genesis of the tumors, but also in identifying family members in the line of descent, who can be advised to have frequent examinations for the earliest possible detection of cancer, which may be life-saving. During the past year five families have been comprehensively studied in this way, and papers have been published or accepted for publication on Hodgkin's disease and various manifestations of immunological disorder among close relatives (5), and familial gastric cancer and immunological abnormalities (6).

Other Childhood Cancers:

Since 1961, the Branch has paid particular attention to the etiology of childhood cancers as revealed by epidemiology -- a subject little studied at that time. During the past year continued use has been made of the Branch's national registry of death certificates for all children who died of cancer in the United States, 1960-1968. Information from these certificates has been coupled with multihospital surveys in which hospital charts for children with a specific neoplasm are abstracted to gain information that is not available

through death certificates. Among the findings made or published during the past year was an excess of duplications or failures in intrapelvic development in children with presacral teratomas (submitted for publication); gonadal dysgenesis as the only congenital anomaly that occurred excessively with ovarian cancers (26); undescended testes and inguinal hernias as the only anomalies associated with testicular cancer, a tumor that exhibits peaks at 2 years of age and in late adolescence among Whites but not among non-whites (25); excessive occurrence of retinoblastoma with mental retardation, possibly due to D-deletion syndrome, and with second primary tumors not attributable to radiation (21); mesothelioma, a rare cause of death under 20 years of age, which showed neither geographical clustering nor the fibrous cell type that would suggest an etiologic role of asbestos, as in adults (20); and two studies on the pathology and relative frequency of ovarian tumors of various cell types under 20 years of age (22, 52).

The mortality from Wilms' tumor under 5 years of age was shown to have declined 40% from 1960 through 1967, the last year for which data were available (17). The decline apparently reflected an improvement in therapy.

Studies of leukemia revealed that among 73 children with the acute myelomonocytic form of the disease 10 had antecedent blood dyscrasias for months or years (27); that for an as yet unknown reason, birth weight was higher than usual among children who subsequently developed acute lymphocytic leukemia (56); and that certain patterns of palm prints and finger prints occurred more frequently than usual among children with acute lymphocytic leukemia, suggesting a prenatal influence in the development of this disease (57).

As therapy increasingly extends the lives of children with cancer, it becomes important to determine adverse effects of treatment, especially chemotherapy, and the health of the offspring of persons who had cancer during childhood. To evaluate the feasibility of a multihospital survey of late effects of chemotherapy, the Carcinogenesis Area, NCI, in consultation with the Epidemiology Branch made a contract for a study based at the Children's Hospital of Philadelphia. Preliminary results showed that, as expected, second primary tumors occurred in relation to radiotherapy. Additional data will be needed to determine if there was an interaction with certain forms of chemotherapy. The most significant finding was that a small sub-group of the children given careful clinical and laboratory examinations revealed a wide range of late effects of therapy which had gone unnoticed or for which no tests were made when the children were routinely followed in the clinic. The feasibility study has thus called attention to a serious deficiency in the routine care of these children. When the series has been extended to evaluate late effects of various treatment modalities, a paper will be prepared for publication of the findings.

Review of the history of medicine reveals that the relationships between certain chemicals or ionizing radiation and specific diseases in man have usually been made initially by alert clinicians. With this in mind, the Branch initiated an Alert Practitioner Program at its Boston Field Station (Children's Cancer Research Foundation) in conjunction with an NIEHS-sponsored contract with the American Academy of Pediatrics for feasibility studies at three pediatric centers (Einstein, Duke and Los Angeles County-USC Medical

Center). The purpose was a) to identify by deep etiological histories, new environmental agents that are harmful to children, b) to increase interest in etiology at medical schools and c) to identify students with an aptitude to think epidemiologically so they might be guided in the development of this talent. In the first year of the study, the program achieved all three objectives. Unexpectedly, the three centers that studied newborn infants each reported a suspicion that progestational agents early in pregnancy may induce certain congenital malformations, a suspicion which arose concurrently at McGill University. The feasibility study also indicated that deep etiologic histories of children with cancer can reveal information of interest with regard to familial aggregation and to chemical agents during pregnancy or early in life.

The American Academy of Pediatrics has invited practitioners to submit their observations suggesting new environmental causes of children's diseases to the Academy's Committee on Environmental Hazards for evaluation. In addition, the Epidemiology Branch will prepare an exhibit sponsored by the Committee for the next annual meeting of practicing pediatricians, illustrating observations made in the past, and inviting visitors to the booth to submit case histories or family histories of interest from their own experience.

The Branch will also play a large role in a symposium concerning the effect of chemical pollutants on the fetus and child, to be held in June 1973 by the American Academy of Pediatrics and supported by NIEHS and NICHD.

Eight reviews or chapters for books were contributed on the etiology of childhood cancer (8, 13, 14, 31, 34, 36, 41, 45). In addition, during the year, members of the Branch have published original research as well as reviews on pediatric diseases other than cancer (11, 24, 33, 39, 47). Among them was a new evaluation of the effect of intrauterine exposure to the radiation of the atomic bombs in Hiroshima and Nagasaki, which showed that the frequency and severity of small head circumference was proportionate to dose and detectable even at a maternal air-dose of 10-19 rads (44). An excess of mental retardation was noted, beginning at about 50 rads (1). An analytical review concerning diseases in domestic animals as models of genetic and teratogenic disorders was published as a major article in Science by one of our young staff members (49).

Other Adult Cancers:

Another valuable resource for studies by the Branch has been the computer tape containing information from the death certificates for the 4.8 million people who died of cancer in the U.S., 1950-1968. During the year, the information, which previously could be studied geographically only by state, was refined so it can now be studied by county. In the first such study, it was found that no excess of deaths from cancer occurred among people in Colorado living in counties where houses were located on uranium-mill tailings (30). In another (preliminary) study of 3 cancer sites marked contrasts were observed in the array of counties with high rates of cancer of the stomach, lung or urinary bladder. The different patterns suggested new clues to etiology, which are being further explored.

In addition, during the year publications based on analysis of the data on the original tape showed a marked preponderance of cancer mortality among U.S. non-whites as compared with whites (3), an incipient more rapid rise in lung cancer death rates among U.S. women as compared with men (2) and an excess of cancer of the gallbladder and bile ducts among American Indians as compared with other ethnic groups (4).

Under a contract with NAS-NRC, study of the relationship of viral diseases among soldiers in World War II to subsequent cancer mortality revealed no relationship to infectious mononucleosis (43) or to yellow-fever vaccination (55). Many variables which are recorded for veterans of World War II can be studied for a relationship to cancer. Among the more promising opportunities recognized by a systematic recent review are studies concerning removal of organs, such as the spleen, because of injury during the war, and certain drug therapy, such as diphenylhydantoin for epilepsy.

Comprehensive reviews were published on drug-induced cancers (18), radiation-induced cancer (42), new hypotheses on the etiology of cancer (38), and persons at high risk of colorectal cancer (19), among other subjects (7, 23, 28, 29, 32, 35).

Veterinary Medical Data Program (VMDP):

In 1961 the Epizoology Section was initiated in the expectation that research into neoplasia among domestic animals would provide new insight into the origins of human cancer. At that time, there was an absence of systematically collected data for epidemiologic research. The Section developed a procedure for making standard observations on a mass scale to permit machine-processing and retrieval. Thirteen veterinary colleges contribute about 14,000 abstracts per month summarizing diagnostic, demographic and identifying information on each animal discharged. Each contributing institution receives a monthly listing of its data; NCI has the pooled data for its use. Since its beginning, the VMDP has accumulated more than 700,000 abstracts, of which about 4% concern tumors.

During the year a report was issued on 2,397 primary skin tumors of domestic animals (53). Boxer dogs had significantly more hemangioblastic tumors than is usual in the canine species; mastocytomas were excessive in Bulldogs, Boxers and Boston Terriers. These findings suggested that immunological studies, for example, may reveal abnormalities in high- vs. low-risk breeds.

A prospective study of feline infectious anemia revealed that 6 of the cases developed leukemia as compared with 0.5 expected, a significant excess (submitted for publication).

Studies of diseases other than cancer have shown that among domestic animals the frequency of congenital ocular defects was highest in dogs, and that certain breeds were at very high risk of specific abnormalities (e.g., microphthalmos-anophthalmos in the Collie and persistent pupillary membrane in the Basenji) (54). Analysis of 700 congenital heart defects in dogs revealed marked similiarities structurally and epidemiologically to defects occurring in man. Examples included patent ductus arteriosus in the

Miniature/Toy Poodle (97 observed vs. 31.8 expected), and mitral valve defect in the Samoyed (5 observed vs. 0.36 expected) (48, 51).

Studies of cancer in progress planned for the immediate future concern multiple primary tumors, tumors in animals under 2 years of age, pancreatic neoplasms and tumors of the blood-forming organs.

Robert W. Miller, M.D.

Conferences

International Commission on Radiological Protection
Co-Chairman, Task Group on Epidemiological Surveys of Human Populations.
U.S.-Japan Cooperative Medical Science Program
Panel on Environmental Mutagenesis and Carcinogenesis. Tokyo, Japan. August 25-27, 1972.
American Academy of Pediatrics, Meeting of Committee on Environmental Hazards. Evanston, Illinois. October 1 and 2, 1972.
NCI-sponsored Conference on Late Effects of Cancer Chemotherapy in Children. Boston, Massachusetts. October 19-20, 1972.
Fogarty International Center
Subcommittee on Prevention of Fetal and Perinatal Disease. Bethesda, Maryland. October 31, 1972.
World Health Organization
Chairman, Group Consultation of Paediatric Oncology. Geneva, Switzerland. December 3-7, 1972.
IARC. Revision of chapter concerning human cancer from polycyclic aromatic hydrocarbons, in preparation for the issuance of a handbook on this subject. Lyon, France. December 8, 1972.
1st Meeting of the Pan-American Society of Pediatric Pathologists. Mexico City, Mexico. January 3-5, 1973.
National Academy of Sciences
Meeting of the Advisory Committee on Veterans Affairs and Epidemiology. Washington, D.C. January 11, 1973.
Panelist, Conference on Biohazards in Cancer Research. Asilomar, California. January 22-24, 1973.
Meeting of U.S.-Japan Cooperative Science Medical Program's Panel on Environmental Mutagenesis and Carcinogenesis. Bethesda, Maryland. February 6, 1973.
Meeting, American Academy of Pediatrics, Committee on Nutrition. Washington, D.C. February 12-13, 1973.
Chairman, Review of the findings in the feasibility study of the Alert Practitioner Program, sponsored by NIEHS through a contract with the American Academy of Pediatrics. Research Triangle, North Carolina. March 8, 1973.
Meeting, American Academy of Pediatrics, Committee on Environmental Hazards. Research Triangle Park, North Carolina. March 9-10, 1973.
Annual Meeting, National Commission on Radiological Protection and Measurements. Bethesda, Maryland. March 15, 1973.
NCI Special Virus Cancer Conference, Human Tumors Associated with Herpesviruses. Discussion Leader, Relationship between Infectious Mononucleosis and Hodgkin's Disease. Bethesda, Maryland. March 26-27, 1973.
FDA - Panel on progestational agents in early pregnancy as a possible cause of congenital malformations. Bethesda, Maryland. March 29, 1973.
American Cancer Society
Planning Committee for National Conference on Childhood Cancer. New York, New York. April 9, 1973.

Fogarty International Center
Subcommittee to prepare book, Prevention of Fetal and Perinatal Disease. Chapter "Epidemiologic Aspects." Bethesda, Maryland. April 24, 1973.
American Academy of Pediatrics
Meeting, Committee on Nutrition. Washington, D.C. May 21-22, 1973.
National Academy of Sciences
Meeting, Advisory Committee on Veterans Follow-up and Epidemiology. Washington, D.C. June 15, 1973.

Consultant

Children's Hospital of Philadelphia. Carcinogenesis Area contract on late effects of chemotherapy on long-term survivors of childhood cancer. Philadelphia, Pennsylvania. August 8, 1972, September 22, 1972 and February 16, 1973.
Atomic Bomb Casualty Commission, concerning NCI contract for research into radiogenic cancer. Hiroshima and Nagasaki, Japan. August 15-24, 1972 and November 8-24, 1972.
Childhood Tumour Registry. Manchester, England. December 13-14, 1972.
National Academy of Sciences, Executive Council Committee to Evaluate the Atomic Bomb Casualty Commission. Washington, D.C. February 21, 1973.
Project site visitor with Dr. Alex Langmuir to advise Commissioner of Health on the quality of research of The New York State Birth Defects Institute. Albany, New York. March 12-13, 1973.
NIEHS, to evaluate trip report, Opportunities in Brazil for Studying the Effects of Hycanthone on Teratogenesis and Carcinogenesis. March 20, 1973.
NICHD, epidemiologic aspects of H. influenza vaccination in Charlotte, North Carolina. April 12, 1973.

Lectures

"New Hypotheses on the Etiology of Cancer (Epidemiologic Studies)," National Cancer Congress. Los Angeles, California, September 27-29, 1972.
"Epidemiology of Congenital Malformations," IV Pan-American Congress of Pharmacology. Caracas, Venezuela, July 9-14, 1972.
"Epidemiology of Cancer," Francis Delafield Hospital. New York City, New York, October 6, 1972.
"New Clues to Etiology through the Observations of Alert Practitioners," Georgetown University Pediatric Grand Rounds. Washington, D.C., October 13, 1972.
"Genetic and Viral Origins of Human Cancer: Epidemiologic Evidence," Workshop on Epidemiologic Aspects of Carcinogenesis. Belmont, Maryland, October 22-25, 1972.
"Persons at Very High Risk of Cancer," Princess Takamatsu Cancer Symposium. Tokyo, Japan, November 25-29, 1972.
"The Alert Practitioner Approach to Etiology of Childhood Diseases," Royal Manchester Hospital, Department of Pediatrics. Manchester, England, December 13, 1972.
"Syndromes of Cancers and Congenital Malformations," Paterson Laboratory. Manchester, England, December 14, 1972.

"Collaboration in Childhood Oncology Among the Epidemiology Branch (NCI), UICC and WHO," AAAS Symposium on International Cancer Epidemiology. Bethesda, Maryland, December 27, 1972.
"The Alert Practitioner Approach to the Etiology of Cancer," Department of Radiotherapy, Children's Cancer Research Foundation. Boston, Massachusetts, February 13, 1973.
"Epidemiology of Childhood Urologic Tumors," National Conference on Urologic Cancer. Washington, D.C., March 30, 1973.
"New Hypotheses on the Etiology of Cancer," Department of Radiotherapy, University of Maryland. Baltimore, Maryland, May 1, 1973.
"General Epidemiological Approach: How Environmental Effects on Health are Recognized," American Academy of Pediatrics, Program Chairman, Symposium on Susceptibility of the Fetus and Child to Chemical Pollutants. Discussion Leader. Brown's Lake, Wisconsin, June 11-13, 1973.

Joseph F. Fraumeni, Jr., M.D.

Committee Membership

Chairman, Panel on Chronic Disease Epidemiology, United States-United Kingdom Cooperative Program in Environmental Health Sciences.
Cancer Cause and Prevention Management Group, National Cancer Institute.
Breast Cancer Working Group, Special Virus Cancer Program, National Cancer Institute.
Scientific Advisory Committee, Cancer Control Bureau, New York State Department of Health.
Cancer Research Training Committee, National Cancer Institute
Epidemiology Committee, Breast Cancer Task Force, National Cancer Institute.
NIH Committee on Transplantation Research.
Program for Evaluation of Carcinogenic Effects of Chemicals in Man, International Agency for Research on Cancer.
Advisory Committee to the American College of Surgeons/National Institutes of Health Renal Transplant Registry.
Program Committee, American Association for Cancer Research.

Consultant

National Institute of Environmental Health Sciences (Program Development).
American Cancer Society (Personnel Grants).
Christie Hospital and Manchester Tumour Registry, Manchester England.
Department of Pediatric Oncology, Institut Gustave Roussy, Villejuif, France.

Lectures

"Genetic Determinants of Cancer," Conference on Host-Environment Interactions in the Etiology of Cancer in Man - Implementation in Research. Sponsored by the John E. Fogarty International Center of the National Institutes of Health and the League for the Fight Against Cancer of the Croatian Republic of Yugoslavia, Primosten, Yugoslavia, August 28, 1972.

"The Epidemiologist's Role in Laboratory Studies of Cancer Etiology." Seventh Annual Joint Working Conference, Special Virus Cancer Program, National Cancer Institute, Hershey, Pennsylvania, October 30, 1972.
"Epidemiology of Childhood Cancer: Recent Developments." Institut Gustave-Roussy, Villejuif, France, November 28, 1972.
"Familial Cancer." Christie Hospital, Manchester, England, December 13, 1972.
"Genetic Determinants of Cancer." Pediatric Grand Rounds, Georgetown University Hospital, Washington, D.C., January 5, 1973.
"Groups at High Risk of Cancer." Joint Center for Radiation Therapy, Harvard Medical School, Boston, Massachusetts, January 23, 1973.
"Epidemiology of Children's Cancer." The Candlelighters, Washington, D.C., January 26, 1973.
"Familial Hodgkin's Disease." Human Tumors Associated with Herpes Viruses. Fogarty International Center, National Cancer Institute, Bethesda, Maryland, March 27, 1973.
"Drug-Induced Cancer." Conference on Carcinogenic Testing in Development of New Drugs, National Academy of Sciences - National Research Council, Washington, D.C., May 24, 1973.
"Known and Suspected Human Teratogens and Transplacental Carcinogens." Conference on Susceptibility of the Fetus and Child to Chemical Pollutants. American Academy of Pediatrics, Brown's Lake, Wisconsin, June 12, 1973.
"Multiple Primary Neoplasms: Relationship to Familial Cancer." Fifth Perugia Quadrennial International Conference on Cancer. Perugia, Italy, June 28, 1973.

William A. Priester, DVM

Committee Membership

Executive Secretary, Biometry and Epidemiology Contract Review Committee. Bioassay Advisory Committee, Etiology Carcinogenesis, NCI.

Consultant

AID (Uruguay) "Animal Disease Reporting and Analysis."

Lectures

"Using Veterinary Clinical Data in Research." Paper presented at the following schools: University of Missouri School of Veterinary Medicine, May 9, 1972; School of Veterinary Medicine, Kansas State University, May 10, 1972; College of Veterinary Medicine, Iowa State University, May 11, 1972; and School of Veterinary Medicine, University of Illinois, May 12, 1972.

"Congenital Heart Disease in Dogs -- An Example of Clinical Studies Using the Veterinary Medical Data Program." Paper presented at the following schools: College of Veterinary Medicine, Michigan State University, May 24, 1972; School of Veterinary Medicine, Purdue University, May 25, 1972.

"The Veterinary Medical Data Program -- its potential for Clinical Studies." College of Veterinary Medicine, Ohio State University, May 26, 1972.

John J. Mulvihill, M.D.

Committee Membership

National Academy of Sciences-National Research Council
Committee on the Biological Effects of Ionizing Radiation
Subcommittee on Growth and Development

Lectures

"Congenital and Genetic Disease in Domestic Animals." Fairleigh Dickinson University, March 15, 1973.

Edward T. Creagan, M.D.

Lectures

"Familial Cancer." Demography Area Meeting, National Cancer Institute, NIH, Bethesda, Maryland, June 19, 1972.
"Cancer Patterns Among American Indians and Family Studies of Malignancy." Etiology Program Advisory Committee, Seventh Meeting, National Cancer Institute, NIH, Bethesda, Maryland, November 9-10, 1972.

Howard M. Hayes, DVM

Lectures

"The National Cancer Institute's Veterinary Medical Data Program: Description and Application." Paper presented at the following schools: Auburn University School of Veterinary Medicine, February 28, 1973; and, University of Georgia School of Veterinary Medicine, March 1, 1973.

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Serial No. NCI-4325

1. Epidemiology Branch, OASDD, DCCP
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Planning and Development in Cancer Epidemiology

Previous Serial Number: Same

Principal Investigator: Robert W. Miller, M.D.

Other Investigators: J. F. Fraumeni, Jr., M.D., W. A. Priester, DVM, and other professional staff on the Epidemiology Branch

Cooperating Units: None

Man Years:

Total	2.0
Professional	2.0
Other	0.0

Project Description

Objectives:

1. To originate new epidemiologic approaches to the study of cancer causation in man and animals.

2. To develop sources of data related to specific epidemiologic problems.

3. To stimulate epidemiologic research in other health agencies.

4. To develop on-the-job training for medical and statistical officers.

Methods Employed:

The Program is based on:

1. Leads from animal experimentation, laboratory research and clinical observations.

2. Prospective studies (in retrospect) which relate cancer occurrence to events recorded prior to the onset of cancer in medical examinations obtained in a standard fashion from large numbers of persons (examples -- clinical health-surveys, and military medical examinations).

3. Retrospective studies based on questionnaires obtained by personal interview or by mail, for a comparison of persons (examples -- clinical health-surveys, and military medical examinations).

4. "Laterospective" studies which concern the detection from clinic records of the excessive concurrence of cancer with pre-existent disease, such as congenital defects or autoimmune disorders.

As specific epidemiologic questions arise from laboratory or clinical observations, sources of field data are developed to answer these. Conversely, the Branch seeks by its surveys to raise questions which can be answered by laboratory or clinical studies. Parallel with this, to fill the tremendous deficiency in qualified personnel, the selection and training of young physicians, veterinarians, and statisticians must take place. The principles they learn from the guided planning, execution, and analysis of a program in cancer epidemiology are applicable to studies of the genesis of congenital malformations, heart disease, arthritis, mental aberrations, or the effects of ionizing radiation.

Significance to Bio-medical Research and the Program of the Institute:

If the example set by the Branch is a good one, interest will be stimulated among medical scientists in the use of epidemiologic methods for their research. In consequence, there may develop a further recognition of the usefulness of office and hospital records for survey studies. With this would go an appreciation for the need to adapt the standard medical records for epidemiologic research. These developments, in turn, could promote an interest in looking beyond the walls of hospitals and medical centers for opportunities in medical research.

Proposed Course of Project:

To develop opportunities in cancer epidemiology as they arise. During the past year an "Alert Practitioner Program" was initiated at the Boston Field Station in conjunction with similar NIEHS-sponsored feasibility studies in the Pediatrics Departments at Montefiore Hospital (New York City), Duke University and Los Angeles County-USC Medical Center. Under faculty supervision, medical students obtain special etiological histories and pedigrees from parents whose children have cancer or other diseases of unknown etiology. The purpose is to increase attention to etiology in medical schools, to seek new clues to causes of disease and to identify students with an aptitude to think epidemiologically, who can then be advised as to further training in this regard.

Serial No. NCI-4371

1. Epidemiology Branch, OASDD, DCCP
2. Epizoology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: National Cancer Institute Veterinary Medical Data Program

Previous Serial Number: NCI-4371

Principal Investigator: W. A. Priester, DVM

Other Investigators: H. M. Hayes, DVM, J. J. Mulvihill, M.D.

Cooperating Units: School and Colleges of Veterinary Medicine: Michigan State University, University of Missouri, University of Minnesota, Iowa State University, Ontario Veterinary College, Purdue University, University of Georgia, Kansas State University, University of Illinois, University of California, Ohio State University, University of Saskatchewan, Colorado State University

Also, see Project Report NCI-4379 and Project Report NCI-4380.

Man Years:

Total	:	2.40
Professional	:	.40
Other	:	2.00

Project Description

Objectives:

To provide animal disease data as a research resource. To coordinate the program with related programs primarily in area of disease nomenclature, terminology and code. To develop ways and means of data utilization applicable to comparative medical studies.

Methods Employed:

Standard data processing methods. Details are described in related Project Reports and Contract Narratives of Epizoology Section.

Major Findings:

Not applicable as this is primarily a service activity. The National Cancer Institute Veterinary Medical Data Program is currently processing data from 11 veterinary colleges in this country and 2 in Canada. The program has been endorsed and implemented in Europe under the auspices of WHO. For the first time, veterinary medicine and the medical sciences have benefit of a national and international animal disease data system.

Significance to Bio-medical Research and the Program of the Institute:

The comparative (veterinary) medical data retrieval system instituted at the College of Veterinary Medicine, Michigan State University on a pilot basis (PH 43-64-85) was undertaken to design a system for collection, storage and retrieval of veterinary medical clinical data. The program is now fully operative and has been extended to 12 additional veterinary colleges. Establishment and maintenance of reliable sources of veterinary medical data provide a research resource, hitherto unavailable, that is applicable not only to cancer research but to research areas such as infectious diseases, congenital defects, and gerontological diseases. Continued NCI Epidemiology Branch animal studies research function depends upon continued availability of valid domestic animal disease data, particularly as regards neoplastic disease. The data bank, which included information on more than 700,000 patients through 1972, is often used as a reference source by scientists at NCI and elsewhere.

Proposed Course of Project:

Additional veterinary schools will be added to the program as interest and capability of the individual applicants are established. It is anticipated that this project will continue for a minimum of two years.

Date initiated: October 22, 1965

Current annual level: $28,000

Publications

Priester, W. A.: The management of clinical and laboratory data in veterinary medicine. Adv. in Vet. Sci. and Compar. Med. 16: 73-102, 1972.

Serial No. NCI-4377

1. Epidemiology Branch, OASDD, DCCP
2. Ecology Studies Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Familial Aggregation of Malignancies

Previous Serial Number: Same

Principal Investigators: Joseph F. Fraumeni, Jr., M.D. and Robert W. Miller, M.D.

Other Investigators: John J. Mulvihill, M.D., Frederick P. Li, M.D., Gordon W. Grundy, M.D., and Edward T. Creagan, M.D.

Cooperating Units: None

Man Years:

Total	:	1.50
Professional	:	1.25
Other	:	.25

Project Description

Objectives:

To document the occurrence of patterns of family aggregation of neoplasms. To study such families by genetic and laboratory investigations, in an effort to elucidate the degree to which heredity and/or the common familial environment contribute to the etiology of neoplasms. To distribute tissue and blood specimens from such families to interested laboratory investigators, for etiologic study by cytogenetic, immunological, viral, and tissue culture methods. To identify genetic disorders predisposing to cancer.

Methods Employed:

Interview of cancer patients with respect to family occurrence of cancer and other disorders, as well as information on prior medical and environmental history; documentation of history by obtaining appropriate vital records and hospital charts; collection and distribution of biological specimens from such families; review of hospital records of series of patients with selected genetic diseases or neoplasms; survey of childhood cancer death certificates in the U.S. since 1960 to identify sib aggregation.

Major Findings:

A number of families have been studied with sites-specific cancers, such as familial lymphoma, breast cancer, ovarian cancer, chronic lymphocytic leukemia, chronic myelogenous leukemia, acute leukemia, cancer of the colon, stomach cancer, and osteogenic sarcoma. Other familial occurrences have represented multiple cancer syndromes, including familial adenocarcinomas of multiple sites, and the familial syndrome of sarcoma, breast cancer and other neoplasms. Many of the tumors which occur together in familial syndromes also coexist excessively in studies of multiple primary cancer, suggesting that a familial cancer syndrome may represent a "scattering" over the family tree of tumors which share etiologic influences. The results of laboratory studies of specimens obtained from members of various cancer families are being assembled.

Submitted for publication were reports on a variety of familial occurrences: a) familial Hodgkin's disease in association with idiopathic thrombocytopenic-purpura and other disorders suggesting immune dysfunction, b) familial arrhenoblastoma of the ovary with thyroid adenoma, c) in a series of children with non-Hodgkin's lymphoma, a tendency for sibship aggregation with lymphoma, leukemia and other tumors, and d) familial gastric cancer with laboratory parameters reflecting cell-mediated immune deficiency and autoimmunity. In addition, two reviews on the genetic determinants of cancer will soon be published.

Significance to Bio-medical Research and the Program of the Institute:

Epidemiologic surveys and detailed studies of families at high risk of cancer may help to detect environmental and genetic influences in carcinogenesis. In addition, identification of these families has therapeutic implications, enabling surveillance and early diagnosis of neoplasms.

Proposed Course of Project:

The same approach will be continued. New Laboratory methods and epidemiologic clues from other sources will be incorporated into the project protocol as necessary.

Publications

Creagan, E. T. and Fraumeni, J. F., Jr.: Familial Hodgkin's disease. Lancet 2: 547, 1972.

Serial No. NCI-4378

1. Epidemiology Branch, OASDD, DCCP
2. Ecology Studies Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: U. S. Cancer Mortality Survey

Previous Serial Number: NCI-4378

Principal Investigator: Thomas J. Mason

Other Investigators: Joseph F. Fraumeni, Jr., M.D., Frank W. McKay, Jr., and Edward T. Creagan, M.D.

Cooperating Units: National Center for Health Statistics

Man Years:

Total	:	3.25
Professional	:	2.75
Other	:	0.50

Project Description

Objectives:

To examine the cancer mortality experience in the United States, 1950-69 relative to cancer etiology for specific races, White, Black, American Indian, Chinese, Japanese, other, and also for individual counties within states.

Methods Employed:

Computer analysis of over 6 million death certificates by site, sex, race, state, and age. The investigation is ongoing, updated year by year, and expanding. Base populations for Whites, Blacks, and all nonwhites are maintained for the entire time period. The system for the total U.S. provides race, sex, ICD, and age specific analyses of cancer mortality consisting of numbers dead, populations at risk, age-specific and age-adjusted rates, a measure of the variability in the age-adjusted rate, and a linear regression of the age-adjusted rates as a function of time. This system is fully operational, and has been providing the required information for a paper comparing White and Black cancer mortality by site for the period 1950-1969 (in preparation).

A system for the analysis of cancer mortality for individual counties with the flexibilities of the national system is currently operational, and was tested

using mortality for White males and females. In order to have access to the basic county data the total U.S. cancer mortality was ordered by state, county, race, and sex. The ICD codes have been combined into 35 "T" codes, and the mortality combined into four pentads (1950-54, 1955-59, 1960-64, 1965-69). The county system also provides a confidence interval test of the hypothesis that a county rate significantly differs from the U.S. rate, as well as a regression analysis and subsequent comparison of county trends with trends for the U.S.

Major Findings:

The following analyses and subsequent results utilized the computer systems which have been developed.

No relationship between cancer mortality and ionizing radiation due to uranium mill tailings was detected in the State of Colorado, where this sand-like residue has been used as construction fill since 1951.

In an analysis of cancer mortality among American Indians, 1950-1967 a reduced risk was found in both Indian males and females for most sites of cancer. Specific sites of interest are the low rates in Indian males for lung, prostate, and colon, and in Indian females for breast cancer. Gallbladder cancer was the only site for which mortality among Indian males and females was significantly higher than that among both White and Black males and females in the United States.

An analysis of familial stomach cancer and immunologic abnormalities suggested that family susceptibility to stomach cancer results, at least in part, from a genetically controlled disorder involving autoimmunity and immune deficiency. This analysis compared the rates of stomach cancer in Floyd County, Virginia, to the total U.S. as well as the Southwestern region of the state (19 counties). An excess of stomach cancer was detected in Floyd County for both comparisons.

Significant variations in mortality for childhood non-Hodgkin's lymphoma occurred between the periods 1950-59 and 1960-69. Lymphosarcoma decreased for both sexes at all ages, while reticulum cell sarcoma increased. For all cell types combined, males had a net increase in mortality, whereas females showed a shift toward higher mortality in late childhood.

For the 8-year period, 1960-67, death rates for Wilms' tumor declined by a third among White children, chiefly those under 5 years. There was a small fall in mortality among nonwhite children.

Study was made of U.S. mortality rates for ovarian cancers among girls ages 0-19 years, 1950-68, and of 59 hospital records for children with these neoplasms. The death rates showed no significant changes over the 18-year period.

A current investigation of stomach cancer has detected a significant excess of mortality in 23 coal-mining counties in which the number of underground

miners of bituminous coal compressed at least 25% of the White male population age 25-59. The mortality experience in these counties was compared to that of a group of counties matched on other variables than coal mining.

An investigation of cancer mortality for Blacks and Whites 1950-1969 provides rates and rates of change by site which had formerly been available only for all nonwhites. Significant differences in the rates of change of bladder cancer in males and colon cancer in females were detected between the races. Additional mortality data is required to determine if these differences are due entirely to improved diagnostic procedures or represent true differences both occupational and/or environmental.

Significance to Bio-medical Research and the Program of the Institute:

This survey provides a continually expanding data set against which specific etiological hypotheses about cancer can be tested. The capability of subdivision of the data set into specific racial and geographical subsets (specifically county level analysis) provides an opportunity to more critically test hypotheses regarding the etiology of cancer than was possible at an earlier point in time.

Proposed Course of Project:

The addition of mortality for all nonwhites and Blacks for individual counties to facilitate racial comparisons by site and sex for individual counties, as well as the creation of a demographic file from the 1960 census with such measures as median school years completed age 25 or greater, occupation and migration will provide the basis for the most extensive analysis of cancer mortality possible to date. The project will provide an investigation of cancer mortality by race, site, and sex testing current etiologic hypotheses utilizing individual counties as a basic unit.

Publications

Mason, T. J., Fraumeni, J. F., Jr. and McKay, F. W., Jr.: Uranium mill tailings and cancer mortality in Colorado. J. Natl. Cancer Inst. 49: 661-664, 1972.

Creagan, E. T. and Fraumeni, J. F., Jr.: Cancer mortality among American Indians, 1950-67. J. Natl. Cancer Inst. 49: 959-967, 1972.

Creagan, E. T. and Fraumeni, J. F., Jr.: Familial gastric cancer and immunologic abnormalities. Proc. Am. Ass. Cancer Res. In press.

Fraumeni, J. F., Jr., Everson, R. B. and Dalager, N. A.: Declining mortality from Wilms' tumour in the United States. Lancet 2: 48, 1972.

Li, F. P., Fraumeni, J. F., Jr. and Dalager, N. A.: Ovarian cancers in the young: epidemiologic observations. Cancer. In press.

Serial No. NCI-4379

1. Epidemiology Branch, OASDD, DCCP
2. Ecology Studies Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Studies of Congenital Defects in Domestic Animals

Previous Serial Number: NCI-4379

Principal Investigator: John J. Mulvihill, M.D.

Other Investigators: William A. Priester, DVM and Howard M. Hayes, DVM

Cooperating Units: See Project Report NCI-4371

Man Years:

Total	:	.70
Professional	:	.30
Other	:	.40

Project Description

Objectives:

To study the characteristics of patients with congenital defects for clues as to possible relationships to cancer in the same clinic-hospital population. To relate these findings to human beings.

Methods Employed:

From case summaries submitted to the Veterinary Medical Data Program (NCI-4371), series of animals with congenital defects are systematically analyzed in relation to the total number of animals seen in the same time period. A general survey of the findings has already been published for cases seen from March 1964 through January 1969. During this year, reports have appeared on cardiovascular defects occurring in dogs and on congenital ocular defects. Currently in progress are studies of progressive retinal atrophy.

Major Findings:

In dogs the frequency of heart defects was 4 per 1,000, with purebreds at 3 times higher risk. For each anatomic site of congenital heart disease, there seems to be a dog breed at special risk: Miniature and Toy Poodles with patent ductus arteriosus and German Shepherd Dogs with persistence of the right aortic arch.

There were 673 diagnoses of congenital ocular defects among 131,453 horses, cattle, cats, and dogs brought to 10 veterinary school clinics in the United States and Canada during the period March, 1964, to January, 1969. The most frequent defects were: ectasia syndrome, entropion, cataract, microphthalmos-anophthalmos, opacity of cornea, lacrimal anomalies, dermoid cyst, persistent pupillary membrane, and ectropion, in descending order of frequency. The relative frequency of defects in dogs was 6 times that for any other species. Certain breeds were at high risk for specific defects. There was no sex predisposition for any of the ocular defects studied.

Significance to Bio-medical Research and the Program of the Institute:

Experiments on animals are essential to progress in medical research. Traditionally, a small number of species is used for laboratory experimentation. Currently popular laboratory animals are generally poor models for genetic disease and congenital malformations in man, and the rarity of these individual conditions in the human population limits clinical research. Our project attempts to detect conditions spontaneously occurring in domestic animals which are more nearly homologous to human afflictions. Taking heart malformations in dogs as an example, we have defined high frequencies of 1) patent ductus arteriosus in Miniature/Toy Poodles, and 2) persistence of the right aortic arch in German Shepherds. Since certain congenital malformations in man are associated with childhood malignancies, we hope to identify domestic animals which may serve as research models to explore the borderland between teratology and oncology.

Proposed Course of Project:

Data will continue to come from the Veterinary Medical Data Program and will be analyzed for breed-specific lesions.

Publications

Mulvihill, J.J.: Congenital and genetic disease in domestic animals. Science 176: 132-137, 1972.

Priester, W. A.: Congenital ocular defects in cattle, horses, cats, and dogs. J. Am. Vet. Med. Assoc. 160: 1504-1511, 1972.

Mulvihill, J.J.: Comments on the epidemiology of congenital heart disease in dogs. Part XV, The Cardiovascular System, In Daniel Bergsma (Ed.) Birth Defects: Orig. Art. Series, The National Foundation, N. Y., Vol. VIII: 5, 175-177, 1972.

Mulvihill, J. J. and Priester, W. A.: Congenital heart disease in dogs: Epidemiologic similarities to man. Teratology 7:73-78, 1973.

Serial No. NCI-4380

1. Epidemiology Branch, OASDD, DCCP
2. Epizoology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Study of the zoographic characteristics of domestic animals with tumors.

Previous Serial Number: NCI-4380

Principal Investigator: W. A. Priester, DVM

Other Investigators: Howard M. Hayes, DVM

Man Years:

Total	:	1.40
Professional	:	.40
Other	:	1.00

Project Description

Objectives:

To provide a reference of the distribution of spontaneous tumors in domestic animals. To search for unusual distribution of tumors, within a large, animal hospital population, which might suggest possible clues regarding the etiology of cancer.

Methods Employed:

Using patients with tumors reported to the Veterinary Medical Data Program as case series, and all patients seen during like periods as population references, the following will be studied:

Characteristics of patients: age, breed/species, sex
Characteristics of tumors: degree of malignancy, site, and histogenic type.

In addition, other factors may be studied as the analyses progress.

Major Findings:

8,634 tumors were reported during the period March 1964 through December 1969 among 202,277 patients seen. The proportion of patients with tumors was

highest among dogs and lowest among horses, when the four major species categories, bovine, equine, feline, and canine, were considered. A significantly increased tumor risk was noted in 1 bovine, 2 equine, and 14 canine breeds as compared to all breeds within each speices category. Large breeds of dogs tended to be at higher risk for tumors than were small dogs. All 4 species showed an increasing risk for tumors with age; the equine species, however, showed much less age dependence than the other 3. Female cattle were at excess risk for malignant tumors.

Significance to Bio-medical Research and the Program of the Institute:

Animal studies done by the Branch have demonstrated several relationships of interest in cancer research -- bone sarcoma and size, canine lymphoma and certain breeds, canine mastocytoma and breed ancestry. This study is designed to detect high risk groups in a systematic fashion. The results may well define animal subgroups suitable for studies of cancer etiology that would be impractical in a human population.

Proposed Course of Study:

It is anticipated that the results from the current population under study, 1964-1969, can be used to generate hypotheses that will be tested in the 1970-1971 study population. It is expected the project will continue for a minimum of 3 years.

Date initiated: May 1970

Current annual level: $19,000

Publications

Priester, W. A.: Skin tumors in domestic animals. Data from 12 United States and Canadian colleges of veterinary medicine. J. Natl. Cancer Inst. 50: 457-466, 1973.

Serial Number NCI-4400
1. Epidemiology Branch, OASSD
DCCP
2. Ecology Studies Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Epidemiology Branch Field Station, Boston, Massachusetts

Previous Serial Number: NCI-4400

Principal Investigator: Frederick P. Li, M.D.

Other Investigators: None

Man Years:

Total	:	1.8
Professional	:	1.0
Other	:	0.8

Project Description

Objectives:

1) To study the epidemiology of human neoplasms and to develop etiologic hypotheses through close observation of individual patients at the bedside.

2) To provide teaching in cancer epidemiology to trainees in clinical cancer treatment programs in several Boston hospitals.

Methods Employed:

Questionnaires were developed over the past year to gather new data on the genetic and environmental factors in the etiology of human cancers. To date, Dr. Li and a medical student have conducted nearly 100 questionnaire interviews on patients with childhood tumors, lymphomas and sarcomas. In addition, a mailed questionnaire study is underway to evaluate the relationship between prior tonsillectomy and Hodgkin's disease. Another questionnaire study is examining the long-term survivors of childhood neoplasms and their offsprings for occurrence of new tumors and other disorders. Collaboration with clinicians and clinical trainees in these studies has stimulated interest in cancer etiology. Dr. Li organized a lecture series on cancer epidemiology attended each session by 30-40 students, house officers and senior physicians.

Major Findings:

Familial aggregates of specific cancers, particularly sarcomas, have been identified for further epidemiologic and laboratory investigations. Environmental agents have been identified in patient interviews for more detailed evaluation regarding their potential role in human carcinogenesis.

Significance to Bio-medical Research and the Programs at the Institute:

Studies at the Field Station generate etiologic hypotheses for clinical and laboratory testing. The studies also help evaluate the relevance for human populations of etiologic data provided by other investigators at the Institute. The teaching on cancer epidemiology provided to medical students and junior house staff may help to stimulate interest in cancer epidemiology and to influence the selection of a career in this field.

Proposed Course of Project:

The Field Station will continue with studies on the patterns of occurrence of human cancers. The emphasis will be on detection of clues to etiology through careful clinical study of the natural history of cancer in individual patients. The new questionnaire studies will hopefully provide clues to cancer etiology which were not evident from retrospective reviews of prior data sources. Dr. Li, who is of Chinese ancestry and speaks Cantonese, is also initiating studies on cancer among the Chinese in the United States, with the goal of developing future international studies on the Chinese.

CONTRACT NARRATIVES
EPIDEMIOLOGY BRANCH, DCCP
Fiscal Year 1973

Special Cancer Studies Section

ALBANY MEDICAL COLLEGE OF UNION UNIVERSITY (NIH-71-2426)

Title: Study of Histopathologic Epidemiology of Childhood Cancer

Contractor's Project Director: Dr. J. N. P. Davies

Project Officer (NCI): Robert W. Miller, M.D.

Objectives: To carry out an international study of the incidence and frequencies of childhood cancer and to determine the causes of the striking variation in incidence from one country to another.

Methods Employed: Data on all childhood cancers is currently being collected from about 20 different countries. This statistical information will be analyzed by cell type and compared with data from other centers. A histologic review of the tumor types will be carried out when specific neoplasms may provide a basis for comparison, such as ovarian tumors and lymphomas which vary greatly from country to country. The principal investigator will request blocks or sections of certain tumors for this purpose.

Major Findings: The original hypothesis, that childhood cancers vary from one country to the next, as to cell type, has proven correct. A prime example of this variation is the very low risk of Ewing's tumor among Blacks in the United States and in Africa. The results have stimulated interest in childhood cancer among the contributing centers, some of which have in consequence published reports of their findings. Also, interest at WHO was stimulated, to the extent that an expert panel was convened on childhood cancer in Geneva in December 1972 to recommend a program to improve care and research concerning childhood cancer. Twenty-one recommendations were made, and efforts are now being made to implement them locally, nationally and internationally.

Significance to NCI Program and Bio-medical Research: Marked variations in the frequency of cancer according to race and geographic origin provide opportunities to identify genetic and environmental factors in etiology. The Epidemiology Branch has been involved in epidemiologic studies of childhood cancer in the U.S. The detection and clarification of international variation in childhood cancer should increase the opportunities for generating etiologic leads. The proposed histopathologic studies should play a very integral part in illuminating these leads.

Proposed Course of Project: As data continues to be received from various cancer registries, hospitals and individual investigators from each country, tabulations and correlations will be made to elicit possible clues to origin and genetic relationships. Future analyses and further data solicitation will be made as deemed necessary to the total project.

Date Contract Initiated: June 24, 1971

Current Annual Level: $34,970

CONTRACT NARRATIVES
EPIDEMIOLOGY BRANCH, DCCP
Fiscal Year 1973

Ecology Studies Section

HARVARD UNIVERSITY SCHOOL OF PUBLIC HEALTH (NIH-71-2179)

Title: Epidemiology and Etiology of Breast Cancer

Contractor's Project Director: Brian MacMahon, M.D.

Project Officer (NCI): Joseph F. Fraumeni, Jr., M.D.

Objectives: To expedite and expand ongoing epidemiologic studies directed towards understanding the etiology of breast cancer and the identification of a preventive measure. At the present time, the studies are focusing on the hypothesis that an important determinant of a woman's lifetime breast cancer risk is the proportion of estrogens that she metabolizes to estriol during the early years of reproductive life.

Methods Employed: Comparisons are being made of urinary estrogen profiles in young women from populations with different breast cancer risks. These studies involve the following contrasts: 1) an international study, in which women from two areas of North America (Vancouver, Boston) are compared with those from three Asian centers (Nagoya, Taipai, Hong Kong); 2) a Hawaiian migrant study in which Hawaiian women of Asian descent (whose breast cancer rates are intermediate between those of North American and Asian women) are compared with Asian women in their homelands and with Hawaiian women of Caucasian descent; 3) an examination of additional U.S. study groups characterized by parity, presence of fibrocystic disease, and family history of breast cancer. Other phases of the project involve a degradation study of frozen urine to evaluate the stability of estrogen fractions, an experimental study of rats to clarify the carcinogenic and protective potential of estrogen fractions, and the identification of prospective study groups for projects to come.

It is planned to expand the international comparisons to certain European populations with varying breast cancer rates. Three northern countries with high breast cancer rates (Denmark, Sweden and Netherlands) are to be compared with two southern countries (Greece and Yugoslavia) where the rates are half those of the north. The three Scandinavian countries will also be compared with Finland, which has breast cancer rates about two-thirds of those in Norway, Sweden and Denmark. In addition, Oslo will be compared with the County of Trams, one of the three Norwegian Counties north of the Arctic Circle where breast cancer rates are about two-thirds of those in the Capital.

Major Findings: Two geographic contrasts have been made to date: women from North America are compared with those from Asia, and Hawaiian women of Asian descent as contrasted with Asian women in homelands and Hawaiian women of Caucasian descent. In both studies, preliminary results have shown parallels between the population's breast cancer risk and the urinary estrogen profile -- in the direction predicted by the hypothesis.

Significance to NCI Program and Bio-medical Research: Epidemiologic characteristics of breast cancer are consistent with hormonal determinants, which can be best evaluated by joint epidemiologic-laboratory investigations of the type planned by this contract.

Proposed Course of Project: Preliminary evidence indicates that the estrogen-fraction hypothesis should continue to be evaluated. Investigations of other hormonal determinants, particularly androgen profiles, will be incorporated into the project.

Date Contract Initiated: June 23, 1971

Current Annual Level: $102,066

CONTRACT NARRATIVES
EPIDEMIOLOGY BRANCH, DCCP
Fiscal Year 1973

Ecology Studies Section

JOHNS HOPKINS UNIVERSITY (NIH-70-2134)

Title: Epidemiological Study of Cancer Mortality Among Diabetics

Contractor's Project Director: Irving I. Kessler, M.D.

Project Officer (NCI): Joseph F. Fraumeni, Jr., M.D.

Objectives: To evaluate over time the cancer mortality experience of diabetics, whose per capita consumption of cyclamates since 1960 has greatly exceeded that for the general population. The study also affords an opportunity of determining cancer risk in a large group of diabetics followed throughout most of their life span.

Methods Employed: Utilizing a group of 21,447 diabetics registered at the Joslin Clinic in Boston over a 26-year period, the risk of deaths from all forms of cancer in the precyclamate and postcyclamate periods are compared with one another and with the rates prevailing in the general Massachusetts population.

Major Findings: Since 1950, the cancer mortality experience of diabetics has been similar to that occurring before 1960. Pancreatic cancer remains the only neoplasm that significantly exceeds the rates in the general population, and confirms the positive relationships previously reported between pre-existent diabetes and subsequent risk of pancreatic cancer. No increases in bladder cancer have been identified over the study period.

Significance to NCI Program and Bio-medical Research: The Epidemiology Branch is concerned with studies relating environmental agents to cancer, and the surveillance of populations exposed to potential carcinogens. A widely used food additive, cyclamates, was found to cause bladder tumors in experimental animals; its carcinogenic potential in man is unknown, but may be clarified by a long-term survey of groups, such as diabetics, with heavier than normal exposure to cyclamates. This study also provides an opportunity to assess the risk of various types of cancer among diabetics.

Proposed Course of Project: The contract will be terminated on June 30, 1973. At that time the analysis should be completed. Results should be submitted for publication in the fall of 1973.

Date Contract Initiated: March 1, 1970

Current Annual Level: $29,200

CONTRACT NARRATIVES
EPIDEMIOLOGY BRANCH, DCCP
Fiscal Year 1973

Office of the Branch Chief

NATIONAL ACADEMY OF SCIENCES - NATIONAL RESEARCH COUNCIL (64-44; T.O. 61)

Title: Epidemiologic research on radiogenic cancer

Contractor's Project Director: LeRoy Allen, M.D.

Project Officer (NCI): Robert W. Miller, M.D.

Objectives: To determine the frequency and nature of cancer among atomic-bomb survivors in relation to radiation dose.

Methods Employed: The Atomic Bomb Casualty Commission has had clinics in Hiroshima and Nagasaki since 1948. Cancer research will continue through a) bi-annual clinical examinations of about 20,000 survivors with a wide range of radiation dosage, b) studies of death certificates for about 100,000 people who were in either of the cities at the time of the explosions, and c) a pathology program relating to both of these study samples. In addition, under the contract, citywide tissue registries are being established under the aegis of the Hiroshima Medical Association and Nagasaki University and one-year visiting scientist programs are offered to Japanese faculty members in Departments of Epidemiology, who consult the program for about 2 months in the United States before spending the balance of the year at the Hiroshima ABCC engaged in cancer epidemiology.

Major Findings: Under the contract, none as yet.

Significance to NCI Program and Bio-medical Research: The contract will permit continuation of cancer research which has revealed that radiation in sufficient dose can induce in man several forms of leukemia and thyroid cancer. Increases in lung cancer and breast cancer are suspected, but more cases must be accumulated before the evidence is conclusive. The ABCC findings do not support claims, based on studies of low-dose diagnostic irradiation, that all forms of childhood cancer are increased by intrauterine exposure. A recent finding at ABCC that persons exposed to the bomb before 10 years of age are showing a marked increase in various forms of neoplasia at 16-33 years of age, indicates that this cohort needs special attention to determine how long elevated cancer rates persist. In addition to the information derived about radiogenic cancer, this contract opens an opportunity for greater interaction between Japanese and American cancer epidemiologists at ABCC and in general.

Date Contract Initiated: February 29, 1972

Current Annual Level: $540,000

CONTRACT NARRATIVES
EPIDEMIOLOGY BRANCH, DCCP
Fiscal Year 1973

Ecology Studies Section

NATIONAL ACADEMY OF SCIENCES (PH43-64-44)

Title: Epidemiologic Studies in Etiology of Cancer in Veterans

Contractor's Project Director: Gilbert W. Beebe, Ph.D.

Project Officer (NCI): Joseph F. Fraumeni, Jr., M.D. and Robert W. Miller, M.D.

Objectives: To develop and conduct a broad program of epidemiologic studies in cancer among veterans.

Methods Employed: The Epidemiology Branch of the National Cancer Institute and the Follow-up Agency of the National Academy of Sciences will develop an epidemiology program designed to make efficient use of the military-veteran population, utilizing medical, demographic, and environmental observations made at entry and during military service, and the subsequent medical history of veterans ascertained through facilities of the Veterans Administration and supplemented by mortality data. The following activities were carried out during the past year: 1) studies of viral and other factors in the etiology of cancer, including surveys to evaluate the influence on cancer risk of ABO blood groups, religion, educational level, intelligence, and body size; 2) preservation on magnetic tape of the Army Diagnostic Index to the medical experience of the Korean War; 3) evaluation of familial factors in cancer by means of a twin registry of 16,000 pairs of veteran twins born in 1917-27; 4) study of respiratory cancer risk in World War I veterans exposed to mustard gas; 5) a search for risk factors in Hodgkin's disease among 700 men under age 31 with at least one year of duty prior to diagnosis in a military hospital; 6) planning activities for follow-up study of 40,000 men who served in the Navy during the Korean War, 1950-54, and were occupationally exposed to microwave radiation (Radar); and 7) planning activities for a series of studies to evaluate groups of veterans whose risk of cancer may be altered by preexisting disease, injury, therapy or environmental exposures in the service.

Major Findings: Reports were published on the lack of a relationship between cancer and histories of 1) infectious mononucleosis and 2) exposure to yellow-fever vaccine containing avian leukosis virus.

Significance to NCI Program and Bio-medical Research: The Epidemiology Branch of the National Cancer Institute is concerned with studies to identify and clarify etiologic factors in cancer, and these objectives can be more readily achieved by coordinating efforts with the outstanding resources and staff of the Follow-up Agency.

Proposed Course of Project: Terminating in the current contract year will be the preservation of diagnostic index to the Korean War, studies of viral and other factors, and the relation of respiratory cancer to mustard gas exposure. Continuing projects will include the studies of cancer in twins and Hodgkin's disease, and a variety of new projects will be undertaken to identify or clarify risk factors in cancer.

Date Contract Initiated: June 28, 1971

Current Annual Level: $99,897

Publications

Miller, R.W. and Beebe, G.W.: Infectious mononucleosis and the empirical risk of cancer. J. Natl. Cancer Inst. 50: 315-321, 1973.

Waters, T.D., Anderson, P.S., Jr., Beebe, G. W. and Miller, R.W.: Yellow-fever vaccination, avian leukosis virus, and cancer risk in man. Science 177: 76-77, 1972.

CONTRACT NARRATIVES
EPIDEMIOLOGY BRANCH, DCCP
Fiscal Year 1973

Ecology Studies Section

TULANE UNIVERSITY SCHOOL OF MEDICINE (NIH-71-2423)

Title: Epidemiology of Lymphomas

Contractor's Project Director: Guy R. Newell, M.D.

Project Officer (NCI): Joseph F. Fraumeni, Jr., M.D.

Objectives: To conduct a multi-hospital and prospective-incidence study of Lymphomas in Louisiana looking for clues to disease etiology.

Methods Employed: A questionnaire was developed for multi-hospital case control study of Hodgkin's disease to investigate relationships to various factors such as tonsillectomy and appendectomy, prior immune status, allergic conditions, smoking, and birth characteristics. Pathology slides were collected for histological classification. In the incidence study all Louisiana residents with Hodgkin's disease were ascertained, to evaluate demographic variables, seasonal variation, clustering, and other factors.

Major Findings: Preliminary results of the case-control study did not confirm the suggestion that tonsillectomy or other conditions mentioned represent risk factors in Hodgkin's disease, but (in collaboration with a similar study in Los Angeles) produced a new finding suggesting that dexedrine use for weight control may be a risk factor.

Significance to NCI Program and Bio-medical Research: The Epidemiology Branch is concerned with studies relating environmental agents to cancer, including identification of etiologic influences in the Lymphomas. There have been recent reports from Albany on the relationship of Hodgkin's disease to tonsillectomy, and a tendency for some cases of Hodgkin's disease to occur in an "extended epidemic." These observations indicate the importance of continued epidemiologic and etiologic studies of this cancer.

Proposed Course of Project: The contract will terminate on June 30, 1973. The project director and staff have demonstrated the feasibility of an epidemiologic study of Hodgkin's disease and other lymphomas in Louisiana, and are seeking grant support to continue the project.

Date Contract Initiated: June 29, 1971

Current Annual Level: $60,045

CONTRACT NARRATIVE
(Summary of Personal Service Contracts)

Animal Studies

VETERINARY MEDICAL DATA PROGRAM

Personal Service Contracts between the Epizootiology Section and Colleges of Veterinary Medicine at the University of Missouri, Michigan State University, Iowa State University, Ontario Veterinary College, and the University of Georgia, University of Illinois, Kansas State University, Ohio State University, University of Saskatchewan, Purdue University, University of Minnesota, University of California, and Colorado State University.

Title: Purchase Veterinary Case Data.

Objectives: To add to volume of stored data, available to Epizootiology Section, in the Veterinary Medical Data Program. To further encourage adoption of standard nomenclature and coding of animal disease data. To make available to veterinary medicine, comparative medical research and the scientific community the developments of the Veterinary Medical Data Program.

Methods Employed: Purchase of completed veterinary medical case abstracts in terms of National Cancer Institute Veterinary Medical Data Program (as detailed in related Contract Narratives).

Major Findings: None, as this is primarily a service activity.

Significance to NCI Program and Bio-medical Research: Increase store of data in the Veterinary Medical Data Program. Of particular importance in terms of study of low prevalence diseases (cancer, etc.), geographical distribution and absolute numbers.

Proposed Course of Project: Minimum of two years.

Dates Initiated:

Michigan State University	- November 1965
Iowa State University	- October 1965
Ontario Veterinary College	- July 1966
University of Georgia	- January 1967
University of Missouri	- February 1967
University of Minnesota	- December 1967
University of Illinois	- January 1968
Kansas State University	- January 1968
Purdue University	- January 1968
Ohio State University	- January 1969
University of Saskatchewan	- January 1969
University of California	- July 1970
Colorado State University	- August 1972

Current Annual Level: $46,000

CONTRACT NARRATIVES
EPIDEMIOLOGY BRANCH, DCCP
Fiscal Year 1973

Ecology Studies Section

WESTERN RESERVE UNIVERSITY (PH 43-64-524)

Title: Mortality Experience of Children who were Inadvertently Inoculated with SV-40 very Early in Life

Contractor's Project Director: Eli Gold, M.D.

Project Officer (NCI): Joseph F. Fraumeni, Jr., M.D.

Objectives: To determine whether the inoculation of human infants with polio vaccine containing SV-40 at birth is associated with an increased mortality due to malignant neoplasms.

Methods Employed: The mortality experience of 1,077 mainly Negro children, born in Cleveland, Ohio, between December 1959 and May 1962 and inoculated with SV-40 shortly after birth, will be followed through time. The location and name of each child are determined annually. Deaths are discovered through the use of a continuously up-dated census of Cleveland school children and from the vital records of the State of Ohio and other states to which the children have migrated. The names in the study group are compared against an alphabetic file of all childhood cancer deaths in the U.S. maintained in the Epidemiology Branch, NCI. The frequency of cancer deaths in the study group will be compared with the expected cancer mortality for children in the State of Ohio.

Major Findings: As yet there have been no cancers or other disorders in the study group attributed to SV-40. An interim report of the findings was published in Science 167: 59-60, 1970.

Significance to NCI Program and Bio-medical Research: Previous investigations by the Epidemiology Branch of school-age children who were inadvertently inoculated with SV-40 will be extended. The known importance of neonatal exposure in the causation of many viral neoplasms of animals suggests the possibility that the cancer mortality of children inoculated at birth might be different from that of children exposed at school age. The present study will extend our present knowledge in this area.

Proposed Course of Project: Since neoplasms with a latent period longer than 8 years could not be detected by the current follow-up, surveillance of the children will continue to be maintained.

Date Contract Initiated: March 9, 1964

Current Annual Level: $9,251

ANNUAL REPORT

CARCINOGENESIS PROGRAM
DIVISION OF CANCER CAUSE AND PREVENTION
NATIONAL CANCER INSTITUTE

July 1, 1972 through June 30, 1973

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I. CARCINOGENESIS PROGRAM SUMMARY REPORT

A. INTRODUCTION

The Carcinogenesis Program is responsible for planning, implementing, and managing the National Cancer Institute's coordinated research program on carcinogenesis by chemical and physical factors and on its prevention. In order to meet this responsibility, five coordinated program approaches have been established:

1. Identification and characterization of *population groups at risk* for different cancers;

2. Identification of carcinogenic activity of selected agents by *bioassays*;

3. Development and selection of *biological models* for bioassay, for the characterization of carcinogenesis processes, and for correlation with man;

4. Identification of *processes* required for carcinogenic action of selected agents as target points for corrective measures in man; and

5. Development, application, and evaluation of *corrective measures* for man and his environment.

The Carcinogenesis Program consists of an *intramural* component devoted to laboratory research, scientific documentation and coordination, and a *collaborative* component devoted primarily to coordinated targeted research implemented through the contract mechanism. There is no programmatic distinction between the two except from an administrative standpoint. They are, in fact, designed to complement each other.

Historically, this Program developed from the Carcinogenesis Studies Branch established in March 1961 as part of the Field Studies Area, for intramural and collaborative investigations on carcinogenic factors, particularly as hazards in the human environment. With the reorganization of NCI and the establishment of the Office of the Scientific Director for Etiology in January 1966, the Carcinogenesis Program was organized under the Office of the Associate Scientific Director for Carcinogenesis (OASDC), with the task of "planning and administering a program of basic and applied research in carcinogenesis leading to the identification or definition of environmental carcinogens, and to the elucidation of carcinogenesis mechanisms." A Biology Branch and a Chemistry Branch were established to implement this program. A contract-supported collaborative program was developed since 1962 to respond to the need for extensive bioassay testing and screening of potential environmental chemical carcinogenic hazards, and for a variety of fundamental research programs on mechanisms of carcinogenesis.

The scope of the whole Carcinogenesis Program was reassessed in 1968. Following an extensive survey of the leading scientific centers in this field and a series of discussion panels convened by NCI at the request of the National Advisory Cancer Council, a Program Plan on Chemical Carcinogenesis and Prevention and Cancers was designed and approved: New directions were thus established for both intramural and collaborative activities.

At the end of FY 1968, the Carcinogenesis Program had a total personnel strength of 105 positions, all but a few totally engaged in laboratory work, often loosely related to the newly established program priorities. The contract program was at a funding level of about $5 million, with extremely limited coordination and management resources. The task of developing a highly coordinated targeted program, requiring full participation of scientific staff, development of new major program areas and establishment of managerial and technical resources, was accepted as a challenge in spite of the limited resources then available.

In 1968-1969 the intramural laboratories and offices were moved from temporary quarters to the new Building 37 on the NIH reservation. Unfortunately at that time when a major expansion in the programs was called for, the Carcinogenesis Program was hit by the reduction in positions that affected all Institutes, and the total position strength underwent a loss of about 20%. Only at the end of FY 1971 did the position ceiling reach back to the FY 1968 level, and in FY 1972 it was raised to about 140. However, in FY 1973, in spite of further increases in program responsibilities and budget, the personnel ceiling was cut down again to 134 positions. This lack of personnel support represents the single most serious problem in the program and a major hindrance to its accomplishments. During these years, the Carcinogenesis staff gave invaluable cooperation to the task of substantially expanding the Carcinogenesis Program activities, both in-house and through contracts, at a time when a rapidly growing public awareness of the problems of environmental cancer hazards was giving a clear indication to the Nation of the increasing needs in this field.

In spite of the critical shortage of positions, by reassigning space, personnel and individual tasks and above all by the untiring efforts and cooperation of many members of the staff, several major new programs were activated. Their viability is now seriously affected by the new personnel cuts.

A new Experimental Pathology Branch was established in FY 1970 besides the previously existing Biology Branch and Chemistry Branch. Special units were set up in the Office of the Associate Scientific Director: a Program and Data Analysis Unit, a Lung Cancer Unit, and an Epidemiologic Pathology Unit (transferred by the Demography Program). In order to provide an effective management structure to the contract-supported program, the Office of the Coordinator for Collaborative Research was established in FY 1971 and eight segments were created to manage and coordinate the expanding contract program, which reached the level of $22 million in FY 1972 and is scheduled to reach a level of approximately $27 million in FY 1973. In FY 1972, the Registry of Experimental Cancers was transferred from the Office of the Director, NCI, to the Carcinogenesis Program and the Office of the Scientific

Coordinator for Environmental Carcinogenesis was established. In FY 1973 a new group of Sections was established in the Experimental Pathology Branch to reflect its emphasis on the major organ systems.

During the current fiscal year a major development of the Carcinogenesis Program has been the establishment of a large operation at the NCI Frederick Cancer Research Center (FCRC). The Carcinogenesis Program has undertaken to support about 40% of the Center's activities including long-term animal bioassays, preparative and analytical chemistry resources, *in vitro* carcinogenesis methods, and studies on intestinal bacterial flora in carcinogen metabolism related to colon cancer. Plans have been made for a major expansion of the Carcinogenesis Program at FCRC through the recruitment of highly qualified scientists in experimental pathology, cell biology, and molecular biology, committed to the field of carcinogenesis research.

Members of the staff have participated in the development of international cooperative programs, related to carcinogenesis. A panel on mutagenesis and carcinogenesis is being set up in conjunction with the National Institute of Environmental Health Sciences for the US-Japan agreement. Doctor Joseph DiPaolo has headed the US delegation to Moscow for the first meeting on cytogenetics in the context of the US-USSR agreement on cancer.

The end of the current fiscal year will coincide with the conclusion of the first five-year period of operation of the Carcinogenesis Program under its Plan. The Program--in spite of two periods of severe restrictions on personnel growth--has developed an articulated structure much more responsive to scientific program needs and priorities. All professional members of the staff share a direct interest and involvement in the total program, participating in varying degrees to both their intramural research activities and the coordination of the collaborative program. Their high degree of scientific productivity--attested by an imposing contribution to the scientific literature, and by their standing in the scientific community--was coupled with an increasing dedication to the development of effective research teams throughout the country, supported by the contract program. The organization, the scientific activities, and the progress of the Carcinogenesis Program are reported in the pages that follow.

B. ORGANIZATION

The organization of the Carcinogenesis Program is presently divided into two major components: Intramural Program and Collaborative Program. The former is articulated in the Office of the Associate Scientific Director for Carcinogenesis with its units and in three branches, each with several sections and units. The latter is articulated into nine segments. In addition to these staff functions, several advisory groups provide scientific consultation and review.

1. INTRAMURAL PROGRAM

The Office of the Associate Scientific Director for Carcinogenesis
(U. Saffiotti, M.D., ASD; J. A. Cooper, II, Ph.D., Acting deputy ASD):

Office of the Scientific Coordinator for Environmental Carcinogenesis
(H. Kraybill, Ph.D., Scientific Coordinator)
Epidemiologic Pathology Unit (J. Berg, M.D., Head)
Lung Cancer Unit (M. B. Sporn, M.D., Head)
Program and Data Analysis Unit (N. P. Page, D.V.M., Acting Head)
Registry of Experimental Cancers

The Biology Branch (H. J. Rapp, Sc.D., Chief):

Cellular Immunity Section (B. Zbar, M.D., Head)
Cytogenetics and Cytology Section (J. A. DiPaolo, Ph.D., Head)
Immunochemistry Section (T. Borsos, Sc.D., Head)
Tumor Antigen Section (E. Leonard, M.D., Head)

The Chemistry Branch (H. V. Gelboin, Ph.D., Chief):

Cell Growth Regulation Section (J. P. Bader, Ph.D., Head)
Molecular Carcinogenesis Section (H. V. Gelboin, Ph.D., Acting Head)
Nucleic Acids Section (C. W. Dingman, M.D., Head)
Protein Section (A. C. Peacock, Ph.D., Head)

The Experimental Pathology Branch (R. R. Bates, M.D., Chief):

Carcinogen Screening Section (E. K. Weisburger, Ph.D., Head)
Digestive System Carcinogenesis Section (R. R. Bates, M.D.,
Acting Head)
Endocrine Carcinogenesis Section (R. R. Bates, M.D.,Acting Head)
Perinatal Carcinogenesis Section (J. M. Rice, Ph.D., Head)
Histopathology Unit (D. G. Kaufman, M.D., Acting Head)
In Vitro Pathogenesis Unit (S. H. Yuspa, M.D., Acting Head)
Ultrastructure Unit (C. C. Harris, M.D., Acting Head)

2. COLLABORATIVE PROGRAM

The Office of the Associate Scientific Director for Carcinogenesis (U. Saffiotti, M.D., ASD; A. H. Heim, Ph.D., Coordinator for Collaborative Research)

[1]*Bioassay Operations Segment* (N. P. Page, D.V.M., Director;
J. Sontag, Ph.D., Acting Manager)

Biological Models Segment (R. R. Bates, M.D., Director;
R. Pledger, Ph.D., Manager)

Biology and Immunology Segment (H. J. Rapp, Sc.D., Director;
A. H. Heim, Ph.D., Acting Manager)

[1]*Carcinogen Metabolism and Toxicology Segment* (E. K. Weisburger,
Ph.D., Director, J. Sontag, Manager)

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[1]These two segments were established in March 1973 from parts of the Bioassay Segment. J. H. Weisburger, Ph.D., served as Director of the Bioassay Segment until his retirement in November 1972.

Chemistry and Molecular Carcinogenesis Segment (A. C. Peacock, Ph.D., Director; A. E. Kaplan, Manager[2])

Colon Cancer Segment (J. Berg, M.D., Director; A. E. Kaplan, Ph.D., Manager[3])

Information and Resources Segment (J. A. Cooper, II, Ph.D., Director; M. D. Litwack, Ph.D., Manager)

Lung Cancer Segment (M. B. Sporn, M.D., Director; C. Smith, Ph.D., Manager)

Tobacco Research Segment (G. B. Gori, Ph.D., Director; T. Owen, Ph.D., Manager)

3. ADVISORY GROUPS AND CONSULTANTS

The *Carcinogenesis Advisory Panel* provides overall discussion and advice to the Carcinogenesis Program staff on the development of the program and its priorities in the context of future activities of the National Cancer Institute.

The *Carcinogenesis Contract Program Management Group* provides review for need, priority and relevance for the whole program.

The *Carcinogenesis Intramural Program Group* provides a coordinating and planning function at the senior staff level. The entire professional staff is now essentially participating in the planning and monitoring of the overall program, providing a vital correlation between intramural and collaborative research programs.

The *Segment Advisory Groups* have been established to provide advice on program needs and priorities as well as technical review of contract proposals. They all include several members from outside NCI and they do not receive contract support from the segment on which they are an advisor.

Close liaison continues to be maintained with the Cancer Cause and Prevention Program Advisory Committee at the Division level, and it is hoped that a more effective liaison will be established with the National Cancer Advisory Board.

a. Carcinogenesis Advisory Panel

Dr. Emmanuel Farber, Professor of Pathology, Fels Research Institute, Temple University, Philadelphia, Pennsylvania

Dr. Theodore Hauschka, Director of Cancer Research, Roswell Park Memorial Institute, Buffalo, New York

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[2]R. Pledger, Ph.D., served as Manager of this Segment until December 1972.

[3]S. Silverman, Ph.D., served as Manager of this Segment until December 1972.

Dr. Maureen Henderson, Professor and Chairman, Preventive Medicine and Rehabilitation, University of Maryland, Baltimore, Maryland

Dr. Paul Kotin, Vice President, Health Sciences Center, Temple University, Philadelphia, Pennsylvania

Dr. Peter Magee, Professor of Biochemistry, Courtauld Institute of Biochemistry, Middlesex Hospital Medical School, London, England

Dr. James Miller, Professor of Oncology, McArdle Memorial Laboratory for Cancer Research, University of Wisconsin, Madison, Wisconsin

Dr. Norton Nelson, Director, Institute of Environmental Medicine, New York University Medical Center, New York, New York

Dr. James Price, Vice President, Experimental Therapy, Abbott Laboratories, North Chicago, Illinois

Dr. Arthur Upton, Dean, School of Basic Health Sciences, State University of New York, Stony Brook, New York

Dr. Gerald Wogan, Professor of Food Toxicology, Massachusetts Institute of Technology, Cambridge, Massachusetts

b. Carcinogenesis Contract Program Management Group

Dr. Umberto Saffiotti, Associate Scientific Director for Carcinogenesis, *Chairman*

Dr. Allen H. Heim, Coordinator for Collaborative Research, *Executive Secretary*

Dr. Richard R. Bates, Director, Biological Models Segment

Dr. John Berg, Director, Colon Cancer Segment

Dr. John A. Cooper, II, Director, Information and Resources Segment

Dr. Thaddeus Domanski, Program Director for Carcinogenesis, Division of Cancer Grants, NCI

Dr. Harry V. Gelboin, Chief, Chemistry Branch, Carcinogenesis

Dr. Gio Gori, Director, Tobacco Research Segment

Dr. Herman Kraybill, Scientific Coordinator for Environmental Carcinogenesis, Office of the Associate Scientific Director for Carcinogenesis

Dr. Norbert P. Page, Director, Bioassay Operations Segment

Dr. Andrew C. Peacock, Director, Chemistry and Molecular Carcinogenesis Segment

Dr. Herbert J. Rapp, Director, Biology and Immunology Segment

Dr. Michael B. Sporn, Director, Lung Cancer Segment

Dr. Elizabeth K. Weisburger, Director, Carcinogen Metabolism and Toxicology Segment

c. Carcinogenesis Intramural Program Group

Dr. Umberto Saffiotti, Associate Scientific Director for Carcinogenesis, *Chairman*

Mr. John Miller, Administrative Officer for Carcinogenesis *Executive Secretary*

Dr. Richard R. Bates, Chief, Experimental Pathology Branch, Carcinogenesis

Dr. John Berg, Head, Epidemiologic Pathology Unit, Office of the Associate Scientific Director for Carcinogenesis

Dr. John A. Cooper, II, Acting duputy Associate Scientific Director for Carcinogenesis

Dr. Harry V. Gelboin, Chief, Chemistry Branch, Carcinogenesis

Dr. Allen H. Heim, Coordinator for Collaborative Research, Office of the Associate Scientific Director for Carcinogenesis

Dr. Herman Kraybill, Scientific Coordinator for Environmental Carcinogenesis, Office of the Associate Scientific Director for Carcinogenesis

Dr. Norbert P. Page, Acting Head, Program and Data Analysis Unit, Office of the Associate Scientific Director for Carcinogenesis

Dr. Herbert J. Rapp, Chief, Biology Branch, Carcinogenesis

Dr. Michael B. Sporn, Head, Lung Cancer Unit, Office of the Associate Scientific Director for Carcinogenesis

Dr. Elizabeth K. Weisburger, Carcinogen Screening Section, Experimental Pathology Branch, Carcinogenesis

d. Segment Advisory Groups

(1) Bioassay Operations Segment

Dr. Norbert P. Page, *Director*

Dr. James Sontag, *Acting Manager*

Dr. Richard Adamson, Head, Pharmacology and Experimental Therapeutics Section, DCBD, NCI

Dr. John P. Gilbert, Staff Statistician, Harvard Computing Center, Cambridge, Massachusetts

Dr. Harold Grice, Head, Pathology Toxicology Section, Food and Drug Directorate, Ottawa, Canada

Dr. Paul Harris, 4114 East 65th Street, Indianapolis, Indiana

Dr. Bernard P. McNamara, Chief, Toxicology Division, Biomedical Laboratory, Edgewood Arsenal, Aberdeen Proving Ground, Maryland

Dr. Paul Newberne, Professor of Nutritional Pathology, Massachusetts Institute of Technology, Cambridge, Massachusetts

Dr. Robert A. Squire, Director, Comparative Pathology Program, Johns Hopkins University School of Medicine, Baltimore, Maryland

Dr. Elizabeth K. Weisburger, Head, Carcinogen Screening Section, Experimental Pathology Branch, Carcinogenesis

(2) Biological Models Segment

Dr. Richard R. Bates, *Director*

Dr. Richard Pledger, *Manager*

Dr. John Berg, Head, Epidemiologic Pathology Unit, Office of the Associate Scientific Director for Carcinogenesis, DCCP, NCI

Dr. Howard Bern, Professor of Zoology, Department of Zoology, University of California, Berkeley, California

Dr. Thomas Dao, Chief, Department of Breast Surgery and Endocrine Research Laboratory, Roswell Park Memorial Institute, Buffalo, New York

Dr. Pietro Gullino, Head, Tumor Physiopathology Section, Laboratory of Biochemistry, DCBD, NCI

Dr. Elizabeth Miller, Professor of Oncology, McArdle Laboratory for Research on Cancer, University of Wisconsin, Madison, Wisconsin

Dr. Jerry Rice, Head, Perinatal Carcinogenesis Section, Experimental Pathology Branch, Carcinogenesis

Dr. Edward Smuckler, Professor of Pathology, Department of Pathology, University of Washington, Seattle, Washington

Dr. Michael B. Sporn, Head, Lung Cancer Unit, Office of the Associate Scientific Director for Carcinogenesis

Dr. Benjamin Trump, Chairman, Department of Pathology, University of Maryland, Baltimore, Maryland

Dr. Paul Webster, Chief, Gastroenterology Division, Veterans Administration Hospital, Augusta, Georgia

(3) Biology and Immunology Segment

Dr. Herbert J. Rapp, *Director*

Dr. Allen Heim, *Acting Manager*

Dr. Gerald Bartlett, Department of Pathology, College of Medicine, Hershey, Pennsylvania

Dr. Tibor Borsos, Head, Immunochemistry Section, Biology Branch, Carcinogenesis

Dr. Joseph DiPaolo, Head, Cytogenetics and Cytology Section, Biology Branch, Carcinogenesis

Dr. Michael Edidin, Department of Biology, Johns Hopkins University, Baltimore, Maryland

Dr. Hugues Ryser, Professor of Pathology and Pharmacology, Department of Pathology, Boston University School of Medicine, Boston, Massachusetts

Dr. Paul Ts'o, Professor of Biophysical Chemistry, Department of Radiological Science, Johns Hopkins University, Baltimore, Maryland

Dr. Willie Turner, Professor and Chairman, Department of Microbiology, Howard University, Washington, D.C.

Dr. Berton Zbar, Head, Cellular Immunity Section, Biology Branch, Carcinogenesis

(4) Carcinogen Metabolism and Toxicology Segment

Dr. Elizabeth K. Weisburger, *Director*

Dr. James Sontag, *Manager*

Dr. Leo Friedman, Director, Division of Toxicology, Food and Drug Administration, Washington, D.C.

Dr. Harold Grice, Head, Pathology Toxicology Section, Food and Drug Directorate, Ottawa, Canada

Dr. Charles Irving, Chief, Cancer Research Laboratory, Veterans Administration Hospital, Memphis, Tennessee

Dr. Larry Keefer, Staff Fellow, Office of the Associate Scientific Director for Carcinogenesis

Dr. Lionel Poirier, Staff Fellow, Carcinogen Screening Section, Experimental Pathology Branch, Carcinogenesis

Dr. Eugene Sawicki, Chief, Laboratory Measurements Research Section, Division of Chemistry and Physics, Environmental Protection Agency, Research Triangle Park, North Carolina

Dr. Bitten Stripp, Staff Fellow, Laboratory of Chemical Pharmacology, National Heart and Lung Institute, NIH

Dr. Floie M. Vane, Research Group Chief, Department of Biochemistry and Drug Metabolism, Hoffman-La Roche, Nutley, New Jersey

(5) Chemistry and Molecular Carcinogenesis Segment

Dr. Andrew Peacock, *Director*

Dr. Ann E. Kaplan, *Manager*

Dr. John Bader, Head, Cell Growth Regulation Section, Chemistry Branch, Carcinogenesis

Dr. Donald Brown, Staff Member, Department of Embryology, Carnegie Institution of Washington, Baltimore, Maryland

Dr. C. Wesley Dingman, Head, Nucleic Acids Section, Chemistry Branch, Carcinogenesis

Dr. Harry V. Gelboin, Chief, Chemistry Branch, Carcinogenesis

Dr. Marie Green, Protein Section, Chemistry Branch, Carcinogenesis

Dr. Dolph Hatfield, Cell Growth Regulation Section, Chemistry Branch, Carcinogenesis

Dr. Robert Holley, Fellow, Salk Institute for Biological Studies, San Diego, California

Dr. Paul Howard-Flanders, Professor of Radiobiology and Molecular Biophysics and Biochemistry, Yale University School of Medicine, New Haven, Connecticut

Dr. Tsuyoshi Kakefuda, Nucleic Acids Section, Chemistry Branch, Carcinogenesis

Dr. Arthur Ness, Protein Section, Chemistry Branch, Carcinogenesis

Dr. Melvin Newman, Professor of Chemistry, Department of Chemistry, Ohio State University, Columbus, Ohio

Dr. James M. Price, Vice-President, Experimental Therapy, Abbott Laboratories, North Chicago, Illinois

Dr. Hewson Swift, Professor of Biology, University of Chicago, Chicago, Illinois

Dr. I. Bernard Weinstein, Associate Professor of Medicine, Columbia University College of Physicians and Surgeons, New York, New York

(6) Colon Cancer Segment

Dr. John Berg, *Director*

Dr. Ann E. Kaplan, *Manager*

Dr. Thomas Almy, Professor and Chairman, Department of Medicine, Dartmouth Medical School, Hanover, New Hampshire

Dr. Richard R. Bates, Chief, Experimental Pathology Branch, Carcinogenesis

Dr. Cecile Edwards, Chairman, Department of Home Economics, Howard University, Washington D.C.

Mr. William Haenszel, Chief, Biometry Branch, Demography, DCCP, NCI

Dr. Edward High, Professor and Chairman, Department of Biochemistry, Meharry Medical College, Nashville, Tennessee

Dr. Alan Hofmann, Professor of Medicine and Physiology, Mayo Clinic and Foundation, Rochester, Minnesota

Dr. Reino Kallio, Professor of Microbiology, University of Illinois, Urbana, Illinois

Dr. Gert Laqueur, Chief, Laboratory of Experimental Pathology, National Institute of Allergy and Infectious Diseases

Dr. James Miller, Professor of Oncology, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, Wisconsin

(7) Information and Resources Segment

Dr. John A. Cooper, II, *Director*

Dr. Marcia Litwack, *Manager*

Dr. Thomas Cameron, Program and Data Analysis Unit, Office of the Associate Scientific Director for Carcinogenesis

Dr. Robert Flynn, Assistant Director for Animal Facilities, Argonne National Laboratory, Argonne, Illinois

Dr. Don Gibson, Health Scientist Administrator, Aging Branch, National Institute of Child Health and Human Development, NIH

Ms. Madeline Henderson, Staff Assistant for Computer Usage Information and Data, Institute for Computer Science and Technology, National Bureau of Standards, Washington, D.C.

Dr. David Kaufman, Lung Cancer Unit, Office of the Associate Scientific Director for Carcinogenesis

Dr. Larry Keefer, Staff Fellow, Office of the Associate Scientific Director for Carcinogenesis

Mr. Terence Kuch, Biometry Branch, Demography, DCCP, NCI

Dr. Norbert Page, Acting Head, Program and Data Analysis Unit, Office of the Associate Scientific Director for Carcinogenesis

Dr. Sidney Siegel, Program and Data Analysis Unit, Office of the Associate Scientific Director for Carcinogenesis

Dr. Charles Warner, Organic Analytical Chemistry, Ayerst Laboratories, Rouses Point, New York

Dr. Elizabeth Weisburger, Head, Carcinogen Screening Section, Experimental Pathology Branch, Carcinogenesis

Dr. Isaac Welt, Director of Scientific and Technical Information System Program, American University, Washington D.C.

(8) Lung Cancer Segment

Dr. Michael B. Sporn, *Director*

Dr. Carl E. Smith, *Manager*

Dr. John Berg, Head, Epidemiologic Pathology Unit, Office of the Associate Scientific Director for Carcinogenesis

Dr. Roswell K. Boutwell, Professor of Oncology, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, Wisconsin

Dr. Jacob Churg, Professor of Pathology, Department of Community Medicine, Mt. Sinai School of Medicine, New York, New York

Dr. John A. Cooper, II, Acting deputy Associate Scientific Director for Carcinogenesis

Dr. Luigi DeLuca, Lung Cancer Unit, Office of the Associate Scientific Director for Carcinogenesis

Dr. Robert T. Drew, Pharmacology and Toxicology Branch, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina

Dr. Emmanuel Farber, Professor of Pathology, Fels Research Institute, Temple University School of Medicine, Philadelphia, Pennsylvania

Dr. Curtis C. Harris, Lung Cancer Unit, Office of the Associate Scientific Director for Carcinogenesis

Dr. David G. Kaufman, Lung Cancer Unit, Office of the Associate Scientific Director for Carcinogenesis

Dr. Hermann Lisco, Associate Professor of Anatomy, Department of Anatomy, Harvard Medical School, Boston, Massachusetts

Dr. Sergei Sorokin, Assistant Professor of Anatomy, Department of Physiology, Harvard School of Public Health, Boston, Massachusetts

(9) Tobacco Research Segment

Dr. Gio B. Gori, *Director*

Dr. Thomas B. Owen, *Manager*

Dr. William Bates, Director, Research Department, Liggett and Myers, Inc., Durham, North Carolina

Dr. Fred Bock, Director, Orchard Park Laboratories, Roswell Park Memorial Institute, Orchard Park, New York

Dr. Roswell K. Boutwell, Professor of Oncology, McArdle Laboratory for Cancer Research, University of Wisconsin, Madison, Wisconsin

Dr. Michael R. Guerin, Analytical Chemistry Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee

Dr. I. W. Hughes, Director of Research and Development, Brown and Williamson Tobacco Corp., Louisville, Kentucky

Dr. Charles Kensler, Senior Vice President, Arthur D. Little, Inc., Cambridge, Massachusetts

Dr. Gardner McMillan, Chief, Arterioschlerotic Disease Branch, National Heart and Lung Institute

Dr. Umberto Saffiotti, Associate Scientific Director for Carcinogenesis

Dr. Marvin Schneiderman, Associate Scientific Director for Demography, DCCP, NCI

Dr. Irving Selikoff, Director, Division of Environmental Medicine, Department of Community Medicine, Mt. Sinai School of Medicine, New York, New York

Dr. Murray Senkus, Director of Research, R. J. Reynolds Tobacco Company, Winston-Salem, North Carolina

Dr. A. W. Spears, Director, Research and Development, Lorillard Research Center, Greensboro, North Carolina

Dr. T. C. Tso, Plant Physiologist, Agriculture Research Service, Crops Research Division, Department of Agriculture, Beltsville, Maryland

Dr. Benjamin L. Van Duuren, Professor of Environmental Medicine, New York University Medical Center, New York, New York

Dr. Helmut Wakeham, Vice-President, Corporate Research and Development, Philip Morris, Inc., Richmond, Virginia

Dr. Ernest Wynder, President, American Health Foundation, New York, New York

e. Other Consultants

Dr. Elvin E. Adams, National Clearinghouse for Smoking and Health, Rockville, Maryland

Dr. Franklin Aldrich, Massachusetts Institute of Technology, Cambridge, Massachusetts

Dr. Alan Armitage, Tobacco Research Council Laboratories, Harrogate, England

Dr. Richard F. Bakemeier, The University of Rochester School of Medicine and Dentistry, Rochester, New York

Dr. Alberto Banfi, Istituto Nazional Tumori, Milano, Italy

Dr. J. George Bekesi, Roswell Park Memorial Institute, Buffalo, New York

Dr. F. M. Berger, Wallace Laboratories, Cranbury, New Jersey

Dr. Peter Bichel, Cancer Research Institute, Aarhus, Denmark

Dr. Richard S. Bornstein, American Oncologic Hospital, Fox Chase, Philadelphia, Pennsylvania

Dr. E. Boyland, 42 Bramerton Street, London, England

Dr. John Burson, Georgia Institute of Technology, School of Chemical Engineering, Atlanta, Georgia

Dr. Robert F. Busse, 509 Brook Forest Lane, Charlotte, North Carolina

Dr. Robert L. Capizzi, Yale University School of Medicine, New Haven, Connecticut

Dr. Merrill Chase, Rockefeller University, New York, New York

Dr. Darwin O. Chee, The Institute for Cancer Research, Philadelphia, Pennsylvania

Dr. Ernest Chu, University of Michigan Medical School, Ann Arbor, Michigan

Dr. Ray G. Crispen, Institution for Tuberculosis Research, Chicago, Illinois

Dr. William D'Aguanno, Food and Drug Administration, Rockville, Maryland

Dr. Tore Dalhamn, Institute of Hygiene, Institute of Uppsala, Stockholm, Sweden

Dr. John P. DaVanzo, Ortho Research Foundation, Raritan, New Jersey

Dr. Lise Davignon, Institute of Microbiology and Hygiene, Quebec, Ontario, Canada

Dr. Gerald Deneau, University of California School of Medicine, Davis, California

Dr. Lieneke den Tonkelaar, Rijks Instituut Voor de Volksgezondheid, Bilthoven, The Netherlands

Dr. F. J. deSerres, Oak Ridge National Laboratory, Oak Ridge, Tennessee

Dr. Edward F. Domino, University of Michigan, Ann Arbor, Michigan

Dr. J. M. Echave Llanos, Universidad Nacional de La Plata, La Plata, Argentina

Dr. O. G. Fahmy, Chester Beatty Research Institute. London, England

Dr. R. E. Falk, University of Toronto, Toronto, Ontario, Canada

Dr. Milton J. Finegold, New York University School of Medicine, New York, New York

Dr. Glenn Fischer, Roger Williams Hospital, Providence, Rhode Island

Dr. Gary Flamm, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina

Dr. John C. Flannagan, National Bureau of Standards, Gaithersburg, Maryland

Dr. Henry S. Fleming, Gulf University Research Consortium, Bay St. Louis, Mississippi

Dr. F. N. Frederickson, University of Connecticut, Storrs, Connecticut

Dr. Henry H. Freedman, Warner-Lambert Research Institute, Morris Plains, New Jersey

Dr. Ernst Freese, National Institute of Neurological Disease and Stroke, Bethesda, Maryland

Dr. Miriam Fukami, Temple University, Philadelphia, Pennsylvania

Dr. Sidney Galler, Department of Commerce, Washington, D.C.

Dr. Robert Greenfield, St. Vincent Hospital, Worcester, Massachusetts

Dr. Warren H. Gullen, Medical College of Georgia, Augusta, Georgia

Dr. Jordan U. Gutterman, M.D. Anderson Hospital and Tumor Institute, Houston, Texas

Dr. Erik Hasselager, National Food Institute, Soborg, Denmark

Dr. Charles Heidelberger, University of Wisconsin, Madison, Wisconsin

Dr. R. Marian Hicks, Middlesex Hospital Medical School, London, England

Dr. Russell Hilf, University of Rochester, School of Medicine and Dentistry, Rochester, New York

Dr. Dietrick Hoffman, American Health Foundation, New York, New York

Dr. Alexander Hollaender, Oak Ridge National Laboratory, Oak Ridge, Tennessee

Dr. Daniel Horn, National Clearinghouse for Smoking and Health, Rockville, Maryland

Dr. Nobuyuki Ito, Mara Medical University, Mara, Japan

Dr. Murray E. Jarvik, University of California School of Medicine, Los Angeles, California

Dr. J. W. Jull, The University of British Columbia, Vancouver, British Columbia, Canada

Dr. Myron Karon, Children's Hospital of Los Angeles, Los Angeles, California

Dr. J. C. Kennedy, Queen's University, Kingston, Ontario, Canada

Dr. Charles M. King, Michael Reese Hospital and Medical Center, Chicago, Illinois

Dr. Edmund Klein, Roswell Park Memorial Institute, Buffalo, New York

Dr. George B. Koelle, University of Pennsylvania, School of Medicine, Philadelphia, Pennsylvania

Dr. S. Landi, Connaught Medical Research Laboratories, Willowdale, Ontario, Canada

Dr. Edgar Lederer, De Chimie des Substances Naturelles, Cif-sur-Yvette, France

Dr. Marvin Legator, Roger Williams Hospital, Providence, Rhode Island

Dr. Paul Lemonde, Institute of Microbiology and Hygiene, Quebec, Ontario, Canada

Dr. William Lijinsky, Oak Ridge National Laboratories, Oak Ridge, Tennessee

Dr. John P. Long, University of Iowa, College of Medicine, Iowa City, Iowa

Dr. G. Michael Loos, J. Gutenberg University, Mainz, West Germany

Dr. Brian MacMahon, Harvard School of Public Health, Boston, Massachusetts

Dr. Veronica Maher, Michigan Cancer Foundation, Detroit, Michigan

Dr. H. V. Malling, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina

Mr. Robert L. Martin, Division of Computer Research and Technology, NIH

Dr. Michael J. Mastrangelo, The Institute for Cancer Research, Philadelphia, Pennsylvania

Dr. Robert McConnell, G. D. Searle and Company, Chicago, Illinois

Dr. Robert A. Moss, Rutgers University, New Brunswick, New Jersey

Dr. M. Lois Murphy, Sloan-Kettering Institute for Cancer Research, New York, New York

Dr. Tetsuichiro Muto, St. Mark's Hospital, London, England

Dr. Larry Nathanson, Tufts University School of Medicine, Boston, Massachusetts

Dr. Mark Nickerson, McGill University, Montreal, Canada

Dr. Kunio Okuda, Chiba University School of Medicine, Chiba, Japan

Dr. George Olah, Case Western Reserve University, Cleveland, Ohio

Dr. Wolfgang Opferkuch, University of Bochum, Bochum, West Germany

Dr. Thomas Osdene, Philip Morris Research Center, Richmond, Virginia

Dr. R. A. Phillips, Ontario Cancer Institute, Toronto, Ontario, Canada

Dr. Roman Pienta, Litton Bionetics, Inc., Frederick, Maryland

Dr. Carl M. Pinsky, Memorial Sloan-Kettering Cancer Center, New York, New York

Dr. Lawrence Plumlee, Environmental Protection Agency, Washington D.C.

Dr. Richmond T. Prehn, Institute for Cancer Research, Philadelphia, Pennsylvania

Dr. Alberto N. Raick, University of Toronto, Toronto, Ontario, Canada

Dr. Manfred Rajewsky, Max-Planck Institut fur Virusforschung, Tubingen, West Germany

Dr. William K. Riker, University of Oregon Medical School, Portland, Oregon

Dr. David Rogers, University of Colorado, Boulder, Colorado

Dr. Eugene Rosenberg, University of Miami, School of Medicine, Miami, Florida

Mr. Howard Rosenberg, 577 14th Avenue, San Francisco, California

Dr. Herbert S. Rosenkranz, Columbia University College of Physicians and Surgeons, New York, New York

Dr. Sol Roy Rosenthal, Box F, Rancho Sante Fe, California

Dr. Hans W. Ruelius, Wyeth Laboratories, Philadelphia, Pennsylvania

Dr. Donald B. Schwartz, Children's Hospital of Los Angeles, Los Angeles, California

Dr. H. F. Seigler, Duke University Medical Center, Durham, North Carolina

Dr. Raymond E. Shapiro, Food and Drug Administration, Washington D.C.

Dr. Edwin Shykind, Department of Commerce, Washington, D.C.

Dr. Richard L. Simmons, University of Minnesota, Minneapolis, Minnesota

Dr. Joseph E. Sokal, Roswell Park Memorial Institute, Buffalo, New York

Dr. Frank C. Sparks, University of California, School of Medicine, Los Angeles, California

Dr. Bernard Stewart, Temple University Health Sciences Center, Philadelphia, Pennsylvania

Dr. Hans Franz Stich, University of British Columbia, Vancouver, British Columbia, Canada

Dr. Douglas Stoltz, Food and Drug Directorate, Ottawa, Ontario, Canada

Dr. Osias Stutman, University of Minnesota Medical School, Minneapolis, Minnesota

Dr. Hector L. Sulit, Institute for Cancer Research, Philadelphia, Pennsylvania

Dr. William C. Summers, Yale University Medical School, New Haven, Connecticut

Dr. Albert Tannenbaum, 6795 Via Estrada, La Jolla, California

Dr. Harold E. Taylor, Medical Research Council, Ottawa, Ontario, Canada

Dr. S. Gale Taylor, Presbyterian-St. Luke's Hospital, Chicago, Illinois

Dr. J. W. Thomas, Vancouver General Hospital, Vancouver, British Columbia, Canada

Dr. Max Tisher, Wesleyan University, Middletown, Connecticut

Dr. Jay A. Tischfield, Yale University, New Haven, Connecticut

Dr. Elliot Vesell, Pennsylvania State University, School of Medicine, Hershey, Pennsylvania

Dr. William D. Vincent, Division of Computer Research and Technology, NIH

Dr. Byron H. Waksman, Yale University, New Haven, Connecticut

Dr. Wolfgang Wechsler, Max Planck Institute for Brain Research, Cologne, West Germany

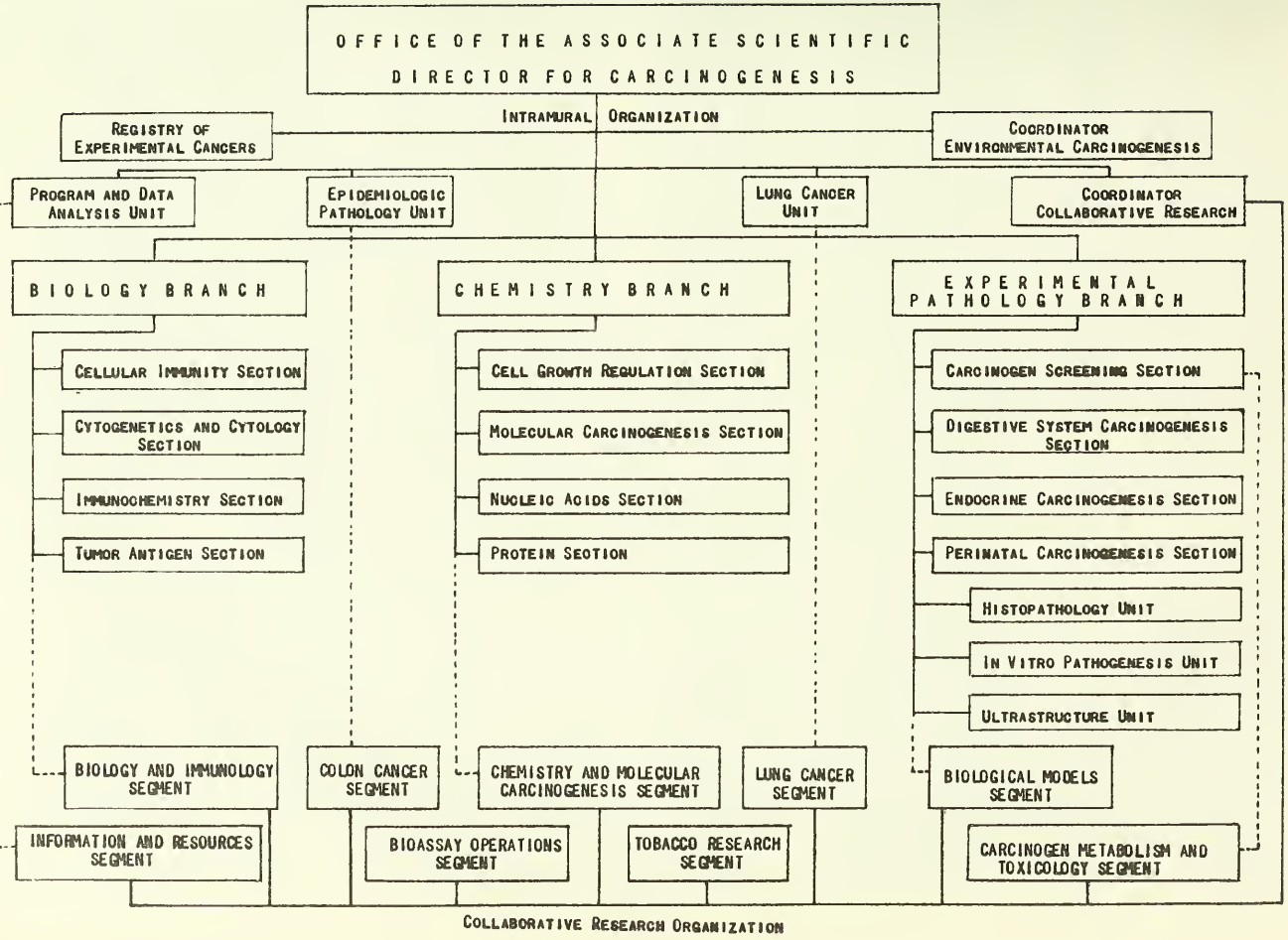
Dr. John H. Weisburger, American Health Foundation, New York, New York

Dr. S. R. Wellings, University of California, School of Medicine, Davis, California

Dr. Janet Wolter, Presbyterian-St. Luke's Hospital, Chicago, Illinois

In the collaborative program, each segment is directed by a senior member of the Carcinogenesis staff, assisted by a segment manager. Other members of the staff serve as project officers on individual contracts. Each segment has a segment advisory group.

Some urgent organizational changes were finally made possible in FY 1972 by the capability for additional recruitment included in the increased appropriation for FY 1972. Top priority was given to the recruitment of competent science managers fully devoted to the coordination of scientific management for the segments. The Office of the Scientific Coordinator for Environmental Carcinogenesis, reporting directly to the ASD, was established.


OFFICE OF THE ASSOCIATE SCIENTIFIC DIRECTOR FOR CARCINOGENESIS
INTRAMURAL ORGANIZATION
REGISTRY OF EXPERIMENTAL CANCERS
COORDINATOR ENVIRONMENTAL CARCINOGENESIS
PROGRAM AND DATA ANALYSIS UNIT
EPIDEMIOLOGIC PATHOLOGY UNIT
LUNG CANCER UNIT
COORDINATOR COLLABORATIVE RESEARCH
BIOLOGY BRANCH
CHEMISTRY BRANCH
EXPERIMENTAL PATHOLOGY BRANCH
CELLULAR IMMUNITY SECTION
CYTOGENETICS AND CYTOLOGY SECTION
IMMUNOCHEMISTRY SECTION
TUMOR ANTIGEN SECTION
CELL GROWTH REGULATION SECTION
MOLECULAR CARCINOGENESIS SECTION
NUCLEIC ACIDS SECTION
PROTEIN SECTION
CARCINOGEN SCREENING SECTION
DIGESTIVE SYSTEM CARCINOGENESIS SECTION
ENDOCRINE CARCINOGENESIS SECTION
PERINATAL CARCINOGENESIS SECTION
HISTOPATHOLOGY UNIT
IN VITRO PATHOGENESIS UNIT
ULTRASTRUCTURE UNIT
BIOLOGY AND IMMUNOLOGY SEGMENT
COLON CANCER SEGMENT
CHEMISTRY AND MOLECULAR CARCINOGENESIS SEGMENT
LUNG CANCER SEGMENT
BIOLOGICAL MODELS SEGMENT
INFORMATION AND RESOURCES SEGMENT
BIOASSAY OPERATIONS SEGMENT
TOBACCO RESEARCH SEGMENT
CARCINOGEN METABOLISM AND TOXICOLOGY SEGMENT
COLLABORATIVE RESEARCH ORGANIZATION

This Office headed by a scientist with extensive experience in environmental carcinogen evaluation, was to be staffed with experts in the fields of food additives, pesticides, drugs, air and water pollution, industrial and occupational exposures, natural products, and tobacco. This group of experts was to provide documentation, identify need and priorities, and essentially provide a liaison between the field of carcinogenesis, with its rapid methodological and scientific progress, and their respective areas of environmental relevance. A particular task of this Office is that of developing a close liaison with other Government agencies and with industry. At this time, the personnel ceiling has totally paralyzed the development of this effort.

Further recruitment was planned (a) to provide adequate staff support to the pressing task of expanding and monitoring the bioassay program, including standardized protocols, data handling system, resources, and biometric evaluation of results and (b) to provide key competence in the Experimental Pathology Branch to implement its commitment to the development of organ-oriented biological models, particularly in the areas of endocrine-related cancers, pancreas, breast and skin cancer. Again the personnel policy has severely blocked these essential developments.

In FY 1973, as in previous years, the Associate Scientific Director for Program, DCCP (Dr. G. B. Gori) has made an outstanding contribution to the Carcinogenesis Program, serving as Segment Director for the Tobacco Research Segment and Chairman of its advisory panel, the Tobacco Working Group, as well as project officer on several important contracts in Carcinogenesis.

C. MANAGEMENT ACTIVITIES

A science-based management system is essential for the effective management of the increasingly complex contract-supported collaborative program. The Carcinogenesis Program established a management staff and separate advisory groups for each specific, major program segment. Senior scientists and staff of the intramural research programs provide the operating nucleus of each segment, thus ensuring a sound scientific base for the contract program. Segment directors, who provide the scientific leadership, and project officers, who provide the constant technical guidance and operational direction of each program area, are members of the intramural staff.

Just as the technical aspects of the Carcinogenesis Collaborative Program are built upon a staff having expertise, research experience, and competence in particular areas, so too the management of the Collaborative Program is built upon scientist-administrators with both research and administrative experience. These scientist-administrators have been designated as segment managers and provide management support to the segment director, project officers, and outside advisors. The nine segments are as follows: Bioassay Operations[1], Biological Models, Biology and Immunology, Carcinogen Metabolism and Toxicology[1], Chemistry and Molecular Carcinogenesis, Colon Cancer, Information and Resources, Lung Cancer, and Tobacco Research.

[1]These two segments were established in March 1973 from parts of the Bioassay Segment.

Each segment has an advisory group made up of the segment director as chairman, the segment manager as executive secretary, senior intramural staff, and at least five advisors drawn from the scientific community. The advisory group either in its entirety or as subgroups participates in segment planning, preparation of requests for competitive proposals, review of proposals and monitoring of contracts.

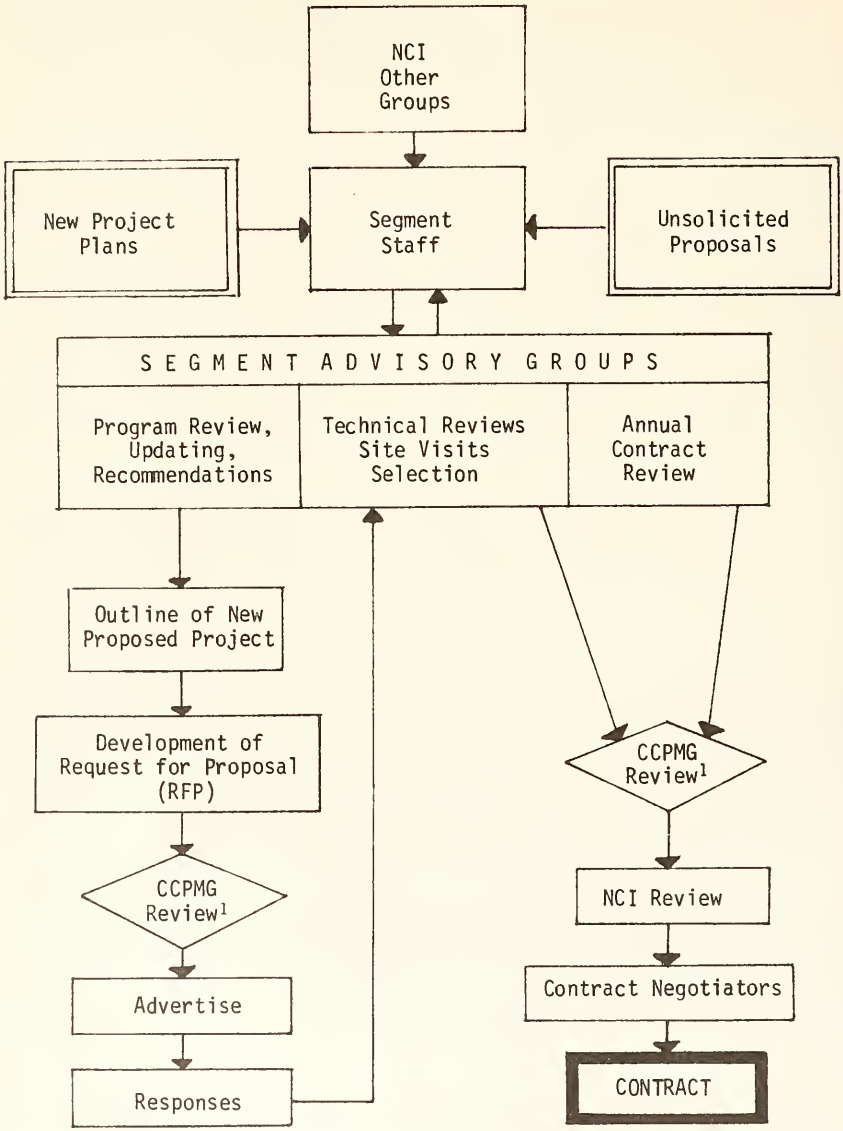
Each segment has the responsibility for developing its own specific program within the framework of the overall Carcinogenesis Program, in keeping with the fundamental objectives of the segment. As a basis for the implementation of its operational plans, each segment receives a preliminary budget apportionment from the Carcinogenesis budget. While this is an essential factor to good planning and program management, it also represents the realistic limiting factor requiring some difficult priority choices between continuing or expanding existing program and initiating new ones. When new ones are initiated, requests for proposals are formulated at the segment level and proposals are reviewed by the segment for technical merit and relevance to the segment program. Recommendations for support of a particular research program and establishment of a contract is made by the segment to the Carcinogenesis Contract Program Management Group (CCPMG)

The CCPMG is an advisory group which includes all segment directors and serves the following functions. It provides review of proposals for need, priority, and relevance within the total Carcinogenesis Program; it functions in an advisory capacity to the Associate Scientific Director for Carcinogenesis; and it reviews "Requests for Proposals" (RFPs) before they are advertised for competitive proposals. This last function is designed to prevent the initiation of inadequately structured RFPs and more importantly, to prevent the initiation of a request for work which may be considered to be of low relevance and priority to the overall program. Membership of the CCPMG is as follows: The Associate Scientific Director for Carcinogenesis (Chairman), the Coordinator for Collaborative Research (Executive Secretary), all of the segment directors and branch chiefs, and the Program Director for Carcinogenesis from the Division of Cancer Grants (to provide liaison with the Grants Program). Segment managers, contract specialists, administrative officers, and appropriate project officers attend the meetings as observers and resource staff. The membership and resource staff of this senior advisory group represents all of the major elements in Carcinogenesis: The Office of the Associate Scientific Director for Carcinogenesis, intramural scientific staff, collaborative program scientific and management staff, contract specialists, and Carcinogenesis' administrative staff, as well as the representative from the Division of Cancer Grants.

The contract development and review process, as presently functioning for the Carcinogenesis Program, is outlined in the following chart.

Following the completion of the dual review process, final recommendation on priority and funding is made by the Associate Scientific Director for Carcinogenesis to the Director of the Division of Cancer Cause and Prevention.

CARCINOGENESIS CONTRACT DEVELOPMENT AND REVIEW PROCESS



[1]CCPMG - Carcinogenesis Contract Program Management Group

The contract specialists (M. Fortin[1], J. L. Tidmore, D. Dougherty, and A. Beatty) assigned to this Program from the Cancer Cause and Prevention Section of the Research Contracts Branch, OD, provided coordination between scientific management and administrative mechanisms of negotiation, award and fiscal monitoring of contracts. They performed an essential and most effective function for the implementation of the complex research programs in the Area.

Responsibility for overall collaborative program management rests with the Coordinator for Collaborative Research who is on the staff of the Associate Scientific Director for Carcinogenesis. Responsibility and legal authority for the administrative aspects of contracting rests with the NCI Contract Officer and with contract negotiators working the Carcinogenesis Program staff. Although program staff and contract staff each has specific areas of responsibility, the two actually work closely together in the conduct of the collaborative program.

In October 1972 the Carcinogenesis Program held its First Annual Collaborative Conference. The Conference, held in San Antonio, Texas, was attended by staff, advisors, contractors and members of other agencies with similar interests. Participants met in several plenary sessions and also in specific segment meetings; an arrangement which provided them with the opportunity to become acquainted with the overall program and its goals as well as the staff, advisors, contractors and objectives of the specific segment which sponsors their research. The Conference was quite successful and served to create an increased awareness on the part of the participants in the Program and its objectives. A second conference in being planned for the Fall of 1973.

Segment activities have increased appreciably during the last year. Approximately 50 unsolicited proposals have been received. Of these, three have been recommended for support. An indication of the extent to which the planning process has progressed and of the degree of concern the Program has for the competitive process can be gotten from the fact that 32 RFPs have been developed and have been issued or will be issued before the end of FY 1973. Because of the multiple responses obtained, this represents reviewing approximately 300-350 new proposals in addition to approximately 130 renewal proposals. A substantial amount of staff time was involved in handling this proposal volume and in setting up and participating in a considerable number of staff and site visits. Outside consultants have been utilized heavily in the review process. The total impact of the management system is best seen in the quantity and quality of work reported by the individual segments.

Not all of the segments have full-time managers. The Chemistry and Molecular Carcinogenesis Segment and Colon Cancer Segment share the services of one Segment Manager; the Biology and Immunology Segment utilizes the services of the Coordinator for Collaborative Research as an acting Segment Manager.

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[1]Was one of the contract specialists until his retirement in February 1973.

The newly established Carcinogen Metabolism and Toxicology Segment shares the services of a Segment Manager with the reorganized Bioassay Operations Segment.

In summary, the Segment system has provided a means of managing a rapidly increasing program responsibility under circumstances which have permitted only minimal staff expansion. It is essential to continue to improve management techniques in order to conserve the efforts and time of the scientific staff upon which the program is built and upon whom much of its ultimate success depends.

D. PROGRAM PLANS

The overall plan for the Carcinogenesis Program was laid down in 1968 and has served as a solid basis for the implementation of new efforts, as a guide for the expansion of the Program and as a reference for its monitoring. Particular emphasis is now given to the development of special programs on the main organ-specific types of cancers, as seen in man. The first of these to be implemented was the Special Lung Cancer Program, developed in FY 1969, partly by the Lung Cancer Task Force and by the Tobacco Working Group. Its general structure was patterned on that of the general Carcinogenesis Plan. An intramural Lung Cancer Unit was established to serve as a research focus in this effort and to provide strong correlation between staff and contractors.

A special plan has been developed on the etiology of colon cancer, through an ad hoc group chaired by Dr. J. W. Berg, with the collaboration of several staff members in close liaison with the National Task Force on Colon Cancer. A contract program has been established in this important area. Two other special programs have been initiated by the Experimental Pathology Branch and the Biological Models Segment on cancer of the prostate and on cancer of the pancreas, again in close liaison with national efforts in these fields.

In response to the planning needs for the development of the new National Cancer Program, the Carcinogenesis staff has outlined a five-year plan and developed a number of supporting documents, including construction plans and plans for the utilization of research facilities at the Frederick Cancer Research Center.

A summary of the general Carcinogenesis Plan is given below.

Program Plan on Chemical Carcinogenesis and Prevention of Cancers

The plan was developed in 1968 through an extensive series of planning sessions conducted by Dr. Carl G. Baker, then Scientific Director for Etiology, Dr. Umberto Saffiotti, Associate Scientific Director for Carcinogenesis, and Mr. Louis M. Carrese, with the participation of Dr. Abraham Cantarow, and Mr. Richard A. Terselic. The Report of the Discussion Groups on Chemical Carcinogenesis, coordinated by Dr. Cantarow for the National Advisory Cancer Council, served as an excellent basis for outlining the research needs in the field of carcinogenesis. The convergence technique was used to identify the major objectives and the linear array of subsequent phases leading to their

implementation. An outline of the program plan is attached (see Chart) and its main phases are discussed below. The program is articulated in a number of segments. The first segment deals with (a) the epidemiologic identification of population groups showing different risks to different types of cancer and (b) to the characterization of such individuals as to their environmental factors and their biological and functional parameters. The program then develops the following distinct approaches:

(1) identification of carcinogenic activity of selected chemicals by bioassay,

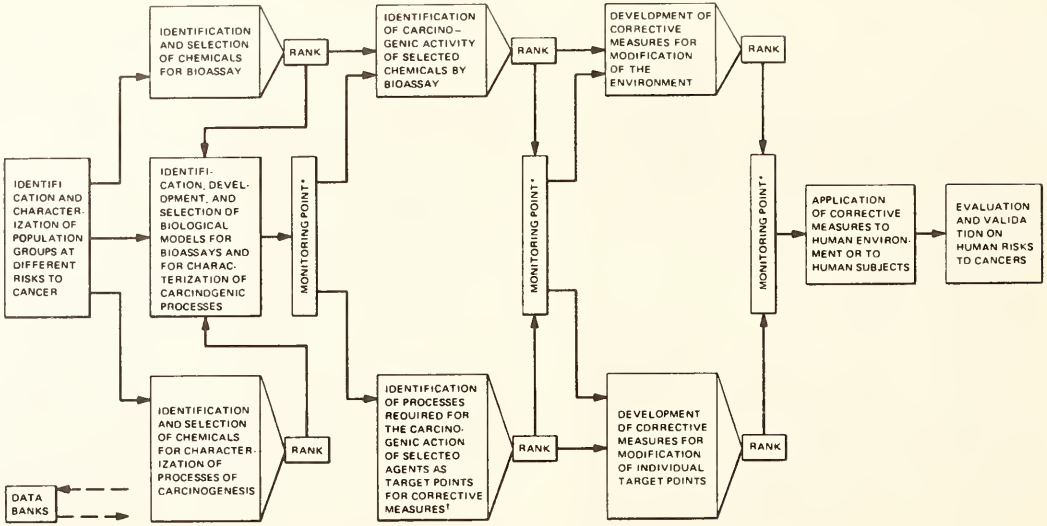
(2) identification, development, and selection of biological models for carcinogenesis bioassays as well as for the characterization of carcinogenesis processes, and

(3) identification of processes required for the carcinogenic action of selected agents as target points for corrective measures.

The first approach requires an initial phase devoted to the identification and selection of chemical agents to be entered in the bioassay systems. The development and implementation of bioassay procedures will require a great deal of accurate standardization so that selected chemical agents will be tested through a battery of precisely defined and reproducible bioassay systems, each well characterized for its sensitivity to specific carcinogenic effects.

After appropriate monitoring for relevance and for program needs, the results of the first phases of the program will lead to the development of corrective measures based, on one hand, on modifications of the environment and, on the other hand, based on modifications of individual target points in the process of carcinogenesis, identified by previous studies. These target points can be visualized as taking place at any one of the several steps required for the process of carcinogenesis to be completed. They include the following: (a) penetration of the chemical agent into the whole organism; (b) transport, retention and elimination of the chemicals in tissues (molecular logistics); (c) metabolic pathways to proximate carcinogen; (d) cell and tissue factors required for the previous steps; (e) penetration into target cells and interaction with cell constituents; (f) neoplastic transformation and conditioning factors; and (g) growth regulation of transformed cells.

The results thus obtained will be in turn appropriately monitored for relevance; the program then considers the development of corrective measures and ultimately suggests application of such corrective measures through the human environment or through the human subject. The last phase of the program deals with the evaluation and validation of the results of such corrective measures in the human situation and is expected to lead to the recognition of decreased cancer risks and in the populations originally studied in Phase I. Data banks will be organized for each of the major phases of the program and they will be a necessary source of information to be used for the monitoring of the whole program.



*Monitoring points: According to program relevence and needs, determine further action and channel through next phases

†Through studies on the penetration of the agents into the organism, their transport, retention, and elimination, their metabolic conversion to the proximete carcinogen: the cellular and tissue factors required for such processes, the penetration into target calls and interaction with cell constituants, the neoplastic transformation, and the factors required for the growth of transformed cells into tumors

CHEMICAL CARCINOGENESIS AND PREVENTION OF CANCER (SUMMARY OR PROGRAM PLANS).

The program is aimed at a comprehensive attack to the problem of chemical carcinogenesis. It recognizes the need for very extensive bioassay efforts of an order of magnitude much greater than that presently available, but also recognizes that the accumulation of information on the carcinogenicity of chemicals in animal systems will not provide a direct extrapolation to the hazards in man. Moreover, it is predictable that a large number of chemical carcinogens will be difficult to remove and, therefore, will remain in the environment of man for a long time to come even after being identified (e.g. combustion products, cigarette smoke, natural food contaminants such as mycotoxins, metals and other air pollutants). It is, therefore, imperative that an effort be made not only to identify and possibly remove an increasing number of carcinogens from the environment, but also, and concurrently, to identify steps in their mode of penetration into the organisms and the target tissues and in their critical interaction with cell constituents so that these steps could be exploited as target points for inhibitory or protective measures. The considerable development of refined technical methods presently available for the identification of these critical steps in carcinogenesis by studies in analytical chemistry, biochemistry, cell biology, molecular biology, and immunology can now be brought to bear on our ability to develop useful, protective measures against the ultimate effects of the carcinogenic process. The identification of critical parameters required for the carcinogenic action of certain groups of chemicals, obtained in animal studies, also represents the only direct link that can be applied to the study of the susceptibility of man to comparable exposure conditions.

For example, the establishment of a strong correlation between the enzymatic activation of certain chemicals to the proximate carcinogen by means of given groups of enzymes, such as the microsomal aryl hydroxylase for polynuclear hydrocarbons may lead to the identification of groups of individuals as being highly susceptible to certain specific carcinogenic exposures, pharmacologic inhibition of some of these enzyme systems may lead to a marked decrease of the cancer risk for such individuals. As other examples, analogous approaches can be made at the level of the control of protein synthesis in differentiation-dependent carcinogenic mechanisms (e.g. hormone-dependent tumors and vitamin-A dependent tumors) and at the level of the immunological control of the growth of transformed cells. A broad front of attack can thus be developed against the sequence of events required for the carcinogenic process to lead to the establishment of progressively growing invasive tumors.

The effective development of such studies depends in part on the availability of appropriate animal models, capable of reproducing tumor types analogous to those observed in man. The development of appropriate biological models is particularly important for the establishment of selectively sensitive bioassay systems. An important phase of the program consists in the selection and standardization of bioassay systems that could be reproduced in several laboratories under strictly controlled standardized conditions. This is an essential requirement if we want to collect and analyse large series of data on carcinogenesis tests. For this purpose, a detailed plan is being worked out for a data retrieval and analysis system for carcinogenesis studies. The present overall Program Plan in Chemical Carcinogenesis

and Prevention of Cancers constitutes the basis for the development of detailed operational plans aimed at the implementation of each of its phases.

E. SCIENTIFIC ACTIVITIES: PROGRESS HIGHLIGHTS

The progress accomplished in the Carcinogenesis Program in FY 1973 continues to cover a broad front of attack to the problem of the identification of causative factors and the prevention of cancers. Many of the projects in this Program have been underway for several years and represent a patient, intensive, and costly long-term effort towards the attainment of our specific program goals. Work in chemical carcinogenesis is characterized to a large extent by the two-three year duration of each lifespan study in common laboratory rodents, with the resulting long-range commitment of efforts and resources. Other projects represent a complex effort to develop and refine the research methods necessary for studying the spectrum of chemical, biochemical, biological, and molecular mechanisms that are responsible for the host response to chemical and physical carcinogens, and for the identification of exploitable preventive measures.

Summary reports of the Office of the Associate Scientific Director, of the branches, and of the segments, as well as detailed reports of individual projects and contracts, will be presented in the following sections of this Annual Report. The highlights of these reports are summarized here in the perspective of the overall plan of operation of the Carcinogenesis Program. The Program Plan in Chemical Carcinogenesis, developed in FY 1969, was the basis for the establishment of new projects. All of the long-term projects that were underway before the Plan was implemented were brought to an orderly conclusion; therefore, the ongoing projects and contracts are all directly related to the Plan's design.

Since 1968 the operation of the Program has been developed along the lines designated in its Program Plan. The major accomplishments over the last five years have been obtained in the following areas of high priority.

A major expansion of the bioassay program took place in FY 1972. In the present fiscal year the emphasis is on strengthening its organization, the standardization, and the monitoring of protocols.

A computerized Bioassay Data System was designed and tried out and is now operative in a majority of the bioassay contracts. It provides, for the first time, an effective centralized monitoring resource and a basis for data analysis.

Several information and resources systems have been set up, including the major first attempt to collect information on levels of exposure for man to environmental chemicals from all sources as a basis for priority ranking in the selection of chemicals for bioassay.

An extensive program has been developed for the chemical analyses and bioassays of tobacco smoke and its constituents, aimed at the development of a less hazardous cigarette.

Major programs have been established for the development of biological models for tumor pathogenesis closely correlated to the main types of cancers in man. Lung cancer and colon cancer programs are well established; pancreas cancer and prostate cancer programs were initiated in FY 1972 and are now developing programs on endocrine cancers and childhood cancers. Their emphasis is on the use of animal models and human material to identify pathogenetic steps susceptible of inhibition or prevention.

In vitro models for neoplastic transformation of cells in culture by chemicals have been developed using cells from hamsters, mice, and guinea pigs. A major advance was the development of a host mediated transformation assay, responsive to carcinogens that require metabolic activation by the host. These new methods are being used to establish their value both as screening techniques and as tools for identifying key steps in carcinogen activation and interaction, susceptible to inhibition. The applicability of these techniques to transformation tests of human cells is currently being investigated. Genetic factors and growth conditioning factors required for neoplastic transformation by chemicals have been identified. Enhancement of chemical cell transformation by radiation and of viral transformation by chemicals have been demonstrated. Transformation of epithelial cells *in vitro* by chemicals has also been obtained in preliminary experiments. A major advance has been made with the transformation of human cells *in vitro* by chemical carcinogens, conditioned by corticosteroids.

New emphasis has been given to the study of carcinogen metabolism and toxicology, and a large collaborative effort is underway in the documentation of the conditions of formation and activation of nitrosamines. Pharmacologic inhibition of the formation of carcinogens has been obtained by blocking nitrosation of amines. Other classes of carcinogens are also under study (e.g. hydrazines, metals).

Major metabolic steps in the enzymatic activation of several classes of carcinogens have been identified with special emphasis on polynuclear hydrocarbons. Their reproducibility in human tissues has already been demonstrated in some cases. Micromethods, applicable to determinations in small samples of human cells, have been developed for measuring carcinogen activation levels. Inhibitors of carcinogen activation have been identified.

Important progress has been made in the application of immunologic methods to the study and control of carcinogenesis and tumor growth in animals and in man. These methods have also contributed valuable knowledge applicable to another program area, i.e. cancer immunotherapy in man.

A major coordination and documentation effort in the field of environmental carcinogenesis has been developed. Extensive consultation and documentation has been provided to DHEW, various governmental and other agencies and to Congress, on many problem of evaluation of environmental carcinogenic hazards and their prevention.

Major accomplishments in the Carcinogenesis Program in FY 1973 are described in the reports from the Office of the Associate Scientific Director for

Carcinogenesis, branches, and segments in the following sections of this report. Highlights of scientific achievements are described in the following paragraphs.

1. BIOASSAYS FOR CARCINOGENIC ACTIVITY

a. Bioassay Operations

Carcinogenesis bioassay testing is currently underway with 445 chemicals including 142 chemical or industrial intermediates, 49 environmental chemicals, 75 food additives, 61 pesticides, 100 drugs and 18 plant extracts. Many of these tests are in an early stage and results will not be known for several months. Most of these studies are for a two-year period using a treatment mode similar to the expected human exposure. During the past year, the results of many studies have shown several classes of chemicals to be active as carcinogens; for example, of 35 drugs tested (mainly cancer chemotherapeutic agents), 25 appear to be carcinogenic. In addition, a number of industrial intermediates and potential environmental contaminants including pesticides and food additives, were also found to be carcinogenic. N-nitroso compounds have been found to be a highly potent class of chemicals.

Two compounds, dibromochloropropane and ethylene dibromide, used in fumigating buildings, foods, and other items which are later used by man, have been found to be highly active in inducing mammary adenocarcinoma and squamous cell carcinoma of the forestomach. These results are now being evaluated for their regulatory significance.

Several aromatic amines and alkylating agents, particularly chloroethers were found to be carcinogenic. As a result, actions have been taken to reduce human exposure to some of the compounds.

Studies are now underway to test the susceptibility of various strains of rats to a series of known carcinogens. These studies may indicate a better strain of animals for bioassay testing than currently used.

Several natural plant extracts have been found active in producing malignant histiocytomas when injected intramuscularly in rats. These materials have not been tested as yet by oral administration.

b. Tobacco Research

The extensive program for the development of a less hazardous cigarette is expanding to include: selection, production and machine smoking of some 40 types of cigarettes; collection of condensates of their smoke, chemical analysis and bioassay by various biological test systems; evaluation of pathologic effects and of inhibitory mechanisms.

Tobacco smoke condensates from 21 different types of experimental cigarettes were collected in mechanical smoking machines and utilized in mouse skin painting bioassay experiments. The initial correlations from these studies with chemical analysis of the tobacco condensate indicate that reconstituted

tobacco sheet show major promise for use in a cigarette which would be less tumorigenic. The manipulation of paper porosity in conjunction with reconstituted tobacco sheet offers a second area of major promise for reduction of tumorigenicity (Several contractors under Tobacco Research Segment).

2. BIOLOGICAL MODELS

a. Organ-Oriented Programs

New special programs aimed at developing specific experimental models for the major types of cancer seen in man, in order to identify pathogenetic steps susceptible of prevention or inhibition, have been started in FY 1972 and developed in FY 1973. They include cancer of the pancreas, endocrine-related cancers (prostate and breast), kidney and bladder, tumors of childhood.

Incorporation of ^{3}H-methyl nitrosourea into RNA and DNA of guinea pig pancreas is significantly increased in a gland which is undergoing regeneration and other toxic damage by ethioine over control levels of incorporation. Thus, there appears to be increased binding of carcinogen during regeneration of the pancreas (University of Kansas, Biological Models Segment).

Exposure of pancreatic dust to dimethylhydrazine resulted in hyperplasia of duct epithelium at 6-9 weeks. Longer term experiments are required to determine whether carcinomas are induced (Boston University, Biological Models Segment).

A definite synergism exists with x-rays and diethylstilbestrol (DES) for mammary carcinogenesis. When early tumors are considered, there is a dosage response in the range of radiations used for tumor induction in DES-treated rats. Using total tumors, the greatest synergism occurred at median dose, 150r. No virus particles have been demonstrated in any of the tumors examined. Preliminary evidence suggests progesteron may inhibit DES-induced mammary cancer and radiation synergism. Overiectomy may alter this progesteron inhibition (Alton Ochsner Medical Foundation, Biological Models Segment).

A cell culture technique for rat mammary epithelium has been developed. The cells replicate in the presence of a mixture of steroid and pituitary hormones until confluent. They can then be maintained at least 60 days in medium supplemented with insulin. These cells do not appear transformed and form normal appearing mammary structures upon transplantation to fat pads (Endocrine Carcinogenesis Section, Experimental Pathology Branch).

Transplantation studies in syngeneic hosts of chemically induced mouse liver tumors have shown the malignant nature of these tumors (often questioned in the evaluation of bioassay results). Several hepatocarcinogens were found to induce early production of alpha-fetoprotein in rats, a useful marker which is common to animals and man.

Transplantable lung tumors in strain C_3Hf are cross-reactive immunologically with each other and with normal lung tissue of lung tumor susceptible strain A, but not with C_3Hf sarcomas or with normal lung tissue from the lung tumor resistant strain DBA/Z. Genetic analysis of susceptibility has indicated that the ability of C_3Hf lung tumors to grow better in allogeneic than in syngeneic mice is due to the action of a single gene and correlates with the presence of strain C_3H lung tissue of an antigen cross reactive with the pulmonary tumors (Perinatal Carcinogenesis Section, Experimental Pathology Branch).

A high incidence of tumors can be induced in kidneys of rats by N-2-(4'-fluorobiphenyl)acetamide. Histologically these have a remarkable resemblance to human clear cell carcinomas of the kidney. The histogenesis and transplantation behavior of these tumors and pre-neoplastic lesions are under investigation. Human kidneys are being searched for early morphologic lesions resembling those shown to be premalignant in the rat (University of Maryland, Biological Models Segment).

b. Lung Cancer

Methods have been developed that permit us to study morphologic and biochemicals events directly on the respiratory epithelium, using *in vivo* or *in vitro* exposure techniques, in animals and in human tissues. Specific early ultrastructural and biochemical changes induced by carcinogens have been identified. The role of anticarcinogenic substances is investigated. A major pathway of action of retinol in mucus synthesis has been identified as the control of transglycosylation reactions: its role in the control of respiratory cancer induction is actively studied.

Systems for the generation of aerosols, vapors, and industrial dusts have been developed for testing the carcinogenicity of many substances by the inhalation route. Among the important substances which have been found to be carcinogenic or co-carcinogenic for the respiratory tract are calcium chromate dusts, sulfur dioxide, dusts formed from polyurethane foam (workers in the building trades industry may be exposed to this type of dust), and bis-chloromethyl-ether. The above findings have been reported to the National Institute of Occupational Safety and Health, and efforts have been made to curtail human exposure. Long-term followup studies of exposed workers have been instituted (New York University, Lung Cancer Segment).

An intensive effort is being made to improve the technique of sputum cytology and to use it effectively for earlier diagnosis of morphological stages in the development of lung cancer in man. A large group of uranium miners is being followed with sputum cytology in order to achieve the most accurate definition of the time and stage of development of lung cancer in this population of workers who are at such high risk for lung cancer. An atlas of human sputum cytology is being compiled that will be of help in diagnostic centers throughout the country. A method for respiratory cytology has been developed for laboratory animals and a good correlation has been found between animals and man, in terms of the cytologic stages that occur during the development of bronchogenic squamous cell cancer. These studies

are important because they indicate that findings made in the animal model should be of direct relevance to study of the human disease (St. Mary's Hospital and AEC-Oak Ridge National Laboratory, Lung Cancer Segment).

Exfoliative cytology studies during the experimental development of bronchogenic carcinoma in hamsters suggest that preinvasive carcinoma can be detected cytologically and that accurate diagnosis of early invasive carcinoma is feasible. This finding is of great importance with respect to the problem of early diagnosis of human lung cancer and for monitoring the effectiveness of inhibitory treatment (AEC-Oak Ridge National Laboratory, Lung Cancer Segment).

The process of development of squamous cell cancer of the lung in the hamster animal model is strikingly similar to that found in man, and the ultrastructure of the tumors caused by either of the above two carcinogens in the animal is very similar to the ultrastructure of comparable human tumors. These findings again indicate that the animal model should be of direct relevance to the human disease (Lung Cancer Unit, Office of the Associate Scientific Director for Carcinogenesis).

The technique of light microscopic autoradiography of the respiratory tract has been improved so that the actual amount of radioactivity in cellular and subcellular compartments of respiratory epithelium can be quantitatively measured. The distribution and binding of labelled carcinogens has been measured in respiratory epithelium (Lung Cancer Unit, Office of the Associate Scientific Director for Carcinogenesis; Veterans Administration Hospital, Lung Cancer Segment).

Using the short-term organ culture technique developed in the Lung Cancer Unit's laboratory, the binding of the carcinogen, benzpyrene, to DNA of tracheal epithelial cells was demonstrated. This binding is inducible by previous treatment of animals with benzpyrene, and can be strongly inhibited by the antimetabolite, 7,8-benzoflavone. In vitamin A-deficient animals, an enhanced binding of benzpyrene to DNA of tracheal epithelial cells was demonstrated. The above findings are of importance because they show that prior exposure to a carcinogen in the respiratory tract may enhance subsequent carcinogenic response, and because they indicate that a nutritional deficiency that occurs commonly in man may be associated with an increased carcinogenic response (Lung Cancer Unit, Office of the Associate Scientific Director for Carcinogenesis).

Since isoenzyme ("isozyme") patterns have been found to change in tissues other than lung during carcinogenesis, new techniques have been developed for measurement in respiratory epithelium of the isozyme pattern of several different enzymes, including lactic dehydrogenase, malic dehydrogenase, aldolase, and hexokinase. The lactic dehydrogenase pattern in normal epithelium was found to be very different from that found in lung tumors induced by benzpyrene and nitrosomethylurea (Lung Cancer Unit, Office of the Associate Scientific Director for Carcinogenesis).

Administration of high doses of vitamin A to rats partially protects the respiratory tract epithelium from the tumorigenic effects of a polynuclear hydrocarbon such as methylcholanthrene (AEC-Oak Ridge National Laboratory, Lung Cancer Segment).

Vitamin A and analogs can prevent the activation of benzpyrene to a presumed "proximate carcinogen". This is an important observation because it indicates that vitamin A and analogs can modify the metabolism of the class of hydrocarbon carcinogens which are believed to be important agents in development of lung cancer in man (Southern Research Institute, Lung Cancer Segment).

States of vitamin A deficiency have been found to be associated with a high rate of cellular proliferation and carcinogen binding in respiratory epithelium. This is an important finding because it suggests that there may be a synergistic effect between vitamin A deficiency and carcinogen action in respiratory epithelium (Veterans Administration Hospital, Lung Cancer Segment).

A new study on the effects of vitamin and vitamin A analogs on epithelial cells in tissue culture has just been started, in collaboration with the Experimental Pathology Branch. It is already clear from these new studies that vitamin A and vitamin A analogs can be shown to have profound effects on the growth and differentiation of epidermal cell cultures. These tissue culture studies are important because they will serve as a primary screen for evaluation of biological activity of new synthetic vitamin A analogs, as well as being useful for further elucidation of the mechanism of action of vitamin A. Since the eventual use of vitamin A analogs for cancer prevention in man is currently under consideration, it is of utmost importance to have the most efficient primary screen for assessment of activity (Lung Cancer Unit, Office of the Associate Scientific Director for Carcinogenesis).

New experiments have indicated that tracheal epithelium can synthesize a mannolipid from retinol and GDP-mannose. Hydrolysis data on the purified mannolipid indicate that it has the structure of a retinyl-phosphate-mannose type of compound. The mannolipid has been shown to function as a carrier for mannose during formation of glycoproteins (Lung Cancer Unit, Office of the Associate Scientific Director for Carcinogenesis).

c. Colon Cancer

A program has been established in FY 1972 in close collaboration with the National Task Force on Colon Cancer. Its focus is on the definition of the correlation of colon cancer morbidity in migrant populations and the pathology of these cancers with environmental dietary factors. The role of bacterial flora in the metabolism of intestinal carcinogens and in the susceptibility of population groups is being studied.

The general consensus about the cause of human bowel cancer is that bacteria in the large bowel produce the actual carcinogen from precursors in the feces. Studies indicate that the fecal microflora of Americans consuming a mixed Western diet (high risk) showed increased ability to hydrolyze glucuronide conjugates as compared to those of American vegetarians, Seventh-Day Adventists, Japanese, and Chinese. Gluconides are a main detoxification product of carcinogens and hence such carcinogens might be preferentially reactivated with the "high risk" diet. Also, Americans

consuming a Western-type diet excreted high levels of bile acids as well as increased amounts of microbially degraded acid and neutral steroids, compared to other groups. *In vitro* incubation studies indicate that acid and neutral steroids were more extensively degraded to various metabolites by the anaerobes isolated from Americans on Western diet compared to vegetarians. One or more of these compounds may be carcinogens; they are now being tested (American Health Foundation, Colon Cancer Segment).

A diet history interview questionnaire has been developed for use in epidemiologic case control studies of cancer of the large bowel. Although specifically developed for contract work, it will be available for use in other diet history studies (Epidemiologic Pathology Unit, Office of the Associate Scientific Director for Carcinogenesis).

Familial polyposis is a cancer-producing disease caused by a dominant gene. In search for the mechanism of gene action, loss of control of DNA synthesis prior to any morphologic changes has been found and what part of the regulating mechanism is defective is now being studied (Memorial Hospital for Cancer and Allied Diseases, Colon Cancer Segment).

Chemically-induced carcinomas of the small intestine and of the colon have been successfully transplanted into weanling rats (Carcinogen Screening Section, Experimental Pathology Branch).

d. *In Vitro* Models for Chemical Carcinogenesis

Much progress has been made in the development and definition of *in vitro* systems for studying neoplastic transformation at the cellular level. Several laboratories in the intramural program and several institutions in the collaborative program have contributed to this field. Plans are presently being made for a working conference of all key investigators in this area of research.

The hamster embryo cell system has now been tested with over 30 carcinogens and analogs and shows a high degree of correlation between *in vivo* carcinogenic activity and *in vitro* transforming activity. The problem posed by those chemicals that require metabolic activation in the whole animal to become reactive as carcinogens has been resolved by the development of a host mediated *in vivo* - *in vitro* combination bioassay. The system differs from the established quantitative *in vitro* assays by *in vivo* exposure of fetal target cells following intraperitoneal injection of pregnant hamsters with chemical compounds as opposed to direct application of chemicals to cells in culture. Fetuses exposed *in utero* are subsequently excised, the cells grown in culture and the presence of transformed cells determined as in the standard *in vitro* systems by study of colony morphology; neoplastic transformation is verified by tumor production. None of the five non-carcinogenic compounds whereas all twelve chemical carcinogens tested including several known to be inactive by direct *in vitro* exposure to cells produce neoplastic transformation. Thus, this *in vivo* - *in vitro* system provides a reproducible bioassay for chemical carcinogens reducing false negatives that may occur because of the requirement for metabolic activation (Cytogenetics and Cytology Section, Biology Branch).

Morphological cellular changes of strain 2 guinea pig embryo cells exposed to chemical carcinogens compatible with *in vitro* chemical neoplastic transformed as observed in Syrian hamster embryo cells have been seen with cells exposed to chemical carcinogen but not to non-carcinogens either while *in utero* or after introduction into culture. Transformed guinea pig cells exhibit *in vitro* properties characteristic of transformed hamster and mouse cells. The incidence of tumor formation following inoculation of transformed guinea pig cells, however, is less in irradiated syngeneic guinea pigs than in irradiated hamsters. Complement dependent microcytotoxicity assay with antisera prepared to cultured untreated fetal cells and to cell strains derived from cells transformed following exposure to chemical carcinogens of different chemical classes indicates cross reactivity between the transformed cells and the untreated cultured guinea pig fetal cells. There is some evidence in addition for antigenic differences in the different cell strains but the differences have yet to be defined (Cytogenetics and Cytology Section, Biology Branch).

After several years of examining chromosomes of tumor cells and transformed cells in our studies of carcinogenesis, it was concluded that chromosome changes appeared to be random. With new techniques for chromosome bands, it has been possible to definitely identify each pair of Syrian hamster chromosomes. The technique has made possible the accurate identification of numerical changes in chromosome groups and of chromosomes with uncommon banding pattern which occurred subsequent to neoplastic transformation. Thus far the following tentative conclusions have been formed: similar banding patterns in transformed cell lines and tumor lines derived from them may occur, providing conclusive evidence that the transformed cells were responsible for tumors obtained. Different carcinogens may produce transformation associated with a specific marker but not all transformed lines have the same marker even with the same chemical. The increase in chromsome number does not involve a specific chromosome group. In some rearrangements heterochromatin may be involved. The significance of the changes observed is debatable but probably reflects secondary alterations (Cytogenetics and Cytology Section, Biology Branch).

A coordinated group of projects has been developed in the area of somatic cell genetics and considerable progress has been made. In the previous year, methods were developed for the induction and isolation of mutants of mammalian cells. Using these procedures, mutants with altered cell membranes have been isolated (Ontario Cancer Institute, Chemistry and Molecular Carcinogenesis Segment).

Since altered cell membranes may be directly responsible for conversion of cells to malignant forms, such mutants will be extremely valuable in studies on carcinogenesis. Temperature sensitive mutants have been isolated, one of which is defective in the processing of ribosomal RNA from a precursor. This single mutant allows the examination of a number of physiological processes related to RNA metabolism and their role in carcinogenesis (New York University, Chemistry and Molecular Carcinogenesis Segment).

Hamster cells treated with certain chemical carcinogens were found to behave as tumor cells at physiological temperature, but as normal cells at a lower

temperature. This work suggests that chemical carcinogens may exert their effects by imposing a genetic change which results in temperature sensitivity of a function required to keep the cell normal. This new hypothesis, which can be tested, may lead to a molecular understanding of chemical carcinogenesis, and also to the development of a rapid test for carcinogenicity (University of Illinois, Chemistry and Molecular Carcinogenesis Segment).

In-house research in this field has defined the metabolic requirements for infection and transformation of cells by RNA-containing tumor viruses. Viral DNA had been shown previously to be the intracellular form of the genome of RNA tumor viruses. Recent results demonstrate that no new proteins are required for synthesis of this DNA, that synthesis of this DNA begins within the first hour after exposure of susceptible cells to virus, and that the DNA itself reproduces within the infected cell. These studies are important to an understanding of how cells become induced to malignancy by viruses (Cell Growth Regulation Section, Chemistry Branch).

Earlier studies from this Section demonstrated that cellular divisions were not a requirement for reproduction of RNA tumor viruses. Further experimentation revealed that both morphological and biochemical changes associated with malignancy could be induced without the necessity of cellular division. These studies suggest that as a general phenomenon, genetic and physiological changes rendering a cell malignant can occur without intervening cellular divisions. Additional studies in this Section concerned the identification of the molecules responsible for malignant transformation. Cells infected with a mutant of a tumorigenic virus behave as tumor cells at 37° but not at 41°. Results of experiments on these cells involving metabolic analogs, cell density measurements, histochemistry, and electron microscopy suggest that the temperature sensitive molecule responsible for the malignant change is a protein, and that this protein is involved in the entry and efflux of ions and water across cell membranes.

In-house research in the Experimental Pathology Branch (*In Vitro* Pathogenesis Section) has continued to develop culture systems for the study of epidermal cells and their transformation. In ordinary culture medium, epidermal cell cultures differentiate with a gradual loss of proliferating cells until the cultures die. Addition of retinyl acetate to early cultures prevents differentiation and results in maintenance of replicating epithelial cultures for prolonged periods. Once differentiation has proceeded beyond a certain point, it cannot be reversed by retinyl acetate. Carcinogen binding continues to be studied in this system.

In contrast to other reported systems of repair of carcinogen-induced damage to DNA, β-propiolactone bound to DNA of mouse skin cells results in repair by insertion of only one type of nucleoside, deoxyguanosine. This suggests a new type of repair in which only a single base to which a carcinogen is bound can be removed. Other reported mechanisms include removal of larger portions of DNA with repair by insertion of all four deoxyribonucleosides.

The system for *in vitro* cultures of liver cells and their transformation by chemicals developed in the Carcinogen Screening Section (Experimental Pathology Branch) was further defined.

3. IDENTIFICATION OF PROCESSES OF CARCINOGENESIS

a. Chemistry, Carcinogen Metabolism and Molecular Carcinogenesis

A large coordinated program has been developed in the field of nitrosamine carcinogenesis with major advances in the analytical method in the study of their metabolic pathways and in their formation by nitrosation of secondary and tertiary amines.

Nitrosation of secondary amines by nitrite ion has been shown to proceed smoothly in neutral and basic medium under catalysis by formaldehyde and other electrophilic species. Although the implications of this finding for the area of environmental carcinogenesis are not yet clear, the data raise the strong suggestion that non-acidic media such as saliva, polluted water, soil, and foodstuffs should not be ignored as possible environments for the synthesis of biologically significant quantities of N-nitroso compounds.

Analytical methods for volatile and non-volatile nitrosamines have been developed and applied preliminarily to meat and fish products, alcoholic beverages, and water samples. Kinetics on nitrosation of amino acids and simple amines have been examined, especially as catalyzed by anions and as such, reactions migh occur in frozen and other multiphase systems, which can lead to marked acceleration. Bacteria responsible for nitrate reduction in human saliva have been characterized (Massachusetts Institute of Technology, Carcinogen Metabolism and Toxicology Segment).

Kinetics of nitrosation of drugs and amino acids have been studied; measurable dimethylnitrosamine is produced *in vitro* from aminopyrine and nitrite within five seconds, but terramycin is nitrosated much more slowly. Vitamin C inhibits the acute liver necrosis in rats otherwise observed on feeding dimethylamine and nitrite and blocks lung adenoma enhancement by piperazine and nitrite in mice. Naturally-occurring ureas and their nitro derivatives are also under investigation; during long-term feeding, nitrosodihydrouracil produced liver tumors in 100% of the rats (University of Nebraska, Eppley Institute for Research on Cancer, Office of the Associate Scientific Director for Carcinogenesis).

Methods have been developed for the analyses of tobacco smoke, fish, and other foodstuffs for nitrosatable secondary and tertiary amines. Steric effects on the synthesis of nitroso piperidines have been charactered. Amino pyrine and heptamethyleneimine produce liver and lung tumors respectively in 100% of the rats fed these materials together with nitrite of 30 to 50 weeks; the former compound was administered with nitrite at dose levels similar to those permitted by FDA regulations (AEC-Oak Ridge National Laboratory, Colon Cancer Segment).

Nitrosamines are among the most ubiquitous and most powerful environmental carcinogens. Hence, eliminating nitrosamine from the environment will not be enough to eliminate nitrosamine cancers if such exist in humans; more specific counteraction will be needed.

The chemical mechanisms of activation of nitrosamines are being studied in the intramural laboratories. Metabolism of other classes of carcinogens have been further defined. Acetanilide and *p*-hydroxyacetanilide were found to inhibit cancer induction by 3'-methyl-4-dimethylaminoazobenzene or the potent hepatocarcinogen, 6-(*p*-dimethylaminophenylazo)quinoline, in the livers of male Sprague-Dawley rats on a 12% protein-low riboflavine diet. Thus, the inhibitory effects of acetanilide and *p*-hydroxyacetanilide for liver carcinogens also hold for certain aminoazo dyes. Two new water soluble metabolites of acetanilide have been identified, through the use of various instrumental techniques, as S-(5-acetamido-2-hydroxyphenyl)mercapturic acid and S-(5-acetamidophenyl)mercapturic acid. The chief biliary metabolite of the carcinogen, 6-aminochrysene, also employed clinically in treatment of mammary cancers was the N-glucuronide of the amine. Since prefeeding *p*-hydroxyacetanilide increased the excretion of the glucuronides of 2-fluorenylacetamide derivatives, the possibility arose that it might act as an enzyme inducer. Liver microsomes from rats fed *p*-hydroxyacetanilide for four to five weeks had a threefold increase in glucuronyl transferase levels over those in controls (Carcinogen Screening Section, Experimental Pathology Branch).

Major emphasis has continued to be given to the identification and control of enzyme systems responsible for the activation and detoxification of polynuclear hydrocarbons (Office of the Chief, and Molecular Carcinogenesis Section, Chemistry Branch).

The vast majority of foreign compounds including carcinogens are metabolized by the microsomal fixed function oxygenase enzyme system. It was found that this enzyme was influenced by a variety of environmental factors such as previous exposure to drugs, pesticides or carcinogens, nutritional and hormonal states, the age, sex and genetic makeup of the organism. This enzyme system is responsible for the conversion of procarcinogens to their ultimate carcinogenic form. In addition, this enzyme was found responsible for the toxic effects of the polycyclic hydrocarbons. The latter important conclusions are derived from the following five lines of evidence developed: (1) the toxicity of benzopyrene (BP) parallels level of enzyme in the cell, (2) the enzyme system catalyzes the formation of BP-DNA complexes, (3) the discovery of an inhibitor of the enzyme system, 7,8-benzoflavone, which prevents BP cytotoxicity, (4) the inhibitor of the enzyme prevents the binding of BP to DNA, RNA, and protein, and (5) the inhibition of the enzyme system reduced dimethylbenzanthracene tumorigenicity by 90%.

The role of aryl hydrocarbon hydroxylase in human carcinogenesis by chemicals is being investigated. A major finding has been that the enzyme system can be identified and measured in a preparation of lymphocytes from human blood. This is the first report of the presence of this enzyme in an easily obtainable human tissue. This finding will be the starting point of an analysis of the enzyme level in a human population and its relevance to chemical carcinogenesis.

A number of different aspects of the P-450 carcinogen metabolizing enzyme systems is being studied in which progress has been made. A highly sensitive and quantitatively reproducible method has been developed for the isolation and identification of eight metabolites of BP. Those include the previously unreported K-region diol which seems to be particularly related to carcinogenic

activity. It was found that the ratio of this possibly ultimate carcinogen may be altered by different enzyme levels and is found in different proportions in different tissues and species. The goal of this project is to relate the metabolic profile of BP to carcinogenicity in different tissues and species.

The regulation of AHH carcinogen metabolizing enzyme system is being intensively studied. A major finding has been that this enzyme is present and highly inducible in a cloned cell line derived from rat liver. This will eventually enable us to study the genetics of this enzyme system. The kinetics of aryl hydrocarbon hydroxylase induction, the nutritional requirements, and the requirement of macromolecule synthesis were described. An important finding has been that temporary inhibition of protein synthesis is followed by a large rise in enzyme level even in the absence of the polycyclic hydrocarbon inducer. This finding indicates that the level of regulation of this enzyme is present at least at two different sites in the cell. Thus enzyme level is controlled by the amount of RNA transcription from the gene and secondly the process regulating the translation of messenger RNA (mRNA) into protein at the level of the cytoplasm.

The regulation of AHH enzyme system was determined in somatic cell hybrids of parent cells that differ both in their basal levels as well as inducible AHH. In certain sets of hybrids the hybrid cell enzyme was considerably less than either parent while in other hybrids the enzyme level was greatly enhanced. Thus these studies indicate the multi-faceted regulation of this enzyme and more importantly demonstrate that cells can be constructed experimentally with unique levels of carcinogen metabolizing activity, either with high, low or intermediate. These cells can then be used for studies in transformation or in an assay for carcinogen activity.

A number of compounds were sought and found which can greatly modify carcinogen metabolism by either inducing the AHH enzyme to high levels or by inhibiting the enzyme system. In one case a compound was found which can enhance enzyme activity by what appears to be an allosteric rather than an induction mechanism. These compounds are being tested for their effect on tumorigenesis. Thus upon learning enough about the role of the enzyme in carcinogenesis, it may be possible to modify the enzyme activity in a specific manner and hence modify the course of carcinogenesis.

The transport of heme synthesized in the mitochondria was studied. Heme synthesized in mitochondria is transferred to the microsomes and has identified soluble substances in the cytosol which function in this transfer. The study affords insight into the complexity involved in the assembly of this important microsomal enzyme (Johns Hopkins University, Chemistry and Molecular Carcinogenesis Segment).

Work in the Carcinogen Screening Section (Experimental Pathology Branch) showed that aryl hydrocarbon hydroxylase activity of rat embryo cells in culture was increased in cultures that had been infected with Rauscher leukemia virus. This may be one explanation for the enhanced sensitivity of RLV infected cells to transformation by polycyclic aromatic hydrocarbon.

In an entirely new development, in a collaboration of the Chemistry Branch and the Biology Branch, it was found that the AHH carcinogen metabolizing enzyme is present and highly inducible in tissues engaged in immunological activity. Thus lymphocytes and macrophages, and other lymphoid tissues contain the enzyme and are highly inducible. This research opens up an entirely new area of investigations of the role of the enzyme system in the processing of the carcinogen as an antigen and in investigations on the role of the immune system in carcinogenesis.

At the molecular level, the problem of DNA damage and repair by carcinogenic agents and the control mechanisms related to this fundamental process were further investigated.

A new host-cell reactivation assay for determining the efficiency of cellular DNA repair mechanisms using Adenovirus-2 was developed. In this assay, damaged (e.g. UV-irradiated) Adenovirus-2 is plated on monolayers of an appropriate susceptible host cell (e.g. normal human fibroblasts or those from patients with xeroderma pigmentosum) and the development of plaques of lysed dead cells is followed. Cells whose DNA repair is inadequate develop fewer plaques than those whose DNA repair is normal. The plating efficiency using unirradiated Adenovirus is the same on all of these cell lines. This assay will enable us to determine the efficiency of DNA repair mechanisms in cells from a variety of human and rodent tissues and thus to further investigate the relationship between the efficiency of DNA repair and the susceptibility to malignant transformation. Further and more extensive evidence was obtained that the initial reverse transcription of an RNA tumor virus genome is carried out in association with the plasma membrane of infected cells. This data will help advance our understanding of molecular and cellular events underlying the malignant transformation of susceptible cells by RNA tumor viruses (Nucleic Acids Section, Chemistry Branch).

Human diploid fibroblasts repair DNA damage induced by the carcinogens N-acetoxy-2-acetylaminofluorene and 7-bromobenz[α]anthracene by the insertion of all four deoxyribonucleosides. Both A-T rich and E-C rich areas of the genome appear to be equally well repaired (Digestive System Carcinogenesis Section, Experimental Pathology Branch).

Work in the Cell Growth Regulation Section (Chemistry Branch) led to an important observation concerning transfer RNA and protein synthesis in mammalial cells. Triplets of nucleotides in sequence determine the beginning, the amino acid sequence, and the end of the protein synthesized by translation of messenger RNA. Transfer RNA's which recognize these triplets can influence the nature and amount of proteins synthesized, and specific transfer RNA's found in microorganisms only under unusual circumstances were found to be regular components of mammalian differentiated cells. This discovery may have important implications with regard to the maintenance of a "normal" physiological state and to biochemical changes responsible for malignancy.

b. Immunology

Polynuclear hydrocarbon carcinogens were found to sensitize guinea pigs so that exposure to the agent can be recognized by an allergic reaction. Specific

antibodies to carcinogens produced with suitable coupling agents have been made (Brandeis University, Biology and Immunology Segment). In addition antibodies have been made specifically to react with nicotine and cotinine An almost complete correlation was found by radioimmunoassay between the presence of nicotine and cotinine and whether the serum came from a smoker or a nonsmoker.

Feeding of diethylnitrosamine (DEN) to guinea pigs results in the virtual elimination of the fourth component (C4) of complement from the blood. This event preceded by many months the appearance of cancer. Treatment of monocytes (cells synthesizing C4) *in vitro* with DEN resulted in no loss of C4 synthesizing capability by these cells. Monocytes removed from animals fed DEN, however, show greatly impaired *in vitro* synthesis of C4. This observation permits the *in vitro* analysis of early *in vivo* carcinogenic events on a cellular and molecular basis (Immunochemistry Section, Biology Branch).

Susceptibility of a cell to killing by specific antibody and complement is a function of availability of critical sites on the cell surface to the ultimate components of complement. This site of attack is independent of the distribution of antigens to which the complement fixing antibodies are bound and is independent of the distribution of complement fixing antibodies. These results revise the presently accepted dogmas of cell killing by immune mechanisms (Immunochemistry Section, Biology Branch).

The addition of antitumor antibody to suspensions of tumor cells causes tumor antigen to move in the plane of the cell membrane. The antigen-antibody complexes coalesce into aggregates and appear to be extruded from the cell. This system will be useful for study of the dynamics of macromolecules in cell membranes. It suggests that antibody *in vivo* may lead to modulation or removal of cell surface antigens (Tumor Antigen Section, Biology Branch).

Murine tumor cell monolayers were destroyed by PPD-stimulated spleen cells from BCG-immune mice and also by supernatant culture fluids from the cells. Tumor cell monolayers were much more sensitive to the toxic action of the supernatants than were mouse fibroblast monolayers. *In vivo* experiments are indicated to determine whether this differential sensitivity applies to all normal cells so that the active substance in the supernatant fluids could be used as an agent specifically cytotoxic for tumor cells (Tumor Antigen Section, Biology Branch).

Studies in an experimental model for immunotherapy of cancer showed the Phipps, Tice and Glaxo substrains of BCG to have equivalent tumor suppressive properties. There was some suggestion that the Pasteur substrain of BCG possessed greater tumor suppressive properties than the other BCG substrains tested. Studies attempting to purify substances with tumor suppressive properties from the BCG cell wall showed that material with tumor suppressive properties could be obtained by extraction of the cell wall with a mixture of chloroform and methanol. An *in vitro* assay for activated "macrophages" was developed. Killing of listeria monocytogenes was mediated by a factor liberated from macrophages into the supernatant fluid and was not primarily an intracellular process (Cellular Immunity Section, Biology Branch).

4. INFORMATION AND RESOURCES

The Carcinogenesis Bioassay Data System is now fully operational in the bioassay contract program. A significant data base has been created with 20 contractors currently having data in the system. Approximately 12,000 records have now been recorded in the data base. The Phase I output in the form of summary reports and simple grafts have been produced during the past year. Several of the experiments are nearing completion allowing data from them to be analyzed in the computer sub-system of Phase II. All new bioassay studies are being entered into the system as they are initiated.

Standardized chemical resources have been developed. In addition to the development of a procurement service for standard samples of purified carcinogens and analogs, analytical resources have been expanded to provide highly characterized chemicals for testing in the bioassay program. The development of a set of guidelines for the safe handling of potentially carcinogenic materials is also underway.

The first monograph of a series entitled "Evaluation of Carcinogenic Risk of Chemicals to Man" covering a number of polyaromatic hydrocarbons has been published by the International Agency for Research on Cancer (WHO) supported under contract. Two additional monographs are in press. One will cover a selected group of inorganic and organo-metallic compounds and the other, polycyclic aromatic hydrocarbons and heterocyclic compounds. These monographs represent critical reviews of the published data on the carcinogenic activity of selected chemicals and they constitute the major reference for carcinogenicity evaluations. As a function of this contract activity, an international registry of chemicals undergoing chronic toxicity tests is now being established.

Publication of the PHS Publication No. 149 series entitled "Survey of Compounds Which Have Been Tested for Carcinogenic Activity" has been brought up to date. The 1961-1967 volume and the 1970-1971 volume have been completed and are now in press.

The Registry of Experimental Cancers (Office of the Associate Scientific Director for Carcinogenesis) has accomplished the preparation and coding of 12,000 records. The printouts are used by members of the Registry and by investigators who visit the Registry and have been supplied to the International Agency for Research on Cancer in Lyon, France, and to the Institute for Experimental Gerontology in Rijswijk, Holland.

The following workshops and conferences were organized and/or sponsored directly by the Carcinogenesis Program and/or in collaboration with other agencies:

First Workshop on Mutagenicity as a Prescreen for Chemical Carcinogens sponsored by the Bioassay Segment held in Bethesda, Maryland, on July 18, 1972

Conference on Nitrosamines for NCI Staff, Contractors, Advisors, and Other Interested Agencies held in Bethesda, Maryland, on July 20, 1972

Conference on Host-Environment Interactions in the Etiology of Cancer in Man--Implementation in Research sponsored by the Fogarty International Center, NIH, the International Agency for Research on Cancer, and the League for the Fight Against Cancer of the Croatian Republic of Yugoslavia held in Primosten, Yugoslavia, on August 27-September 2, 1972

First Annual Collaborative Conference of the Carcinogenesis Program held in San Antonio, Texas, on October 2-4, 1972

Conference on the Use of BCG in Experimental and Clinical Immunotherapy of Neoplastic Diseases sponsored by the Division of Cancer Cause and Prevention and the Division of Cancer Treatment held in Bethesda, Maryland, on October 5-6, 1972

Workshop on Late Effects of Cancer Therapy in Children and Young Adults sponsored by the National Cancer Institute and the Memorial Hospital for Cancer and Allied Diseases held in Boston, Massachusetts, on October 19-20, 1972

World Symposium on Model Studies in Chemical Carcinogenesis sponsored by the National Cancer Institute, Atomic Energy Commission, and Johns Hopkins University held in Baltimore, Maryland, on October 31-November 3, 1972

Working Group on The Scientific Basis for the Delaney Amendment convened by the New York Academy of Sciences in New York, New York on January 15-16, 1973

Workshop on Environmental Considerations for the Conduct of Chronic Long-Term Studies with Rodents sponsored by the Information and Resource Segment held in Bethesda, Maryland, on April 4-5, 1973

Conference on Carcinogenesis Testing in the Development of New Drugs sponsored by the National Academy of Sciences--National Research Council with the participation of the National Cancer Institute to be held in Washington, D.C. on May 23-25, 1973

F. FISCAL SUMMARY

The following charts and tables report the fiscal history and the present distribution of funds in the Carcinogenesis Program, including Direct Operations and Contracts.

CARCINOGENESIS PROGRAM - FISCAL HISTORY

DESCRIPTION	JUNE 1968	JUNE 1969	JUNE 1970	JUNE 1971	JUNE 1972	JUNE 1973 PROJECTED
PERSONNEL[1]	109	102	106	126	180	172
(FULL-TIME PERMANENT)	(107)	(98)	(96)	(110)	(145)	(148)
(OTHER FULL TIME)	(1)	(1)	(8)	(9)	(23)	(10)
(PART TIME)	(1)	(3)	(2)	(7)	(12)	(14)
SPACE (IN SQ. FT.)	15,885	26,829[2]	26,112[3]	28,337	31,041[4]	38,164[5]
LEVEL OF EXPENDITURE OF IN-HOUSE OPERATION	$1,636,000	$1,802,600	$1,997,000	$2,340,000	$3,538,000	$4,440,000
NUMBER OF CONTRACTS	33	40	51	69	133	169
LEVEL OF EXPENDITURE OF CONTRACTS	$5,602,000	$8,466,600	$7,080,000	$10,598,000	$21,985,813	$26,661,000

1 INCLUDES ALL PERSONNEL ACTUALLY ASSIGNED TO THE CARCINOGENESIS PROGRAM.

2 IN NOVEMBER 1968, OFFICES OF THE OASDC, LOCATED IN THE WISCON BG., AND OFFICES AND LABORATORIES OF THE BIOLOGY BRANCH, LOCATED IN THE AUBURN BG., MOVED TO BUILDING 37. IN AUGUST 1968, OFFICES AND LABORATORIES OF THE CHEMISTRY BRANCH, LOCATED IN THE AUBURN BG., MOVED TO BUILDING 37.

3 EXPERIMENTAL PATHOLOGY BRANCH AND UNITS WITHIN THE OASDC WERE ESTABLISHED IN JANUARY 1970.

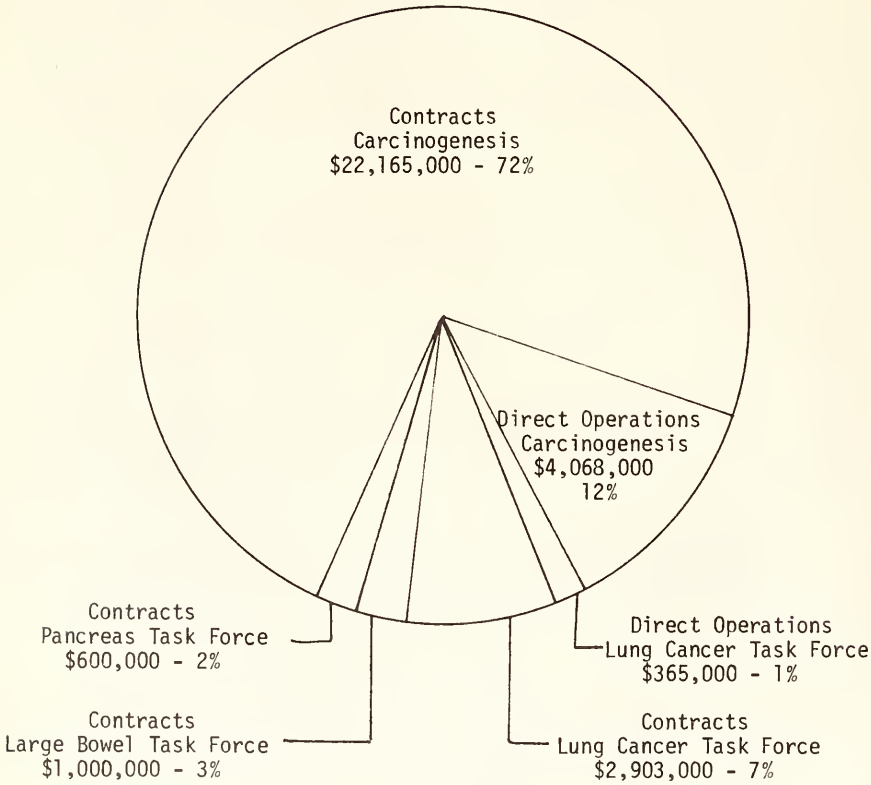
4 THIS AMOUNT INCLUDES SPACE ACQUIRED FROM THE REGISTRY OF EXPERIMENTAL CANCERS WHICH WAS TRANSFERRED FROM THE OD, NCI, AND THE SPACE ACQUIRED IN THE EYE RESEARCH FOUNDATION.

5 IN DECEMBER 1972, OFFICES OF THE OASDC, NOT ENGAGED IN LABORATORY WORK, LOCATED IN BUILDING 37, THE EYE RESEARCH FOUNDATION, AND THE FEDERAL BUILDING, MOVED TO THE LANDOW BG.

CARCINOGENESIS PROGRAM

Allocations - Direct Operations and Contracts

Fiscal Year 1973

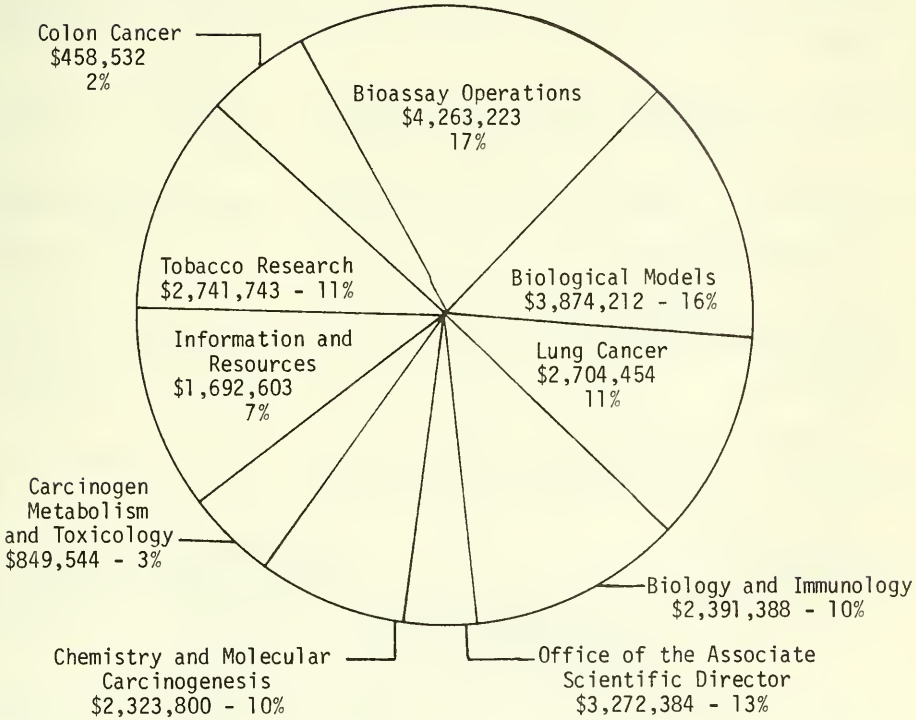


Total Contracts:	$26,668,000
Total Direct Operations:	4,433,000
TOTAL FUNDING:	$31,101,000

CARCINOGENESIS PROGRAM

Distribution of Contract Funds[1]

Fiscal Year 1973



[1]Total Contract Funds: $24,571,833

Based on information available as of March 31, 1973

TABLE I

ANALYSIS OF CONTRACT ACTIVITIES BY SEGMENT
IN THE CARCINOGENESIS PROGRAM

SEGMENT	NO. OF CONTRACTS	TOTAL AMOUNT[1]	PERCENT
TOTAL	169[2]	$24,571,883	(100%)
1. OASDC	6	3,272,384	13
2. Bioassay Operations	23	4,263,223	17
3. Biological Models	30	3,874,212	16
4. Biology and Immunology	23	2,391,388	10
5. Carcinogen Metabolism and Toxicology	8	849,544	3
6. Chemistry and Molecular Carcinogenesis	19	2,323,800	10
7. Colon Cancer	10	458,532	2
8. Information and Resources	22	1,692,603	7
9. Lung Cancer	14	2,704,454	11
10. Tobacco Research	14	2,741,743	11

Allocations:		
	Carcinogenesis Contracts	$22,165,000
	Lung Cancer Task Force	2,903,000
	Pancreas Task Force	600,000
	Large Bowel Task Force	1,000,000
	TOTAL	$26,668,000

[1]Estimation based on information available as of March 31, 1973.
[2]Several contracts are listed more than one time.

TABLE II

ANALYSIS OF CONTRACTS BY ACTIVITY
IN THE CARCINOGENESIS PROGRAM

SEGMENT CONTRACT	TITLE	TOTAL AMOUNT[1]
1. OASDC		($3,272,384)
AEC-NCI INTERAGENCY AGREEMENT (Oak Ridge Natl. Lab.) FS-64-13	NCI-AEC Carcinogenesis Program	(866,000)
COLUMBIA UNIVERSITY 72-3234	Development of a Tissue Culture Transformation System for Aromatic Amine Carcinogens	131,384
FREDERICK CANCER RES. CENTER	Frederick Cancer Res. Center Operation	2,141,000
MEMORIAL HOSPITAL 72-3286	A Study of Oncogenesis and Other Late Effects of Cancer Therapy	80,000
NEBRASKA, UNIVERSITY OF (Eppley Inst. for Res. on Cancer) 68-959	A Resource for Carcinogenesis Bioassays and Related Research	(2,000,000) 820,000
TO BE AWARDED	Conduct Carcinogenesis Research Associated With Construction Money	100,000
2. BIOASSAY OPERATIONS SEGMENT		($4,263,223)
BIO-RESEARCH CONSULTANTS 68-1311	Determination of the Carcinogenicity of Several Chemicals Present in Man's Environment	24,447
CHARLES RIVER BREEDING LABS. 72-2004	Production of Animals - Supplement to DCT Contract	119,985
CINCINNATI, UNIVERSITY OF 73-3203	Study of the Carcinogenic and Co-Carcinogenic Properties of Industrial Chemicals	79,199
DOW CHEMICAL CO. 72-3254	Carcinogenesis Bioassay of Environmental Chemicals	22,587

TABLE II

ANALYSIS OF CONTRACTS BY ACTIVITY
IN THE CARCINOGENESIS PROGRAM

SEGMENT CONTRACT	TITLE	TOTAL AMOUNT[1]
FREDERICK CANCER RES. CENTER Task Order #8	Large Scale Bioassay	[2]
GEORGIA, MEDICAL COLLEGE OF 72-3256	Lifetime Carcinogenic Bioassays on Small Rodents	0
GULF SOUTH RES. INST. 70-2210	Carcinogenesis Bioassay of Pesticides and Other Environmental Chemicals	295,490
HAZLETON LABS. 73-3225	Carcinogenesis Bioassay of Pesticides and Other Environmental Chemicals	413,392
HAZLETON LABS. 72-3278	Carcinogenesis Bioassay of Environmental Chemicals	392,770
HOWARD UNIVERSITY 71-2167	Chemical and Biological Investigation of Potential Carcinogens from Plants	60,979
IIT RES. INST. 71-2338	Carcinogenesis Bioassay of Chlorinated Dibenzodioxins and Related Chemicals	299,052
LITTON-BIONETICS, INC. 69-2085	Carcinogenicity of Chemicals Present in Man's Environment	0
LITTON-BIONETICS, INC. 71-2146	Carcinogenesis Bioassay of Environmental Chemicals	370,260
LITTON-BIONETICS, INC. 72-3252	Lifetime Carcinogenic Bioassays on Small Rodents	0
MASON RES. INST. 71-2144	Carcinogenesis Bioassay of Environmental Chemicals	388,152
MASON RES. INST. 72-3255	Lifetime Carcinogenic Bioassays on Small Rodents	0
MIDWEST RES. INST. 72-3270	Analytical Chemistry Resource	161,215

TABLE II

ANALYSIS OF CONTRACTS BY ACTIVITY
IN THE CARCINOGENESIS PROGRAM

SEGMENT CONTRACT	TITLE	TOTAL AMOUNT[1]
NEBRASKA, UNIVERSITY OF (Eppley Inst. for Res. on Cancer) 68-959	A Resource for Carcinogenesis Bioassays and Related Research	1,000,000
NEW YORK UNIVERSITY 71-2020	Alkylating Agents as Carcinogens and Anti-Carcinogens	118,349
PAPANICOLAOU CANCER RES. INST. 72-3253	Lifetime Carcinogenic Bioassays on Small Rodents	0
SAN FRANCISCO, UNIVERSITY OF 73-3229	Studies of Carcinogenicity of Metallo-Organic Compounds	48,128
SOUTHERN RES. INST. 73-3214	Carcinogenic Studies of Chemo-therapeutic Agents and Related Chemicals	325,218
WOLF RES. AND DEVELOPMENT CORP. 72-3302	Support Contract to Provide Data Control Operations Services and Microfilming Services to the Carcinogenesis Bioassay Data System	144,000
3. BIOLOGICAL MODELS SEGMENT		($3,874,212)
ALTON OCHSNER MEDICAL FDN. 71-2131	Carcinogenesis by Radiation Plus Estrogen	15,000
BOSTON UNIVERSITY 72-3297	Controlled Methods for the Delivery of Chemical Carcinogens to the Pancreas	125,300
CASE WESTERN RESERVE UNIVERSITY 72-3284	Enhanced Induction of Guinea Pig Pancreatic Adenocarcinoma	105,000
CHICAGO, UNIVERSITY OF 72-3290	Route of Carcinogen Administration in Pancreatic Adenocarcinoma Induction	154,800

TABLE II

ANALYSIS OF CONTRACTS BY ACTIVITY
IN THE CARCINOGENESIS PROGRAM

SEGMENT CONTRACT	TITLE	TOTAL AMOUNT[1]
CHICAGO, UNIVERSITY OF 73-3287	Definition of Sensitivity of Carcinogenesis Bioassay Systems	276,068
CORBEL LABS. 72-3299	Provide Animal Holding Facilities and Service	0
DARTMOUTH COLLEGE 72-3296	Enhanced Delivery of Synthetic Nitroso Compounds to the Pancreas in Rats	86,700
HAZLETON LABS. 72-3287	Induction of Adenocarcinoma in Dog Prostate	155,000
KANSAS, UNIVERSITY OF 72-3271	Histogenesis of Guinea Pig Pancreatic Adenocarcinoma	83,300
LITTON-BIONETICS, INC. 72-2063	Preparation and Examination of Experimental Biological Material	104,481
MARYLAND, UNIVERSITY OF 72-3206	Studies of the Histogenesis of Renal Carcinoma	99,925
MASON RES. INST. 72-3238	Uptake and Excretion of Carcinogens in and Their Effect on the Pancreas	150,800
MICHIGAN, UNIVERSITY OF 72-3216	Isolation and Purification of Epidermal Chalone	112,793
MICROBIOLOGICAL ASSOC. 70-2199	Laboratory Service for Support in Carcinogenesis Bioassay and Related Activities	714,703
MICROBIOLOGICAL ASSOC. 72-3300	Preparation of Cell Strains from Human and Animal Prostate	94,000
NEBRASKA, UNIVERSITY OF (at Lincoln) 72-3212	Chemical Carcinogen-Induced Noduligenesis and Tumorigenesis in Whole Mouse Mammary Gland Organ Culture	62,300

TABLE II

ANALYSIS OF CONTRACTS BY ACTIVITY
IN THE CARCINOGENESIS PROGRAM

SEGMENT CONTRACT	TITLE	TOTAL AMOUNT [1]
NEBRASKA, UNIVERSITY OF (Eppley Inst. for Res. on Cancer) 68-959	A Resource for Carcinogenesis Bioassays and Related Research	150,000
PAPANICOLAOU CANCER RES. INST. 72-3288	Induction of Prostatic Adenocarcinoma in the Rat	23,700
ST. LOUIS UNIVERSITY 72-3274	Synthetic Nitroso Derivatives as a Means of Concentrating Carcinogens in the Pancreas	59,100
SOUTHWEST FDN. FOR RES. AND EDUCATION 72-3291	Gonadal Hormone Effects on the Prostate	126,200
STANFORD RES. INST. 71-2166	Combined Effects of Chemical Carcinogens and Other Chemicals	634,662
TEMPLE UNIVERSITY 73-3262	Biochemical and Morphological Components of Hepatic Carcinogenesis	42,467
TENNESSEE, UNIVERSITY OF 69-2077	Carcinogenic Studies of Polyurethanes	0
TENNESSEE, UNIVERSITY OF 72-3282	Maintenance of Organ Explants from Rodent Pancreas	108,900
WEST VIRGINIA UNIVERSITY 72-3283	Relationships of Pituitary Hormones and Androgens on Prostate Metabolism	0
WRIGHT STATE UNIVERSITY 72-3281	Cell Culture Development of Human and Guinea Pig Pancreatic Cells	0
TO BE AWARDED	Etiology of Medullablastoma and Other Brain Tumors	92,000
TO BE AWARDED	Organ Culture of the Prostate	100,437

TABLE II

ANALYSIS OF CONTRACTS BY ACTIVITY
IN THE CARCINOGENESIS PROGRAM

SEGMENT CONTRACT	TITLE	TOTAL AMOUNT[1]
TO BE AWARDED	Neutron-Hormone Co-Carcinogenesis	98,459
TO BE AWARDED	Neutron Hormone Co-Carcinogenesis	98,117
4. BIOLOGY AND IMMUNOLOGY SEGMENT		($2,391,388)
AEC-NCI INTERAGENCY AGREEMENT (Oak Ridge Natl. Lab.) FS-64-13	NCI-AEC Carcinogenesis Program	146,000
BECTON, DICKINSON RES. CENTER 71-2168	Carcinogens as Allergens: Detection of Exposure to Carcinogens by Cell-Mediated Immunologic Reactions to the Carcinogens	88,000
BIOLABS, INC. 71-2164	*In Vitro* Study of Interaction between Chemical and Viral Carcinogens	237,204
BRANDEIS UNIVERSITY 72-3243	Production and Detection of Antibodies to Chemical Carcinogens and Other Small Molecules	97,507
CALIFORNIA, UNIVERSITY OF (at La Jolla) 72-3258	Significance and Relationship of Fetoglobulins to the Induction of Hepatomas by Chemical Carcinogenesis	146,962
CASE WESTERN RESERVE UNIVERSITY 72-3220	Specific Immunological Unresponsiveness to Chemical Carcinogenesis and Its Influence on Tumorigenesis	109,171
CHILDREN'S HOSPITAL MEDICAL CENTER 71-2278	Effects of Carcinogens on *In Vitro* Synthesis of Complement Components	93,370
FREDERICK CANCER RES. CENTER Task Order #10	*In Vitro* Bioassay	[2]

TABLE II

ANALYSIS OF CONTRACTS BY ACTIVITY
IN THE CARCINOGENESIS PROGRAM

SEGMENT CONTRACT	TITLE	TOTAL AMOUNT[1]
ILLINOIS, UNIVERSITY OF 72-3205	Transfer of Tumor Immunity by Cell-Free Extracts of Immune Lymphoid Cells	155,350
JOHNS HOPKINS UNIVERSITY 72-1074	Model Studies on Chemical Carcinogenesis	0
MALLORY INST. OF PATHOLOGY FDN. 71-2276	Detection of Carcinoembryonic Antigens in Humans	114,051
OHIO STATE UNIVERSITY RES. FDN. 72-2047	In Vitro Study of the Nature of Interaction between Chemical and Viral Carcinogens	93,964
RES. FDN. OF CHILDREN'S HOSPITAL OF WASHINGTON D.C. 72-2071	Immunologic Reactivity of Pediatric Cancer Patients	21,187
SCRIPPS CLINIC AND RES. FDN. 72-2046	Isolation and Chemical Characterization of Soluble Human Tumor (CEA) Specific Antigens	73,778
SORVALL, IVAN, INC. NIAID-73-2513	Fractionation of BCG Cell Walls	39,676
TEMPLE UNIVERSITY 73-3200	Induction of Malignant Melanoma in Guinea Pigs	99,612
TEXAS, UNIVERSITY OF 72-3210	Development of In Vitro Methods for the Detection of Cell-Mediated Immunologic Reactivity to Chemical Carcinogens	87,034
TRUDEAU INST., INC. 72-3221	Tumor Inhibition by Mycobacteria: Standardization of Mycobacteria Preparations	175,128
UTAH, UNIVERSITY OF 71-2272	Carcinogenesis Bioassay Resource for Determining and Effect of Chronic Immunosuppression on Physical and Chemical Carcinogenesis	211,176

TABLE II

ANALYSIS OF CONTRACTS BY ACTIVITY
IN THE CARCINOGENESIS PROGRAM

SEGMENT CONTRACT	TITLE	TOTAL AMOUNT[1]
TO BE AWARDED	Response of Peripheral Blood Monocytes from Patients with Neoplastic Disease to Chemotactic Factors	67,218
TO BE AWARDED	Evaluation of a Sensitive, Reproducible Quantitative In Vitro Bioassay for Detecting Chemical Carcinogenesis	200,000
TO BE AWARDED	BCG Vaccine for Cancer Research and Therapy	60,000
TO BE AWARDED	Nicotine in Blood: Detection by Radioimmunoassay	75,000
5. CARCINOGEN METABOLISM AND TOXICOLOGY SEGMENT		($849,544)
CALIFORNIA, UNIVERSITY OF (at San Diego) 73-3232	Pulmonary Tumors in Mice for Carcinogenic and Co-Carcinogenic Bioassay	176,924
MASSACHUSETTS INST. OF TECHNOLOGY 70-2180	Environmental Occurrence of N-Nitroso Compounds	108,186
MASSACHUSETTS INST. OF TECHNOLOGY 73-3217	Toxicity and Carcinogenicity Associated with Fungal Growth of Foodstuffs	191,348
MASSACHUSETTS INST. OF TECHNOLOGY 73-3238	Interactions between Diet and Chemical Carcinogenesis: A Bioassay System	83,125
TEMPLE UNIVERSITY 65-1029	A Search for Carcinogens among Mycotoxins	89,961
TO BE AWARDED	Use of Lymphoma Cells In Vitro and in Host-Mediated Bioassays as a Prescreen for Chemical Carcinogens	50,000

TABLE II

ANALYSIS OF CONTRACTS BY ACTIVITY
IN THE CARCINOGENESIS PROGRAM

SEGMENT CONTRACT	TITLE	TOTAL AMOUNT[1]
TO BE AWARDED	In Vitro DNA Repair as a Potential Prescreen for Chemical Carcinogens	50,000
TO BE AWARDED	In Vitro and Host-Mediated Mutagenicity as a Prescreen for Chemical Chemical Carcinogens	100,000
6. CHEMISTRY AND MOLECULAR CARCINOGENESIS SEGMENT		($2,323,800)
AEC-NCI INTERAGENCY AGREEMENT (University of California at Berkeley) FS-71-58	Molecular Processes Involved in the Carcinogenic Action of Polycyclic Aromatic Hydrocarbons	166,000
AEC-NCI INTERAGENCY AGREEMENT (Oak Ridge Natl. Lab.) FS-64-13	NCI-AEC Carcinogenesis Program	118,000
CONNECTICUT, UNIVERSITY OF (at Storrs) 72-3268	Development of New Methods for Isolating Non-Histone Proteins with Affinity for Homologous DNA	0
HOME FOR THE JEWISH AGED 71-2269	Study of Serum Haptoglobin Types in Patients with Carcinoma of the Pancreas	36,435
ILLINOIS, UNIVERSITY OF 72-3303	Temperature Sensitive Mutants in In Vitro Carcinogenesis	189,765
JOHNS HOPKINS UNIVERSITY 71-2169	Studies on the Regulation of the Heme Moiety of P-450 in Relationship to the Carcinogen Metabolizing Activity	84,780
MCGILL UNIVERSITY 72-3295	Isolation, Purification, and Characterization of Human Prolactin	0
NEW YORK UNIVERSITY 71-2183	The Isolation, Propagation, and Storage of Mutant Vertebrate Cells with Specific Biochemical Lesions	128,949

TABLE II

ANALYSIS OF CONTRACTS BY ACTIVITY
IN THE CARCINOGENESIS PROGRAM

SEGMENT CONTRACT	TITLE	TOTAL AMOUNT[1]
ONTARIO CANCER INST. 72-2051	The Isolation, Propagation, and Storage of Mutant Vertebrate Cells with Specific Biochemical Lesions	64,936
POLYSCIENCES, INC. 72-3245	Optimizing Electrophoretic Separation of Proteins and Nucleic Acids with New Hydrogels	58,702
TEXAS, UNIVERSITY OF (M. D. Anderson Hospital and Tumor Inst.) 71-2268	Study of Serum Haptoglobin Types in Patients with Carcinoma of the Pancreas	19,303
TEXAS, UNIVERSITY OF (M. D. Anderson Hospital and Tumor Inst.) 72-3269	Non-Histone DNA Binding Proteins from Normal Rat Liver and Chemically Induced Rat Hepatomas	0
WEIZMANN INST. OF SCIENCE 70-2217	The Role of the Enzyme Aryl Hydrocarbon Hydroxylase and Its Induction in Polycyclic Hydrocarbon Carcinogenesis	141,930
TO BE AWARDED	Alterations in Translation of Genetic Messages Induced by Viruses and Carcinogens	265,000
TO BE AWARDED	Organic Synthesis of Cold and Tritiated Polycyclic Hydrocarbon Derivatives	350,000
TO BE AWARDED	Selection and Propagation of Haploid or Partial Monosomic Cell	100,000
TO BE AWARDED	Selection and Propagation of Somatic Cells Having Specific Physiological Mutations	100,000
TO BE AWARDED	Mammalian Cell Transport System	400,000

TABLE II

ANALYSIS OF CONTRACTS BY ACTIVITY
IN THE CARCINOGENESIS PROGRAM

SEGMENT CONTRACT	TITLE	TOTAL AMOUNT[1]
TO BE AWARDED	Nature of the Polycyclic Hydrocarbon Nucleic Acid Compound in Hydrocarbon Carcinogenesis	100,000
7. COLON CANCER SEGMENT		($458,532)
AEC-NCI INTERAGENCY AGREEMENT (Oak Ridge Natl. Lab.) FS-72-204	Role of Nitrosamines in Carcinogenesis	0
AMERICAN HEALTH FDN. 71-2310	Experimental Large Bowel Carcinogenesis	0
FREDERICK CANCER RES. CENTER Task Order #7	Selected Bacteria Species	2
GEORGIA, MEDICAL COLLLEGE OF 72-3280	Epidemiologic Study of Colon Cancer among Blacks	0
KAISER RES. FDN. 73-3215	Epidemiologic Study of Colon Cancer in Blacks	45,480
MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES 72-2041	Regulatory Control of Cell Proliferation in Colonic Tissue (In Familial Polyposis)	20,000
OXFORD, UNIVERSITY OF 72-3215	Procurement of Data for Determinations of Disease Linkages	12,000
TO BE AWARDED	Preparation of Bile Acids and Their Derivatives	12,700
TO BE AWARDED	Effect of High Meat Diet on the Bacterial Flora and Chemical Components of Feces	231,435
TO BE AWARDED	Fecal Flora Study	136,917

TABLE II

ANALYSIS OF CONTRACTS BY ACTIVITY
IN THE CARCINOGENESIS PROGRAM

SEGMENT CONTRACT	TITLE	TOTAL AMOUNT[1]
8. INFORMATION AND RESOURCES SEGMENT		($1,692,603)
AEC-NCI INTERAGENCY AGREEMENT (Oak Ridge Natl. Lab.) 72-203	Environmental Mutagen Information Center	61,250
ASH STEVENS 72-3293	Synthesis of Purine and Pyrimidine Nucleotides	149,779
FRANKLIN RES. INST. 73-3309	Carcinogenesis Abstracts, Vol. XI	99,691
FREDERICK CANCER RES. CENTER Task Order #9	Preparation and Characterization of Carcinogens	[2]
FREDERICK CANCER RES. CENTER Task Order #12	Production of Inbred and Hybrid Laboratory Animal Strains	[2]
IIT RES. INST. 70-2245	Production and Characterization of Particulate Materials for Studies in Respiratory Carcinogenesis	97,863
INTERNATIONAL AGENCY FOR RES. ON CANCER 70-2076	Epidemiological Study of the Incidence of Esophageal Cancer	45,000
INTERNATIONAL AGENCY FOR RES. ON CANCER 70-2076	Program on the Evaluation of Carcinogenic Risk of Chemicals to Man	0
MASSACHUSETTS GENERAL HOSPITAL 71-2128	Atlas on Comparative Morphology and Classification of Spontaneous Neoplasma in Dog, Cat, and Man	56,634
MEDIZINISCHE HOCHSCHULE-HANNOVER 71-2148	Development of a Lung Tumor Model with Large Wild Hamsters (Cricetus cricetus)	57,300

TABLE II

ANALYSIS OF CONTRACTS BY ACTIVITY
IN THE CARCINOGENESIS PROGRAM

SEGMENT CONTRACT	TITLE	TOTAL AMOUNT[1]
MIAMI, UNIVERSITY OF 71-2274	Search for Possible Plant Causes of Esophageal Cancer	30,000
SOUTHWEST RES. INST. 72-2065	Development and Application of Analytical Methods of Volatile Nitrosamines in Complex Mixtures	16,285
STANFORD RES. INST. 72-3285	Information System on the Production, Distribution, and Exposure to Man of Environmental Substances	520,556
STARKS ASSOC. 72-3203	Standards for the Carcinogenesis Testing Program - Supplement to DCT Contract	173,479
THOMPSON, JOHN I., CO. 71-2266	Literature Search and Retrieval and Compilation of Data Relating to Chronic Tests in Experimental Animals	0
WOLF RES. AND DEVELOPMENT CORP. 71-2270	Programming Support for the CBDS, Computer Subsystem, Phase I	16,332
TO BE AWARDED	Stability of Benzo[a]pyrene on Particulates Utilized in Respiratory Tract Studies	38,434
TO BE AWARDED	Partial Support for the Institute of Laboratory Animal Resources (Part of the National Academy of Sciences)	20,000
TO BE AWARDED	Animal Pathology Support	100,000
TO BE AWARDED	Development of Small Marsupials	50,000
TO BE AWARDED	Production of 1972-1973 Volume of "Compounds Which Have Been Tested for Carcinogenic Activity"	120,000

TABLE II

ANALYSIS OF CONTRACTS BY ACTIVITY
IN THE CARCINOGENESIS PROGRAM

SEGMENT CONTRACT	TITLE	TOTAL AMOUNT[1]
TO BE AWARDED	Production of Machine Readable Indices of the "Compounds Which Have Been Tested for Carcinogenic Activity"	40,000
9. LUNG CANCER SEGMENT		($2,704,454)
AEC-NCI INTERAGENCY AGREEMENT (Oak Ridge Natl. Lab.) FS-64-13	NCI-AEC Carcinogenesis Program	602,000
HARVARD UNIVERSITY, SCHOOL OF PUBLIC HEALTH 71-2136	Factors Influencing Experimental Respiratory Carcinogenesis by Alpha Radiation and Chemical Carcinogens	85,586
IIT RES. INST. 69-2148	Role of Vehicles and Particulates in Respiratory Carcinogenesis Bioassays	64,077
IIT RES. INST. 72-3292	Susceptibility States and Modulating Factors in Respiratory Carcinogenesis	346,000
MASSACHUSETTS INST. OF TECHNOLOGY 69-2083	Role of Vitamin A in the Control of Differentiation and Carcinogenesis in the Respiratory Tract	200,853
NEBRASKA, UNIVERSITY OF (Eppley Inst. for Res. on Cancer) 69-959	A Resource for Carcinogenesis Bioassays and Related Research	80,000
NEW YORK UNIVERSITY 73-3260	Pulmonary Carcinogenesis	455,773
OHIO STATE RES. FDN. 69-2144	Study of the Role of Vehicles and Particulates in Respiratory Carcinogenesis Bioassay	55,209

TABLE II

ANALYSIS OF CONTRACTS BY ACTIVITY
IN THE CARCINOGENESIS PROGRAM

SEGMENT CONTRACT	TITLE	TOTAL AMOUNT[1]
ST. MARY'S HOSPITAL 72-3233	Morphogenesis of Lung Cancer	140,000
SOUTHERN RES. INST. 72-2064	Organ Culture Assay of Vitamin A Analogs	185,000
STANFORD UNIVERSITY SCHOOL OF MEDICINE 73-3207	Studies on Oat-Cell Carcinoma of the Lung	54,368
VETERAN ADMINISTRATION HOSPITAL (Tampa, Florida) FS-73-206	Autoradiographic Study of the Cellular Response of the Respiratory Tract in Chemical Carcinogenesis	85,588
TO BE AWARDED	Studies of Carcinogenesis in Organ Culture of Trachea and Bronchi	150,000
TO BE AWARDED	Polycyclic Hydrocarbon Metabolism in the Respiratory Tract	200,000
10. TOBACCO RESEARCH SEGMENT		($2,741,743)
AEC-NCI INTERAGENCY AGREEMENT (Oak Ridge Natl. Lab.) FS-40-117-67	Collection, Separation and Elucidation of the Components of Cigarette Smoke	340,000
AMERICAN HEALTH FDN. 73-3305	Evaluation of Carcinogenic Agents in Cigarette Smoke: Biological and Chemical Assays and Epidemiological Studies	728,969
ARTHUR D. LITTLE, INC. 73-3284	Bioassay of the Cytotoxicity of Cigarette Smoke and of Its Effects on Ciliary Function	166,446
BATTELLE-NORTHWEST LABS. 69-1372	Inhalation Co-Carcinogenicity of Industrial Pollutants and Cigarette Smoke	0

TABLE II

ANALYSIS OF CONTRACTS BY ACTIVITY
IN THE CARCINOGENESIS PROGRAM

SEGMENT CONTRACT	TITLE	TOTAL AMOUNT[1]
CENTER FOR DISEASE CONTROL 72-205	Study of Smoking Intervention Techniques	100,000
HAZLETON LABS. 69-2145	Carcinogenicity Bioassay by Intragastric Intubation of Cigarette Smoke Condensates in Experimental Animals	0
HAZLETON LABS. 69-2149	Skin Carcinogenesis Bioassay of Cigarette Smoke Condensates in Mice	298,573
HAZLETON LABS. 72-3275	Chronic Carcinogenesis Bioassays by Intratracheal Instillation of Cigarette Smoke in Syrian Hamsters	0
MELOY LABS. 69-2084	Preparation and Analysis of Cigarette Smoke Condensate Samples	459,294
NEW YORK UNIVERSITY 73-3241	Studies on Carcinogenesis Principles of Processed Tobacco and Tobacco Smoke	101,024
VETERANS ADMINISTRATION HOSPITAL (East Orange, New Jersey) FS-72-66	Test Effects of High and Low Nicotine Cigarettes on Male Beagle Dogs	277,437
TO BE AWARDED	Feasibility Study on Smoke Intake in Humans	70,000
TO BE AWARDED	Lung Pellet Implantation in Cigarette Smoke Condensate	100,000
TO BE AWARDED	Management Support Services to the Tobacco and Health Program of the NCI	100,000
GRAND TOTAL		$24,571,883

[1]Estimated FY 1973 Obligations, as of March 31, 1973
[2]Total Amount Reported under Office of the Associate Scientific Director for Carcinogenesis (1)

II. OFFICE AND BRANCH REPORTS

SUMMARY REPORT

OFFICE OF THE ASSOCIATE SCIENTIFIC DIRECTOR FOR CARCINOGENESIS

July 1, 1972 through June 30, 1973

The direct operation of the Office of the Associate Scientific Director for Carcinogenesis includes the immediate Office of the Associate Scientific Director for Carcinogenesis (U. Saffiotti, Associate Scientific Director; J. A. Cooper, II, Acting deputy Associate Scientific Director; J. Baldeschwieler, Special Consultant); the Coordinator for Collaborative Research (A. H. Heim); the Scientific Coordinator for Environmental Carcinogenesis (H. Kraybill); the Registry of Experimental Cancers (H. L. Stewart, Consultant); the Program and Data Analysis Unit (N. P. Page, Acting Head); the Lung Cancer Unit (M. B. Sporn, Head); and the Epidemiologic Pathology Unit (J. Berg, Head). An Analytical Chemistry Unit is being established.

The planning and coordination of the whole Carcinogenesis Program has continued to take the major effort of the Office. This Office has continued to serve as a focal point for the development of documentation on the assessment of environmental carcinogenesis hazards and on the principles for evaluation of environmental carcinogens, in collaboration with the Demography Program, with the Director, DCCP, NCI, and with the Director, NCI. In this capacity this Office has participated in a number of review and policy meetings at the request of other government departments and agencies, including the following: The Council on Environmental Quality, the President's Science Advisory Committee, and the Office of Science and Technology, Executive Office of the President; Office of the Surgeon General, USPHS; the Office of the Commissioner and the Bureaus of Foods and of Drugs, FDA; Advisory Groups for the National Center for Toxicological Research, FDA; the Drug Advisory Board, National Research Council--National Academy of Sciences; and several committees and ad hoc groups with FDA, EPA, USDA, and other agencies.

Testimonies were submitted by the Associate Scientific Director for the hearings of the Subcommittee on Intergovernmental Relations of the Committee on Government Operations, House of Representatives (Chairman: Rep. L. H. Fountain) on Regulation of Diethylstilbestrol and for the hearings of the Select Committee on Nutrition and Human Needs, United States Senate (Chairman: Senator G. McGovern) on Nutrition and Human Needs.

The Office also participated in the activities of the Working Group on the Evaluation of Carcinogenic Risks of Chemicals to Man, International Agency for Research on Cancer, Lyon, France, and in the Conference on Epidemiological Approaches to Carcinogens sponsored by the Fogarty International Center, NIH, the International Agency for Research on Cancer, and the League for the Fight

Against Cancer of the Croatian Republic of Yugoslavia. This Office also participated in the organization of several meetings as reported in the Carcinogenesis Program Summary Report in Section E (4).

These and other related activities in the area of evaluation of environmental hazards have required a large share of the resources of this Office, including communication with congressional committees, other government agencies, the press, and the information offices at NCI, NIH, and DHEW. Major emphasis has been given in all these activities to the unique role of NCI in providing documentation of carcinogenesis bioassay studies and scientific criteria for evaluation of carcinogenic hazards for man.

The major effort of this Office has been devoted to the scientific planning, development, coordination, and implementation of a considerable expansion in resources and activities assigned to the Carcinogenesis Program as a result of the projected increase in funding level for NCI in FY 1973. Documentation of this effort is provided throughout this Report.

Office of the Scientific Coordinator for Environmental Carcinogenesis: The primary goal of this new Office is to develop and provide mechanisms for implementation of a coordinated program on environmental carcinogenesis. This may be accomplished through diverse approaches, which include collaboration with other governmental agencies (especially those having programmatic responsibilities in environmental health and controls), industrial organizations, manufacturing association, and academic research institutes involved in carcinogenesis research. The Scientific Coordinator also provides documentation and identifies needs and priorities for work on and assessment of environmental agents for carcinogenesis. On particular relevance to the general population, although not all inclusive, are such categories as food additives, food contaminants, drugs, industrial and agricultural chemicals (i.e., pesticides, polychlorinated dioxins, flame proofing materials, plasticizers, etc.), natural toxins, tobacco and tobacco products, physical and biological agents, and the spectrum of environmental contaminants in air and water. Finally, for those in the working environment, there is an added exposure to chemicals and physical agents.

The National Cancer Act of 1971, section 407(b), provides emphasis through legislative authority in the execution of the National Cancer Program that "with the advice of the National Cancer Advisory Board, the National Cancer Institute shall plan and develop an expanded, intensified, and coordinated cancer research program, encompassing the programs of the National Cancer Institute, related programs of the other research institutes, and other Federal and non-Federal programs." In the area of environmental carcinogenesis, this implies an accentuated collaborative program and effort. Additionally, the National Cancer Plan Research Strategy Hierarchy directs attention to components in the plan which relate to a coordinated effort in a national program on environmental carcinogenesis. Briefly, these items related to the following: (a) investigation and characterization of the role of physical agents, chronically administered drugs and dietary factors as contributors to human cancer, (b) establishment of a resource for interagency liaison between the NCI and Federal and State agencies that have responsibility for regulatory action to insure maximum utilization of

presently reliable information about carcinogens and timely action with respect to emergency data on carcinogens, and (c) determination of carcinogenic risk following non-occupational exposure to exogenous chemicals (e.g. in the home) and to identify high risk groups. From the latter, identify and characterize groups at high risk to cancer because of occupational or community non-occupational environmental exposure to carcinogens.

To accomplish the mission in environmental carcinogenesis, one must first identify and classify the various chemicals and agents that are potential carcinogens and experimentally proven carcinogens in test animals. Beyond this, an effective monitoring and surveillance system must be available to provide an intelligence network on levels of these "carcinogens" and trends in exposure in the working and general population (non-occupational) environment. This necessitates close collaboration with those governmental and non-governmental groups which systematically carry out such environmental monitoring by surveillance on exposure routes such as air, water, diet, drugs, and tobacco and the exposures in the work place.

From the intelligence network, it is planned to delineate areas of high exposure to environmental carcinogens, initially on a very selective basis. Interagency collaboration, to begin with such groups as FDA, EPA, and NIOSH, enlisting their help for resource and input on such data, is one of the major goals in assessment of environmental carcinogenesis. Therefore, a data base on environmental exposures to carcinogens computed in many cases from an integrated exposure (air, water and food) can be correlated with data from dose-response relationships on those same chemicals or agents well characterized in experimental animals.

As briefly indicated previously, another important function in interagency collaboration is to provide assistance in the way of documentation, and review of petitions and standards submitted by FDA, NIOSH, EPA, and other Federal and non-Federal groups relevant to evaluations on carcinogenesis and carcinogenic potential. Continuous communication provides such a mechanism for liaison. Interagency collaborative group meetings and workshops provide other means for this accomplishment.

Within the short period of time this office has been in operation, contacts have been established with about 12 Federal agencies and a few academic groups and manufacturing associations that relate to this program area. Within this fiscal year, three meetings of an Interagency Collaborative Group on Environmental Carcinogenesis have been held to discuss problems in environmental carcinogenesis with the view to identifying exposure areas for concentrated studies on the human population.

Assistance and advice has been given to Federal agencies on such matters as (a) toxic substances lists, (b) review of petitions and standards and/or guidelines. Some reviews in this area with recommendations relating to environmental carcinogenesis were as follows: (1) petition on melengestrol acetate, (2) water quality status report for EPA, (3) review of standards on benzene in occupational carcinogens to OSHA and NIOSH, (4) review of document on lead standard submitted to EPA, (5) review of pending legislation on food

additives, FDA, and (6) review of document from NIOSH on "Recommended Coke Oven Work Practices for Reduction of Exposure to Coke Oven Emissions". Some assistance was given to the Calorie Control Council on diet carcinogens and matters relevant to non-nutritive sweeteners.

Papers have been presented at national and international meetings relevant to the broad area of environmental health and environmental carcinogenesis.

It was indicated in last year's Annual Report that this Office would be staffed with experts in the field of food additives, pesticides, drugs, air and water pollution, natural products, tobacco and industrial and occupational exposures. Until this staffing objective is fulfilled, it is anticipated that there will be severe limitations on the mission which was described for the Office of the Scientific Coordinator for Environmental Carcinogenesis.

Analytical Chemistry Unit: This Unit, although not yet formally established has been working as such within the Office of the Associate Scientific Director for Carcinogenesis. It is presently staffed with two Ph.D. chemists.

The purpose of this Unit as presently constituted is threefold: (1) to collaborate with other members of the Carcinogenesis Program in matters requiring chemical expertise, especially in problems of the identification of nonpolymeric organic molecules; (2) to participate in the technical review of contract activities involving chemistry; (3) to conduct independent research on the chemistry on N-nitroso compounds and to serve as a focal point for the exchange of information concerning this important class of carcinogens.

To prepare for the first of these goals, a small but fairly well equipped organic analytical chemistry laboratory has been established during the current fiscal year. Renovations on an instrument room and a bench laboratory were completed in late September and requests for equipping an adjacent, recently acquired module as a utility spectrophotometry laboratory have been placed.

The cornerstone of the Unit's intramural service effort is a high resolution mass spectrometer, the installation of which was completed at the end of February. The collection of reference spectra on compounds of interest to the Carcinogenesis Program is beginning. Current personnel ceilings have blocked the projected hiring of an electronics technician to assist with the maintenance and operation of this sophisticated instrument; the viability of the new mass spectrometry program depends upon the recruitment of such an individual, and a full-time, permanent personnel slot had been designated for this purpose. This situation will severely undermine the ability of the Carcinogenesis Program to cope with the pressing needs and high priorities related to identifying the hazards related to the recently discovered class of carcinogens, the nitrosamines.

Arrangements have been made with the Drug Development Branch, Division of Cancer Treatment, for the use of other needed equipment, including infra-red

and nuclear magnetic resonance instruments and a polarimeter; certain other pieces of equipment have been made available by other members of the Carcinogenesis Program. The Unit is now ready to begin active collaboration on the identification of nonpolymeric organic molecules of interest in carcinogenesis research.

Unit personnel have intensively participated in review and development of proposals for new work under the collaborative program, and have undertaken major responsibility for program developments related to nitrosamines.

As a mechanism for defining the Carcinogenesis Program's priorities in the nitrosamines area, the Unit arranged a conference in July 1972. Carcinogenesis staff, advisors, contractors, and members of government agencies involved with the question of nitrosamines as potential environmental carcinogens were invited. A series of recommendations proposed by the participants were discussed; five research and service activities were identified as of high priority for inclusion in the Carcinogenesis Program; of these, one will be conducted at the Frederick Cancer Research Center, one will be incorporated into a larger information resource contract, and three constitute the basis for individual competitive requests for proposals. To assist in the planning of these programs, as well as to publicize the Carcinogenesis Program's interest in this area, questionnaires were prepared and distributed to several hundred individuals known to be active in this area of research. The responses have been compiled and are expected to broaden the base for direction of these activities considerably.

An important gap in our understanding of the possible role of N-nitroso compounds in environmental carcinogenesis resides in the fact that their simple organic chemical properties have never been fully characterized. The Unit's research activities are aimed at plugging this gap. It is expected that the findings will provide important background and leads for developing analytical methods, for elucidating the environmental distribution of these compounds, and for understanding the biochemical and biological consequences of exposure to them.

In addition to these original research activities, continuing communications have been arranged with others in the field. Mechanisms for accomplishing this have been provided in the following: (a) nitrosamines conference mentioned previously, (b) semi-annual conferences on "Nitrosamines in Foods" arranged by the FDA, (c) tour of fourteen European laboratories involved in this area, (d) site visits to contractors and other nitrosamines laboratories in the United States, and (e) scientific collaboration with investigators at several of these organizations.

Registry of Experimental Cancers: The Registry was formally transferred to the Carcinogenesis Program from the Office of the Director, NCI. It is headed by H. L. Stewart, former Chief of the Laboratory of Pathology, Division of Cancer Biology and Diagnosis, now Consultant to NCI.

The objectives of the Registry are (1) the storage and retrieval of pathological material and data on cancers and other lesions of laboratory animals (primarily rodents), and (2) the use of such information as reference

standards for diagnostic tumor pathology in carcinogenesis studies, as well as for research and educational purposes.

The Registry possesses a large collection of protocols, pathologic material, including histologic slides, paraffin blocks, and gross specimens of spontaneous and induced cancers and other lesions and has a total of 126 single or group accessions from investigators outside of NCI. Approximately 12,000 records have been prepared for coding and coded. The printouts are used by members of the Registry and by investigators who visit the Registry. Three investigators have spent varying periods of time in the Registry studying some of the available material. Forty-six investigators have come to the Registry for consultation.

Epidemiologic Pathology Unit: This Unit represents the major link between the Carcinogenesis Program and the Demography Program and provides expertise and advice to the whole Carcinogenesis Program. Administrative activities of the Colon Cancer Segment have occupied most of the Unit's time and almost all of its staff. However, in the latter part of the year it has been possible to initiate the kind of project work that is needed for further program development. Though most of the Unit's work is developed through contracts, these cannot arise in a vacuum. Relative priorities for all studies must be based on as many available facts as possible. Epidemiologic studies can only be designed when one has some knowledge of the kinds and value of available data. Large-scale laboratory studies cannot be justified without some judgments on feasibility and cost-benefit likelihoods.

On the epidemiologic side, work is underway on techniques of correlation analysis, since the first test of a hypothesis that "X" causes bowel cancer is to determine how closely "X" is associated with bowel cancer in different populations. Along with developing and testing the proper tools for these analyses, studies on disease relationship, food distributions, and occupations have begun. The Unit is also designing second-generation diet studies: Given the fact that a food such as beef is associated with bowel cancer, the next step is to get precise information about types of beef, methods of cooking, quantity of intake, and also similar information about other food items that contain suspect components.

With staffing becoming more complete at the Frederick Cancer Research Center, a collaborative research program is being developed there that gives the Unit, for the first time, a chance for direct involvement in the laboratory investigations that lie at the heart of the Colon Cancer Segment. Work is now well underway to determine what strains of bacteria do to suspect precursors such as tryptophane. (Half of this essential amino acid's immediate metabolic products are carcinogenic.) In addition, a rapid screen for mutagenesis as a parallel reaction to carcinogenesis has been established.

To provide a literature retrieval system for the scientific literature of special interest to the Unit, the Wylbur test-editing system of NIH has been adapted for this prupose. References can be retrieved by key word, combinations of key words, author(s), reference number, journal title, reference title, and date of publication. Output listings can include not only references but summaries of references where these were available from

the article or from an abstract source. The system has not only been helpful to staff members, but lists of references have been furnished upon request to contractors.

Links with general epidemiology have been maintained through technical rather than scientific activities. Within NCI this has consisted in part of continued service as assistant project officer for several Demography contracts, active participation in analysis of material from those contracts, provision of pathology consultation to the Third National Cancer Survey, and when time permits, participation in collaborative studies with the Demography Program. Externally, it has meant maintenance of the collaborative link with the Memorial Hospital for Cancer and Allied Diseases, participation in both the United States and the World Health Organization's work on revision of the International Classification of Diseases, and representation of the Carcinogenesis Program in other meetings.

Technically, the prime achievement of this work has come with the provisional acceptance of a revised International Classification of Neoplastic Disease. This includes new overall concepts about tumors of uncertain malignancy; many decisions based on documentation by the Unit, and a major policy decision whereby the World Health Organization will officially sponsor and publish, as their histologic supplement, the American Cancer Society's code previously developed with substantial participation by this Unit. The Unit has also prepared the documentation needed for the conversion of the End Results Group records to this new code.

Scientifically, the Unit is working to promote the full utilization of pathological data for epidemiologic purposes. Another in the series of epidemiologic-pathology reports, on pancreatic cancer, is in press. This is the second of a series done in collaboration with the End Results Group. Two others, on bowel cancer and ovarian cancer, are in progress. Re-analysis of autopsy data from Boston has shown that some of the reported 41% misdiagnoses of cancer is due to deaths prior to complete workup, but it also showed that a substantial 21% of cancer still was not correctly diagnosed during life.

Continuation of the study of correlations between water supply trace metals and cancer confirmed for the United States a generally overlooked association between low molybdenum levels and esophageal cancer noted once in Africa.

Multiple studies of bowel cancer pathology and epidemiology have been carried out but usually just far enough to yield information usable for program planning for the Colon Cancer Segment. None has yet been developed sufficiently for formal publication. Analysis of mortality data, however, did show an unusually high rate of bowel cancer in rural Scotland. Elsewhere bowel cancer is a distinctly urban disease. This was called to the attention of Scottish investigators through a published letter and personal visits.

There are two major program problems: one is that the comparative pathology program has lost its link with the Armed Forces Institute of Pathology. Without such a collaborative source, the program seems below critical mass

and *comprehensive* projects cannot be undertaken. A realignment that provides more resources for the program is badly needed.

The second problem is the conflict between the Unit's original broadly-based liaison with epidemiology on one hand and the demands of the Colon Cancer Segment on the other, given the Segment's presumably permanent lack of adequate administrative support in the form of a full-time segment manager. The scientific aspects of the two activities overlap so much that these create no problem; what is learned in one area almost always has an important analogy in the other. However, to the extent that consultative and administrative efforts take priority in these areas, the two operations are in competition.

Lung Cancer Unit: The intramural program of the Lung Cancer Unit, which is closely coordinated with the contract program of the Lung Cancer Segment, is devoted to studies of the pathogenesis of lung cancer and its prevention. Current studies include investigations on the carcinogenic effects of different chemicals on the respiratory epithelium in the whole animal, the use of short-term and long-term organ culture systems to study the effects of carcinogens and anti-carcinogens on respiratory epithelium, investigation of the molecular mechanism of action of lung carcinogens in cell-free systems, and investigation of the molecular mechanism of action of the anti-carcinogenic substance, vitamin A. Studies are pursued within the Unit in a manner that allows correlation of findings at the molecular, cellular, and whole animal level. Since the intramural program of the Lung Cancer Unit is closely coordinated with the contract program of the Lung Cancer Segment, a concerted effort is being made to achieve the most rapid dissemination and utilization of new information obtained at the basic level, in order to use this information where it will ultimately be of most direct human benefit.

The following is a summary of the investigations within the Unit:

(1) Animal Studies of Respiratory Carcinogenesis - Studies have been continued on the morphogenesis of squamous cell carcinoma of the respiratory tract of the hamster, using both benzo[a]pyrene and N-nitroso-N-methyl urea as carcinogens. The morphological picture is very similar with both carcinogens; the data suggest that basal cell hyperplasia preceded squamous metaplasia, which in turn is followed by squamous neoplasia. The process of development of squamous cell cancer of the lung in the hamster animal model is strikingly similar to that found in man, and the ultrastructure of the tumors caused by either of the above two carcinogens in the animal is very similar to the ultrastructure of comparable human tumors. These findings are of importance because they indicate that findings made in the animal model should be of direct relevance to the human disease.

In addition to morphological assessment of the state of respiratory epithelial cells, efforts have been made during the past year to make biochemical measurements of enzyme activity in these cells as they progress from normal to malignant. Since isoenzyme ("isozyme") patterns have been found to change in tissues other than lung during carcinogenesis, new techniques have been developed for measurement in respiratory epithelium

of the isozyme pattern of several different enzymes, including lactic dehydrogenase, malic dehydrogenase, aldolase, and hexokinase. The lactic dehydrogenase pattern in normal epithelium was found to be very different from that found in lung tumors induced by benzpyrene and nitrosomethylurea.

Since these isozyme measurements can be made on very tiny amounts of tissue, they represent a basis for identifying neoplastic development by a specific cellular marker and offer some eventual promise of being of use in human lung cancer diagnosis.

(2) Studies on the Isolated Tracheal Epithelium of the Hamster (Short-Term and Long-Term Organ Culture - In last year's report, the short-term (up to hours) organ culture of hamster trachea was reported. Many new studies within the Unit have continued to use this technique. In addition, the long-term (one to two weeks) organ culture of hamster trachea was begun on an intensive basis during the past year. This extension of time for organ culture experiments will enable the examination of a much greater range of effects and interactions of carcinogens and anti-carcinogens on respiratory epithelium. Using the short-term technique, the binding of the carcinogen, benzo[*a*]pyrene, to DNA of tracheal epithelial cells was demonstrated. This binding is inducible by previous treatment of animals with benzpyrene, and can be strongly inhibited by the antimetabolite, 7,8-benzoflavone. In vitamin A-deficient animals, an enhanced binding of benzpyrene to DNA of tracheal epithelial cells was demonstrated. The above findings are of importance because they show that prior exposure to a carcinogen in the respiratory tract may enhance subsequent carcinogenic response, and because they indicate that a nutritional deficiency that occurs commonly in man may be associated with an increased carcinogenic response. The optimal conditions for long-term organ culture of tracheobronchial epithelium are currently being defined. Isozyme measurements, of the type described above, as well as classical morphology, are being used to standardize optimal culture conditions. These studies are of importance, because they will enable studies on human bronchial material to be performed in organ culture during the coming year in the Lung Cancer Unit. Such studies will measure the direct effects of various carcinogens, as well as anti-carcinogenic substances such as vitamin A, on human respiratory tissue.

(3) Studies on the Cellular and Biochemical Mechanism of Action of Vitamin A - Since vitamin A is a required substance for normal differentiation of respiratory epithelium, and since it inhibits the effects of carcinogenic substances such as benzpyrene, both *in vivo* and *in vitro*, studies on the mechanism of action of this substance are being actively pursued. New experiments have indicated that tracheal epithelium can synthesize a mannolipid from retinol and GDP-mannose. Hydrolysis data on the purified mannolipid indicate that it has the structure of a retinyl-phosphate-mannose type of compound. The mannolipid has been shown to function as a carrier for mannose during formation of glycoproteins. Further studies are in progress to characterize the specificity of structure of this type of glycolipid, both in terms of the vitamin A moiety and the sugar moiety. In addition to the above studies of vitamin A in cell-free systems, a new study on the effects of vitamin A and vitamin A analogs on epithelial cells in tissue culture has just been started in collaboration with the Experimental

Pathology Branch. It is already clear from these new studies that vitamin A and vitamin A analogs can be shown to have profound effects on the growth and differentiation of epidermal cell cultures. These tissue culture studies are important, because they will serve as a primary screen for evaluation of biological activity of new synthetic vitamin A analogs, as well as being useful for further elucidation of the mechanism of action of vitamin A. Since the eventual use of vitamin A analogs for cancer prevention in man is currently under consideration, it is of utmost importance to have the most efficient primary screen for assessment of activity.

Program and Data Analysis Unit: During this fiscal year the mission of the Unit has broadened considerably and now consists of (1) coordination and management of the Carcinogen Bioassay Program, and (2) support to the whole Carcinogenesis Program in research resources. The reorganization and central management of the Bioassay Operations Segment under the responsibility of the Unit's staff should result in more efficiency in providing resources to the bioassay program. The Unit provides the Associate Scientific Director and his staff with support in program planning and evaluation, information and data processing, the development and management of animal and chemical resources, and radiation biology.

Much of the Unit's staff activities are related to the collaborative contract program of two separate segments, that of the Bioassay Operations and the Information and Resources Segments. N. P. Page, Acting Unit Head, was appointed Director for the Bioassay Operations Segment in March 1973. T. Cameron was recruited to coordinate animal resources in August 1972.

The Unit currently has responsibilities in the following three main activities:

(1) Carcinogen Bioassay Operations - This program directed by N. Page is responsible for the design and conduct of standardized bioassay tests to detect carcinogenic hazards of chemical and physical agents. This entails (a) identifying and selecting chemical and physical agents for bioassay, (b) establishing logistical capabilities for testing, (c) acquiring, characterizing and purifying these agents, (d) identifying, developing and selecting biological models for carcinogenesis bioassay including improved animal models and short-term bioassay procedures, (e) identifying carcinogenic activity of selected agents by the appropriate bioassay tests, (f) monitoring testing progress performance, (g) developing a data bank to include results of bioassay testing and information on use and characteristics of the agents being tests, (h) analyzing and evaluating the test results, and (i) deciding on further action required of the tested agents.

Many of these activities have previously been conducted by the Program and Data Analysis Unit. For example, data retrieval by the Carcinogenesis Bioassay Data System (CBDS), animal and chemical resources, and data analysis. Actual testing is conducted by contractor laboratories. The separation of the management of the bioassay program from management of resources and data analysis made the necessary coordination difficult. Assigning director responsibility for the Bioassay Operations Segment to the Unit Head should resolve much of these difficulties.

(2) Information Resources - The Carcinogenesis Bioassay Data System (CBDS) designed by this Unit in collaboration with the NIH, Division of Computer Research and Technology, is used to collect, monitor, and store experimental data and results from bioassay operations. It is designed for the complete or selective recall of bioassay data. The system is now in full operation with new bioassay experiments being entered as they are initiated. Data from about 20 bioassay contractors are now on file with several other contractors preparing data for entry into the system. During the coming year the staff will expand the system to permit the analysis of data using those methods best suited to the interpretation of carcinogenesis data. In addition, the Unit maintains chemical and bioassay contract information sub-systems which list data on material presently under test or projected for study, date initiated, etc. The routine data entry, SNOP coding, etc. are provided under contract (Wolf Research and Development Corp., Bioassay Operations Segment).

The staff provides editorial advice and collaboration for the following publications produced under contract: (a) *Survey of Compounds Which Have Been Tested for Carcinogenic Activity* (PHS Publication No. 149) - This publication is produced in a series of volumes and represents a standard reference data base in the field of chemical carcinogenesis bioassays. Volumes are now available for literature published prior to 1960 and 1968-1969. Volumes covering 1961-1967 and 1970-1973 are now being processed (John I. Thompson Co., Information and Resources Segment) and (b) *Carcinogenesis Abstracts* - This is a monthly publication which provides the scientific community with current abstracts on all aspects of carcinogenesis research (Franklin Research Institute, Information and Resources Segment). The volume of information deemed relevant has increased dramatically in the last fiscal year necessitating expansion of this information activity.

Searches are conducted as requested by Carcinogenesis staff utilizing data bases such as the Smithsonian Information Exchange; MEDLARS; National Library of Medicine, Toxicology Information Program; and the Selective Dissemination System of the USDA.

(3) Animal/Chemical Resources - T. Cameron coordinates the animal resources while M. Litwack acts as coordinator of chemical resources. The Unit recognized a need to develop a more extensive program to meet the animal requirements of the Carcinogenesis Program and is assisting the program directors in planning their animal needs as far in advance as possible, coordinating these needs with the animal suppliers and providing information on the characteristics and availability of laboratory animals. The Unit assists Carcinogenesis staff with animal health and husbandry problems, plans animal facility modifications and plans and supports the development of animal models for cancer research. With the recently enacted Animal Welfare Act, assurances of compliance by both contract and intramural programs are necessary. N. Page is currently Chairman while T. Cameron is a member of a NCI ad hoc group appointed for that purpose. Capabilities have been developed to provide standard reference samples of many carcinogens for use in program activities.

The Unit works closely with the Information and Resources Segment which is expected to have 25 contracts in effect by the end of the fiscal year. Unit scientists act as project officers on the majority of those contracts. A discussion of these projects can be found in the narrative description of the Segment.

While these are the three activities within the Unit structure, the success of the bioassay program requires the close coordination and support of all personnel in the Unit. Additional discussion on this can be found in the Summary Report on the Bioassay Operations Segment.

Other activities of the Unit has been their assistance in the analysis of data as requested by program leaders in Carcinogenesis and provides general staff support as requested by the Associate Scientific Director. The Carcinogenesis Program has a limited but significant program in radiation carcinogenesis on the interactions of radiation and chemicals. N. Page acts as coordinator and project officer for these studies.

To effectively manage and monitor the bioassay program and other Unit activities requires expertise in various disciplines including animal care, chemistry, toxicology, biometry and data analysis, pathology, information handling, and fiscal operations. While the Unit possesses several of these disciplines, the need for more staff is urgent in view of the projected further expansion of bioassay and carcinogenesis activities. Several additional positions were planned for the Unit staff to meet current and anticipated needs. Present personnel ceilings do not allow for adequate functioning of this Unit at the level required by program needs.

Serial No. NCI-4696
1. Office of the Associate Scientific Director for Carcinogenesis, DCCP
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Evaluation of Carcinogenic Hazards

Previous Serial Number: Same

Principal Investigator: Umberto Saffiotti, M.D.

Other Investigators: Collaboration from the Staff of the Carcinogenesis Program

Cooperating Units: Collaboration from the Staff of the Demography Program and Outside Advisors

Man Years:

Total:	0.6
Professional:	0.3
Other:	0.3

Project Description

Objectives:

To examine current knowledge in the field of carcinogenesis and related fields and to identify criteria for the evaluation of carcinogenic hazards by chemical and physical agents and for the prevention of their carcinogenic effects in man. To examine laboratory findings on the biological action of chemicals and to review data on exposures for man in order to formulate a scientific evaluation of their carcinogenic risk.

Methods Employed:

Scientific coordination of intramural and collaborative programs in the Carcinogenesis Program, DCCP, NCI. Convening expert advisory groups. Consultation with other government agencies and with members of the scientific community. Review of scientific information. Individual analysis of data collected in this field and particularly results obtained in carcinogenesis experiments.

Major Findings:

A study was made of the carcinogenesis data and of the pathology submitted by the Environmental Protection Agency on the pesticide, Dieldrin. Several other documentations were studied and reviewed for other Federal agencies (Food and Drug Administration and National Institute of Occupational Safety and Health)

Numerous contributions have been made towards the establishment of national policies in the field of carcinogenesis hazards, through discussions and documentation provided to Congressional Committees, government agencies, and offices, and to scientific societies.

Significance to Biomedical Research and the Program of the Institute:

The national policies on environmental health and cancer prevention should be based on sound scientific grounds. The Carcinogenesis Program at NCI provides a central source of information and competence in this field.

Proposed Course of Project:

Continuation and expansion of these functions.

Honors and Awards

Participant, Carcinogenesis Advisory Panel, Division of Cancer Cause and Prevention, National Cancer Institute

Chairman, Carcinogenesis Contract Program Management Group, Division of Cancer Cause and Prevention, National Cancer Institute

Chairman, Carcinogenesis Intramural Program Group, Division of Cancer Cause and Prevention, National Cancer Institute

Member, Cause and Prevention Executive Staff, Division of Cancer Cause and Prevention, National Cancer Institute

Member, Tobacco Working Group, Lung Cancer Task Force, National Cancer Institute

Member, Ad Hoc Committee on Smoking and Health, National Cancer Advisory Board, National Cancer Institute

Member, Ad Hoc Advisory Committee for the Frederick Cancer Research Center, National Cancer Institute

Member, Ad Hoc Committee on Testing for Environmental Chemical Carcinogens, National Cancer Institute

Consultant, Advisory Group on Drug-Related Carcinogenesis, Food and Drug Administration

Participant and Representative for the National Cancer Institute at the Policy Board Meetings and observer to the Scientific Advisory Board Meetings of the National Center for Toxicological Research, Food and Drug Administration

Member, Interagency Panel on Environmental Mutagenesis

Member, Intra-agency Committee to the Board of Advisors of the International Advisory Committee to the Fogarty International Center

Councillor, Society for Occupational and Environmental Health and Member of the Committee on Publications and Awards

Participant, Ad Hoc Subcommittee on Clinical Relevance of Carcinogenicity Testing with Drugs of the Committee on Problems of Drug Safety, Drug Advisory Board, National Research Council, National Academy of Sciences

Participant and Liaison for the National Cancer Institute, Workshops on Carcinogenesis Organized by the Interdisciplinary Communications Program, Smithsonian Institution

Guest Speaker, Roswell Park Memorial Institute, Buffalo, New York, June 28, 1972

Member, Program Committee, and Speaker at the 1972 Conference on Epidemiological Approaches to Carcinogenesis sponsored by the Fogarty International Center, NIH, the IARC, and the League for the Fight Against Cancer of the Croatian Republic of Yugoslavia held in Primosten, Yugoslavia on August 27-September 2, 1972

Participant, Workshop on Late Effects of Cancer Therapy in Children and Young Adults sponsored by the National Cancer Institute and the Memorial Hospital for Cancer and Allied Diseases held in Boston, Massachusetts, on October 19-20, 1972

Speaker and Participant, World Symposium on Model Studies in Chemical Carcinogenesis sponsored by the National Cancer Institute, Atomic Energy Commission, and Johns Hopkins University, held in Baltimore, Maryland, on October 31- November 3, 1972

Speaker, Research and Development Steering Committee of the Pharmaceutical Manufacturer's Association held in Chicago, Illinois, on February 1, 1973

Publications

Saffiotti, U.: Mechanisms of cancer induction in relation to the problem of environmental cancer. In Environment and Cancer (A Collection of Papers Presented at the University of Texas at Houston, M.D. Anderson Hospital and

Tumor Institute, 24th Annual Symposium on Fundamental Cancer Research 1971). Baltimore, Williams and Wilkins Co., 1972, pp. 190-197.

Saffiotti, U.: The laboratory approach to the identification of environmental carcinogens. In Scholefield, P. G. (Ed.): Proceedings of the Ninth Canadian Cancer Research Conference. Toronto, Canada, University of Toronto Press, 1972, pp. 23-36.

Saffiotti, U., Montesano, R., Sellakumar, A. R., Cefis, F., and Kaufman, D. G.: Respiratory tract carcinogenesis in hamsters induced by different numbers of administrations of benzo[a]pyrene and ferric oxide. Cancer Res. 32: 1073-1081, 1972.

Saffiotti, U., Montesano, R., Sellakumar, A. R., and Kaufman, D. G.: Respiratory tract carcinogenesis induced in hamsters by different dose levels of benzo[a]pyrene and ferric oxide. J. Natl. Cancer Inst. 49: 1199-1204, 1972.

Kaufman, D. G., Baker, M. S., Smith, J. M., Henderson, W. R., Harris, C. C., Sporn, M. B., and Saffiotti, U.: RNA metabolism in tracheal epithelium: Alteration in hamsters deficient in vitamin A. Science 177: 1105-1108, 1972.

Kaufman, D. G., Baker, M. S., Harris, C. C., Smith, J. M., Boren, H., Sporn, M. B., and Saffiotti, U.: Coordinated biochemical and morphologic examination of hamster tracheal epithelium. J. Natl. Cancer Inst. 49: 783-792, 1972.

Sellakumar, A. R., Montesano, R., Saffiotti, U., and Kaufman, D. G.: Hamster respiratory carcinogenesis induced by benzo[a]pyrene and different dose levels of ferric oxide. J. Natl. Cancer Inst. 50: 507-510, 1973.

Saffiotti, U.: Metabolic host factors in carcinogenesis. In International Agency for Research on Cancer Monograph. (Proceedings of the Conference on Host-Environment Interactions in the Etiology of Cancer in man--Implementation in Research 1972) (In Press).

Saffiotti, U.: Comments on the scientific basis for the "Delaney Clause". Preventive Med. (In Press).

Harris, C., Kaufman, D., Sporn, M., Smith, J., Jackson, F., and Saffiotti, U.: Ultrastructural effects of N-methyl-N-nitrosourea on the tracheobronchial epithelium of the Syrian golden hamster. Int. J. Cancer (In Press).

Harris, C., Kaufman, D., Sporn, M., and Saffiotti, U.: Histogenesis of squamous metaplasia and squamous cell carcinoma in an animal model. Cancer Chemother. Rep. (In Press).

Serial No. NCI-4542
1. Office of the Associate Scientific Director for Carcinogenesis, DCCP
2. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Chemistry of N-Nitroso Compounds

Previous Serial Number: None

Principal Investigator: Larry K. Keefer, Ph.D.

Other Investigators: Peter Roller, Ph.D., Walter Zielinski and Yuki Oshiro (Frederick Cancer Research Center), William Lijinsky (Biology Division, Oak Ridge National Laboratories), and George Olah (Case Western Reserve University).

Cooperating Units: None

Man Years

Total:	1.6
Professional:	1.3
Other:	0.3

Project Description

Objectives:

(1) To investigate factors such as catalysis which influence the environmental distribution of potentially carcinogenic N-nitroso compounds; (2) To study the deuterium isotope effects on chemical, biochemical, and biological properties of N-nitroso compounds; (3) To prepare crystalline derivatives of non-crystalline dialkylnitrosamines using reversible synthetic procedures as a possible means for separating conformers and investigating structures by x-ray crystallography; (4) To devise a means for chemically suppressing the volatility of low molecular weight nitrosamines to increase recoveries during evaporation of their solutions; and (5) To determine the position of protonation of dialkylnitrosamines.

Methods Employed:

Many standard methods of chemistry have been utilized in this project, including chemical synthesis using superacids and isotopically labeled starting materials.

Major Findings:

(1) A new type of catalysis for the N-nitrosation reaction has been identified. Previously, acidic conditions were thought to be an absolute prerequisite to the synthesis of significant amounts of nitrosamines, and *in vivo* synthesis of these potential carcinogens was considered a possibility only in the stomach. Under catalysis by formaldehyde or other electrophilic species, however, nitrosamine production occurs rather smoothly even under neutral and basic conditions. This finding is of great potential interest to the study of environmental carcinogenesis, as formaldehyde is used in the preservation of fish and in the preparation of slow release nitrogen fertilizers, and it and its analogs may be widely distributed in foodstuffs and other factors in the human environment.

(2) Nitrosomorpholine-3,3,5,5-d_4 has been prepared in high isotopic purity, and is now under test at the Oak Ridge National Laboratory as a liver carcinogen in rats.

(3) Dimethylnitrosamine-d_6 has been prepared in high isotopic purity, and studies of the deuterium isotope effect on the induction of pulmonary adenomatosis in Swiss mice will be begun as soon as possible at Frederick Cancer Research Center.

(4) Tritiated dimethylnitrosamine and ^{14}C-labeled dimethylnitrosamine-d_6 have been synthesized, and studies of the deuterium isotope effect on the methylation of liver RNA and other known metabolic reactions of dimethylnitrosamine are beginning at the Frederick Cancer Research Center.

(5) Dimethylnitrosamine has been found to be surprisingly basic. Stable, crystalline salts have been prepared with perchloric acid and other moderately strong acids. Solutions of dimethylnitrosamine in large volumes of methylene chloride can be subjected to the coarsest evaporation procedures with no loss of nitrosamine if trichloroacetic acid is added first in molar excess. Apparently, molecular association, if not full proton transfer, is occurring in non-polar solutions of this type; this phenomenon has been used to advantage in recovering radioactively labeled dimethylnitrosamine following a synthetic manipulation, and may also permit high yield recoveries of this and related compounds without loss during the analysis of foodstuffs and other complex environmental mixtures. Preliminary evidence has been collected in collaboration with Case Western Reserve University that nitrosamines protonate at oxygen.

Significance to Biomedical Research and the Program of the Institute:

With growing public attention focusing on the possibility that N-nitroso compounds might be responsible for some human cancer, collection of data on the chemical and physical properties of this hitherto little investigated class of compounds has become an urgent matter. Certain aspects of

the studies reported here may aid in developing methods for predicting and determining the environmental distribution of these potential carcinogens, while other aspects are aimed at shedding light on the biochemical mechanisms by which these compounds exhibit their untoward effects.

Proposed Course of Project:

The studies described above will be completed and published, and important implications thereof will be investigated as appropriate. In addition, new studies will be initiated. One program planned for the immediate future will be aimed at the synthesis of C-hydroxy-dimethylnitrosamine or a derivative thereof for use in additional studies of the metabolism and activation of dialkylnitrosamine carcinogens.

Honors and Awards

Member, Information and Resources Segment, Carcinogenesis Program, DCCP, NCI

Invited Speaker, University of New Hampshire Chemistry Department, April 1973

Invited Speaker, Westchester State College Chemistry Department, May 1973

Publications

Keefer, L. K. and Johnson, D. E.: Magnesium hydroxide as a thin-layer chromatographic adsorbent III. Application to separations of vitamin A and related carotenoids. *J. Chromatogr.* 69: 215-218, 1972

Serial No. NCI-4397

1. Office of the Associate Scientific Director for Carcinogenesis, DCCP
2. Epidemiologic Pathology Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Comparative Epidemiology of Malignant Neoplasms of Man and Animals

Previous Serial Number: Same

Principal Investigator: Carolyn H. Lingeman, M.D.

Other Investigators: None

Cooperating Units: Registry of Experimental Cancers and Experimental Pathology Branch, Carcinogenesis

Man Years:

Total:	0.7
Professional:	0.5
Other :	0.2

Project Description

Objectives:

To evaluate systematically the epidemiologic, clinical and morphologic characteristics of the most prevalent malignant neoplasms of man and their counterparts in animals in order to better understand the pathogenic and etiologic mechanisms involved in the processes of carcinogenesis.

Methods Employed:

For each of the site-specific cancers selected for study, all relevant information concerning clinical and epidemiologic characteristics of both human and animal neoplasms was collected from published and unpublished sources. Histologic material from humans and animals with spontaneous and experimental neoplasms of selected sites were examined to determine if morphologic features are comparable.

Because of the need for efficient means for storing, analyzing and retrieving the large volume of bibliographic information and other data that was accumulated during the course of the study, methods for using data processing systems (WYLBUR and CPS) were developed.

Major Findings:

Detailed accounts of findings from earlier studies of neoplasms of the hematopoietic and digestive systems (stomach and intestines) can be found in Annual Reports of previous years. Because this year's studies have emphasized cancers of the pancreas and female genital system (including mammary glands) information obtained about neoplasms occurring in those sites are described in detail:

(1) Pancreas: Adenocarcinomas of exocrine portions of the pancreas, usually originating in ducts or ductules, are among the most frequent human malignant neoplasms in the United States, ranking fourth in males and fifth in females as causes of cancer deaths. Moreover, the incidence of this cancer is increasing yearly, particularly in American Black males. Causes are presumed to be environmental, yet there are no clues as to the etiology(ies). To date, none of the many chemical substances known to be carcinogenic for rodents have induced acceptable examples of pancreatic carcinomas. In man, carcinomas originating in islet cells are less frequent than those of exocrine origin.

Neoplasms of the pancreas are rare in most species of animals and in most laboratory and domesticated animal tumor surveys they account for fewer than 1% of total malignant neoplasms. They were most often reported in dogs and cats; however, authors were not always specific as to whether observed neoplasms originated in islets or exocrine portions. Neoplasms arising in islet cells occur in significant numbers in dogs and their frequency may actually exceed that of neoplasms originating in other areas of the pancreas. There are few convincing reports of duct cell or acinar cell carcinomas of pancreas of dogs and cats, although there are several well-documented examples reported in rodents.

(2) Ovary: Neoplasms of the ovary are the fourth most frequent human female fatal cancer in the United States. They occur at all ages, including young children. Neoplasms of germ cell origin predominate at younger ages, and in orientals; epithelial neoplasms predominate in older individuals and in other ethnic groups that have been studied. There are no hypotheses as to etiology(ies). Benign neoplasms, mostly tubular adenomas or granulosa-luteal cell tumors, have been induced in mice by irradiation, chemical carcinogens, and of ovaries implanting into spleens of gonadectomized hosts. Rodents or other animals only occasionally develop spontaneous malignant epithelial neoplasms of ovaries, although occasional examples of many types, including stromal and germinal epithelial neoplasms, have been observed in one or more species of animals. Particularly interesting are reports of spontaneous adenocarcinomas in ovaries of a high per cent of old chickens of certain flocks. Another animal model worthy of attention is the proliferative lesion of surface epithelium of ovaries of dogs caused by diethylstilbesterol and other female hormones; although claims of metastases by some observers have led to speculation about the possible malignancy of these lesions, some are reversible after discontinuing the medication. At any rate, their value as an experimental model in studies of ovarian reactivity to these widely-used substances warrants further investigation.

(3) Uterus: a. Endometrium - Adenocarcinomas of the endometrium are among the more prevalent human female cancers in the United States. There are no proven etiologic factors except that estrogenic stimulation plays a significant role and host factors influence susceptibility. A search of the literature indicates th t spontaneous carcinomas of the uterine endometrium are rare in most species of animals except cattle and rabbits. The fairly frequent endometrial adenocarcinomas of cattle metastasize to lungs and other sites. The epidemiology and clinical features of the neoplasm have not been defined and there are no hypotheses concerning etiology. In contrast to human endometrial adenocarcinomas which occur in proliferative endometrium, and are frequently associated with estrogenic stimulation, the rabbit neoplasm occurs in atrophic endometrium and growth is not stimulated by estrogenic hormones. The neoplasm is observed with fairly high frequency in older domestic rabbits of several breeds.

b. Cervix - Squamous cell carcinomas of the uterine cervix are among the most prevalent human malignant neoplasm in the United States and many other countries of the world. The epidemiologic features have been well-defined and several hypotheses concerning etiologic factors have been advanced. Most evidence suggests a major role of an infectious agent, possibly a virus of the Herpes group. Neoplasms of the uterine cervix are rare in animals but have been reported in older mice that have been injected as newborns with synthetic estrogens including diethylstilbesterol.

c. Other female genital cancers are infrequent in animals.

(4) Mammary gland: Neoplasms of the mammary glands are among the most frequent neoplasms of man, cat, dog, mouse and rat. The epidemiology of the human neoplasm has been well studied, and the importance of hormonal stimulation or imbalance has been documented. Viruses and hormones interact with genetic factors to cause epithelial mammary gland neoplasms in mice but chemical carcinogens and irradiation can also cause them. Chemical carcinogens also cause adenocarcinomas of mammary glands of rats. Viruses have not been shown to be involved in mammary gland carcinomas of other species. Hormonal factors do affect growth of some canine neoplasms, but have not been proven to be important in those of the cat. To date, no precise morphologic counterpart of the frequent human infiltrating duct cell carcinoma has been found. The dog develops a wide spectrum of epithelial and connective tissue neoplasms some of which are adenocarcinomas. Most of the mammary gland neoplasms of cats are papillary adenomas or adenocarcinomas originating in ducts. Although they sometimes metastasize to lungs and other structures, their behavioral characteristics have not been well studied nor has hormone dependence been established. The papillary type of adenocarcinoma is comparatively infrequent in man.

(5) Hematopoietic Neoplasms: Details collected during the early years of the study were published in a monograph (1969). Interest in neoplasms of this group has continued, particularly plasma cell myeloma. At the present time there is little information about the epidemiology of this disease

although many reports have confirmed a significantly higher frequency in American Blacks, particularly Black males. Surveillance of mortality from myeloma for two states (Indiana and Virginia) since 1950 and 1962 has suggested a tendency to geographic-temporal clustering, but because of the small numbers of cases involved, such tendencies are difficult to prove. There are no hypotheses for etiology and no completely comparable animal models.

(6) Colo-rectal cancers: Because of the Unit's commitment to studies of this most prevalent human fatal cancer in the United States at the present time searches for valid animal models has continued. A paper describing the rare spontaneous intestinal adenomas and adenocarcinomas in animals was published previously. Subsequently, several hundred histologic sections from rats injected with the potent intestinal carcinogen dimethylhydrazine (DMH) have been examined. Small and large intestines of these rats contained a spectrum of mucosal epithelial neoplasms from adenomas, some containing foci of in situ carcinomas, to invasive carcinomas showing all degrees of differentiation. Some carcinomas invaded all layers of intestinal walls, metastasized to lymph nodes, liver and other structures in a manner similar to the human neoplasm. A hypothetical sequence of the pathogenic sequence of formation of the experimental adenocarcinomas, originating in adenomas or in "normal" mucosa provided support for proposed pathogenic sequences for the human counterparts.

Significance to Biomedical Research and the Program of the Institute:

As part of the commitment of the Carcinogenesis Program to correlate epidemiologic information and laboratory experimentation in evaluating action of environmental carcinogens, this project seeks to provide a link between epidemiologist and laboratory investigator by using the special skills and techniques of the pathologist and morphology as the basis for comparisons.

Proposed Course of Project:

The systematic comparisons of human and animals will be continued with increasing attention to searches for valid experimental models and use of data-processing systems for improved dissemination of information.

Serial No. NCI-4398

1. Office of the Associate Scientific Director for Carcinogenesis, DCCP
2. Epidemiologic Pathology Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Registry Based Pathology Studies of Cancer

Previous Serial Number: Same

Principal Investigator: John W. Berg, M.D.

Other Investigators: Guy F. Robbins (Memorial Hospital for Cancer and Allied Diseases), Stephen Baylor, J. David Godwin and Constance Percy (Biometry Branch), Frank McKay (Epidemiology Branch, Demography, NCI), and Fredrick Bauer (Greater Baltimore Medical Center).

Cooperating Units: None

Man Years:

Total	0.4
Professional	0.2
Other	0.2

Project Description

Objectives:

(1) To analyze pathology data now existing in tumor registries by study of epidemiology and survival correlations; (2) To utilize existing registry and related data in the solution of specific pathology problems such as the significance of specific histologic patterns and the behavior of rare tumors; and (3) To undertake studies in quantitative cancer pathology and to base as many of these as possible on registry-defined data.

Methods Employed:

Computer surveys of large record files, record review of specific sets of cases, statistical analyses, and qualitative and quantitative microscopic studies of particular pathology material all are used when appropriate.

Major Findings:

(1) Studies on nomenclature and classification of tumors have been expanded. Collaboration with the National Office of Health Statistics continues in

preparing recommendations for the forthcoming 9th Revision of the International Classification of Diseases. The Unit's unique contribution has been to furnish and interpret real data on tumor types and locations so that decisions will be based on facts rather than on unsubstantiated theorizing, as has been usually true in the past. The past year has seen adoption by WHO and the End Results Group of the 1968 Edition of the American Cancer Society's Manual of Tumor Nomenclature and Coding, as well as WHO use of other results for the 9th Revision.

(2) Epidemiological-pathological studies of specific types of cancer are continuing. A report has been completed on the different histologic varieties of pancreatic cancer. Over 5,000 histologically confirmed cases of cancer of the pancreas from the End Results Group of cancer registries were examined for differences in behavior by epidemiologic and pathologic variables. Islet cell carcinomas were unique, occurring at a much younger age, having a locational preference for the body and/or tail, and a relatively favorable prognosis (14% survival at 15 years). Of cancers of the exocrine pancreas, those with papillary histology were most distinct, two-thirds being found in women, 18% instead of 10% being localized. Survival was somewhat favorable: 30% at one year, 5% at three years. Cancers with squamous metaplasia occurred in general at older ages and led to unusually poor survival. Analysis of "adenocarcinomas" indicated that grade may carry some prognostic information not explained by difference in stage. A striking gradient of decreasing survival with increasing age did not explain, and was not explained, by survival gradients in other variables. Studies of ovarian cancer are now in progress.

(3) Consultations with Dr. Bauer, begun when he was a USPHS pathologist, have led to collaboration in his continuing analysis of cancer at autopsy. He had reported previously that 41% of all cancers in a large autopsy series were incorrectly diagnosed before death. We have now detected a direct association between accurate clinical diagnoses of cancer and increasing length and number of hospital admissions. The prevalence of incorrectly diagnosed cancer progressively decreased from a high of 53% to a low of 30% when both the number and length of hospitalizations increased. However, these relationships were limited to the first five hospital admissions and the first 20 hospital days. Beyond these limits, no further improvement in diagnostic accuracy was noted, and undiagnosed cancer caused 11% of the deaths of patients. Further analyses are in progress.

(4) In Africa testicular cancer in blacks is a rare disease, 1/20th as common as it is in whites. The End Results Group data, the preliminary results of the 3rd National Cancer Survey, and the U. S. death certificate data from the Epidemiology Branch all showed the same finding; namely, that testis cancer in U. S. Blacks neither is as rare as in Africans nor as common as in U. S. Whites. The relative rate is about 30% of the white rates. This work is important scientifically because it raises the question of environmental involvement in the cause of testis cancer but, methodologically, we also hope it will furnish a precedent for more extensive studies pooling NCI data resources.

Significance to Biomedical Research and the Program of the Institute:

While individual studies hopefully have intrinsic value, the prime aim of the project is to provide a broad factual framework for use in planning the programs of the Division of Cancer Cause and Prevention. In particular, it aims for a more specific identification of the heterogenous assortment of diseases including "cancer" of the various anatomic sites and for development of more precise and rigorous techniques for quantitative description of human cancers.

Proposed Course of Project:

As studies now underway are completed, others will be implemented with consideration to the priorities within the Carcinogenesis Program and the Division of Cancer Cause and Prevention and the very limited capabilities of the Unit.

Honors and Awards

Member, Task Force on Melanoma, American Joint Committee on Cancer Staging and End Results Reporting

Member, Task Force to Review "Manual of Tumor Nomenclature"; Member, Clinical Investigation Advisory Committee; Member, Bowel Cancer Pathology Advisory Committee; all American Cancer Society

Consultant, Epidemiologic Pathology, Memorial Hospital for Cancer and Allied Diseases

Member, Working Party on Classification of neoplasms of the Revision of the International Classification of Diseases, U. S. National Committee on Vital and Health Statistics; WHO Working Group on Chapter II: Neoplasms of the 9th Revision of the International Classification of Diseases

Member, Board of Editors, Gastroenterology Section Excerpta Medica

Member, Carcinogenesis Contract Program Management Group, DCCP, NCI

Member, Lung Cancer Segment and Biological Models Segment, Carcinogenesis Program, DCCP, NCI

Invited Speaker, 7th National Cancer Congress, September 1972

Publications

Berg, J. W., Huvos, A. G., Axtell, L. M., and Robbins, G. F.: A new sign of favorable prognosis in breast cancer: Hyperplastic reactive lymph nodes in the apex of the axilla. Ann. Surg. 177: 8-12, 1973.

Bauer, F. W., Robbins, S. L., and Berg, J. W.: An autopsy study of cancer patients II. Hospitalizations and Accuracy of Diagnoses (1955 to 1965) Boston City Hospital. JAMA 223: 299-301, 1973.

Baylor, S. M. and Berg, J. W.: Cross-Classification and survival characteristics of 5,000 cases of cancer of the pancreas. J. Surg. Oncol. (In press).

Serial No. NCI-4538

1. Office of the Associate Scientific Director for Carcinogenesis, DCCP
2. Epidemiologic Pathology Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Correlations between Trace Metals in Water Supplies in Cancer Mortality

Previous Serial Number: None

Principal Investigator: John W. Berg, M.D.

Other Investigators: Frank McKay (Epidemiology Branch, Demography)

Cooperating Units: None

Man Years:

Total	:	0.1
Professional	:	0.1
Other	:	0.0

Project Description

Objectives:

To correlate data on water supply components with geographic variations in mortality from specific cancer types.

Methods Employed:

Various sources of information on water supplies are collected and grouped geographically to coincide with geographic cancer data.

Major Findings:

With a survey of carcinogenic metals complete, the look at "non-carcinogenic" elements was begun. Low molybdenum levels in water supplies of regions and cities was correlated with high esophageal cancer mortality. The effect appeared independent of other race-sex related factors as well as smoking and alcohol consumption.

Significance to Biomedical Research and the Program of the Institute:

Several elements found in water supplies are known to affect carcinogenesis in animals and could play a similar role for humans. These preliminary correlations may lead to more detailed analysis of human intake and animal experiments designed to test human type exposures.

Proposed Course of Project:

A full documentation of this work. Analyses of other metals and correlations with other sources of cancer data will be done to the extent time permits.

Publications

Berg, J. W. and Burbank, F.: Correlations between carcinogenic trace metals in water supplies and cancer mortality. Ann. N.Y. Acad. Sci. 199: 249-264, 1972.

Berg, J. W., Haenszel, W., and Devesa, S. S.: Epidemiology of gastrointestinal cancer. Proceedings of the 7th National Cancer Congress (in press).

Serial No. NCI-4543
1. Office of the Associate Scientific Director for Carcinogenesis, DCCP
2. Epidemiologic Pathology Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Epidemiologic and Pathological Studies on Bowel Cancer

Previous Serial Number: None

Principal Investigators: J. W. Berg, M.D. and Margaret A. Howell, Ph.D.

Other Investigators: None

Cooperating Units: J. David Godwin (Biometry Branch, Demography, NCI), Grant Stemmermann (Kuakini Hospital, Honolulu, Hawaii), Nobuki Sasano (Tohoku University, Sendai, Japan), Edward Mason (University of Iowa, Iowa City, Iowa), Ronald Welch (Louisiana State University, New Orleans, La.), Roland Phillips (Loma Linda Hospital, Loma Linda, Calif.), John Baldwin (Oxford University, London), John Lee (University of Washington, Seattle, Washington), and Manning Feinlieb (NHLI, NIH).

Man Years:

Total	:	1.2
Professional	:	0.5
Other	:	0.7

Project Description

Objectives:

To assemble and analyze bowel cancer data relevant to the NCI Bowel Cancer Programs.

Methods Employed:

Record abstracting and analysis, microscopic examination, literature, and data file reviews.

Major Findings:

(1) Different histological types of bowel cancer show different epidemiologic patterns. Analysis of American and Japanese data with particular

emphasis on the End Result Group material shows that colloid carcinoma, as well as malignant carcinoids, occur in different subpopulations than ordinary adenocarcinomas. The material is awaiting final analysis.

(2) Different populations have quite different distributions of lower bowel cancer. The classical divisions into colon and rectal cancer are weak reflections of this fact. Actual measurements show this much more clearly. For example, lower rectal cancer has not increased in Japanese migrants to Hawaii, while cancer of the upper rectum has increased three-fold. Similarly, the increase of bowel cancer in Iowa farmers has been accompanied by a shift from low-rectum to high rectum cancer predominance. Data have been collected for a similar across-time study of New Orleans Black and Whites.

(3) Analysis of Scottish data (published as a letter to Lancet) has shown a unique pattern of disease. In other countries bowel cancer has been much less common in rural than in urban areas. Scotland, which has had the world's highest mortality from bowel cancer, shows a rural predominance. Contacts have been made with about a dozen interested Scottish investigators and it is hoped that more detailed studies have been stimulated.

(4) Seventh Day Adventists have shown lower death rates from bowel cancer. Since many have been strict vegetarians for long periods, their experience is particularly relevant to the dietary hypothesis of bowel cancer. Provisional incidence rates show an even greater difference with respect to bowel cancer between the Adventists and other Californians. Current studies are updating the mortality analysis and, where possible, relating mortality to details of diet.

(5) World-wide, bowel cancer incidence usually is associated with heart disease in particular and with diabetes, gallbladder disease, varicose veins, etc. Beginning with an analysis of Connecticut mortality, a series of studies are underway to determine if these associations hold for individuals and so point to common etiologic factors. Data is being collected for the Unit's analysis under contract in Oxford, England, while Dr. Feinlieb has agreed to perform the analyses on data now being collected from the NHLI's prospective studies in Honolulu and Framingham. Preliminary analyses in Connecticut show no special associations, but the Oxford data suggest only one association: with cholecystitis.

(6) An increasing excess of bowel cancer in men compared to women raises questions about possible occupational hazards. Working with old American data (all that is available) and more recent British data, we have identified a few occupations with a consistent high risk (analyses in progress) and hope to explore further the implications of the associations.

(7) Review of international data shows substantial inconsistencies that need to be resolved if practical use is to be made of such comparisons. Death rates can be higher than incidence rates (implying incomplete reporting). Teaching hospitals, the source of most published hospital data, are biased towards rectal as opposed to colon cancer, while death certification in many, but not all, areas is biased the other way. In Connecticut, for example,

there was a net transfer of 30% of the rectal cancer cases to colon cancer at the time of certification. Dr. John Lee, among other observations, has described a strong correlation between marital status and bowel cancer death rates in women: spinsters are at highest, currently married at lowest risk.

Significance to Biomedical Research and the Program of the Institute:

The concept of a comprehensive attack on the cause of a cancer or set of cancers implies integration of many types of information. Some, though published, are not in the most useful form. Other facts must be gathered in response to particular needs of the central group. The projects herein are designed either to solve small scale problems within the bowel cancer programs or to furnish the basis for establishing larger efforts by outside groups.

Proposed Course of Project:

Although priority will continue to be given to assembly of these data for in-house use, it is hoped that time will eventually become available to turn most into formal reports.

Honors and Awards

Director, Colon Cancer Segment, Carcinogenesis, NCI

Member, Working Cadre, National Large Bowel Cancer Project

Serial No. NCI-4544

1. Office of the Associate Scientific Director for Carcinogenesis, DCCP
2. Epidemiologic Pathology Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Food Consumption Patterns in Population Groups Contrasting in Incidence and Mortality for Cancer of the Large Bowel

Previous Serial Number: None

Principal Investigator: Margaret A. Howell, Ph.D.

Other Investigators: E. Cuyler Hammond, M.D. (American Cancer Society)

Cooperating Units: American Cancer Society
Department of Agriculture

Man Years:

Total	0.7
Professional	0.4
Other	0.3

Project Description

Objectives:

To determine if population groups differing in incidence and mortality for cancer of the large bowel show dietary differences consistent with findings from a case-control study of Japanese migrants to Hawaii.

Methods Employed:

Food consumption patterns are being or have been studied in three sources of dietary information: the questionnaire used by the American Cancer Society (ACS) in its 1959 prospective study of over one million people, published reports of the Department of Agriculture based on dietary surveys, and per capita food consumption across countries, which is available from the Food and Agriculture Organization (FAO) of the United Nations.

Preliminary results from the Japanese study suggested meats and particularly beef were dietary items which differentiated sharply between colorectal cancer cases and controls. For this reason primary interest in the present analysis has been in the consumption of meat or related dietary components.

From the ACS questionnaire, regional differences in food consumption have been determined. Figures on meat consumption are being compiled from the Department of Agriculture publications by region and also, where possible, by urban-rural and Black-White breakdowns. For the most recent Agriculture data (1965-66), it may be possible to obtain computer tapes for re-analysis of the data in relation to breakdowns in the Third National Cancer Survey incidence data. Based on the FAO data, correlations between dietary components and incidence and mortality rates are being calculated.

Major Findings:

The ACS questionnaire showed the South (which has comparatively low incidence and mortality from colorectal cancer) to differ from the North and other areas of the country in the consumption of a number of food items. One of these was meat or poultry which showed lower consumption in the South than elsewhere.

Findings from other phases of the project are not yet available, but preliminary results from the FAO data suggest that, while beef consumption is highly correlated with both mortality and incidence rates, total meat consumption produces somewhat higher correlations.

Significance to Biomedical Research and the Program of the Institute:

Diet is the most suspect of environmental factors affecting the development of cancer of the large bowel. Yet, only one case-control study, that in Japan, has yielded positive findings in the comparison on dietary histories of individuals with the disease and those free of it. If similar dietary differences can be confirmed in population groups contrasting in incidence and mortality rates for colorectal cancer, these provide supportive evidence for implicating specific dietary components. If specific components can be identified, laboratory testing of potential carcinogenic agents can be better targeted.

Proposed Course of Project:

Completion of the analysis on meat and related dietary components with the possibility of repeating the analysis on other dietary components if indicated by new results from case-control studies sponsored by the Biometry Branch of the Demography Area. At a future time, but not during this fiscal year, case-control data will become available from contracts sponsored by Carcinogenesis (Medical College of Georgia, Contract No. NIH-NCI-E-72-3280 and Kaiser Foundation Research Institute Contract No. NIH-NCI-E-73-3215).

Serial No. NCI-4545

1. Office of the Associate Scientific Director for Carcinogenesis, DCCP
2. Epidemiologic Pathology Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Development of a Diet History Questionnaire for Use in Case-Control Studies of Colon Cancer

Previous Serial Number: None

Principal Investigator: Margaret A. Howell, Ph.D.

Other Investigators: Warren H. Gullen, M.D. (Medical College of Georgia), Gary D. Friedman, M.D. (Kaiser Foundation Research Institute), and Cecile H. Edwards, Ph.D. (Howard University)

Cooperating Units: None

Man Years:

Total	:	0.7
Professional	:	0.5
Other	:	0.2

Project Description

Objectives:

To develop a diet history questionnaire of suitable length for case-control interviews with content appropriate to the study of cancer of the large bowel.

Methods Employed:

Techniques for eliciting diet history information were reviewed, and the most suitable methods for recall were selected for use in a questionnaire. A pool of dietary items was initially developed through (1) a review of the epidemiologic literature to assure adequate item coverage of major dietary hypotheses concerning colorectal cancer, (2) identification and inclusion of items differentiating between colorectal cases and matched hospital controls in a questionnaire used by the Biometry Branch, Demography Area in studies in Hawaii, (3) inclusion of items from other research which were suggestive of differences either in case-control studies or population-based studies, (4) review of items in other diet questionnaires, particularly those used by

Department of Agriculture in order to increase the comprehensiveness of coverage of foods.

An initial pool of demographic items was also screened from the epidemiologic literature pertinent to cancer of the large bowel. Refinement, rephrasing, and final selection of items to determine the content of the questionnaire were undertaken as a collaborative effort between the principal investigators and their co-workers involved in case-control contract work.

Major Findings:

Findings with regard to the development of the questionnaire cannot be reported until pre-testing is performed.

Significance to Biomedical Research and the Program of the Institute:

Since food is considered the most likely source of environmental carcinogens affecting development of cancers of the colon and rectum, a standard, well-developed diet history questionnaire is important. The collaborative effort means greater comparability in dietary information from different sources and different locales. The questionnaire is available upon request, with appropriate clearances, for use in work by other investigators.

Proposed Course of Project:

Through contracts at the Medical College of Georgia (NIH-NCI-E-72-3280) and the Kaiser Foundation Research Institute (NIH-NCI-E-73-3215) the questionnaire will be pretested and revised on the basis of test results. A final form will be used in case-control studies.

Serial No. NCI-4546

1. Office of the Associate Scientific Director for Carcinogenesis, DCCP
2. Epidemiologic Pathology Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Factor Analysis of Mortality and Incidence Data

Previous Serial Number: None

Principal Investigator: Margaret A. Howell, Ph.D.

Other Investigators: John W. Berg, M.D.

Cooperating Units: None

Man Years:

Total	:	0.4
Professional	:	0.1
Other	:	0.3

Project Description

Objectives:

Through the use of factor analysis, to search for epidemiologic clues to the etiology of cancer of the various organ sites, with particular emphasis on cancer of the large bowel.

Methods Employed:

Mortality data from 41 countries, states within the United States, and prefectures in Japan are being analyzed in an exploratory effort to determine if the factors identified suggest new etiological hypotheses which can be tested by other methods. Similar analyses will be made of available inter- and intra-country incidence data.

Major Findings:

The project is in early stages so that little can be reported at the present time. A draft of results based on the mortality data is undergoing technical

review to determine if other approaches more statistically appropriate to the data, such as cluster analysis, should also be employed as an exploratory tool.

Findings thus far indicate that the widely reported negative relationship between gastric cancer and cancer of the large bowel is not supported by the data. The negative association has been viewed as an important epidemiologic lead suggesting opposing etiologies for cancers of the two sites.

Another widely reported positive association, that between breast cancer and cancer of the large bowel, is supported by data from all sources except Japan where mortality rates for both types of cancer are low. The association with breast cancer may be one of the best clues with respect to development of carcinoma of the colon and rectum.

Significance to Biomedical Research and the Program of the Institute:

Confirmation or negation of associations which have been presumed to have etiologic significance is an important contribution to orderly progress in the accumulation of scientific information. If new hypotheses concerning etiology are suggested by exploratory efforts, these can result in re-direction of research.

Proposed Course of Project:

To complete the technical review of the results from mortality data with a view towards other types of analysis, and to extend the methods selected for use in further analysis to incidence data.

If findings warrant publication, a journal article will be prepared.

Serial No. NCI-4547

1. Office of the Associate Scientific Director for Carcinogenesis, DCCP
2. Epidemiologic Pathology Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Study of Selected Bacterial Species

Previous Serial Number: None

Principal Investigator: Sidney J. Silverman, Ph.D.

Other Investigators: Milton Slein (Litton-Bionetics)
King-Thom Chung (Litton-Bionetics)

Cooperating Units: Anaerobe Laboratory, Virginia Polytechnic Institute

Man Years:

Total	10.6
Professional	2.6
Other	8.0

Project Description

Objectives:

(1) To study the metabolism and physiology of intestinal bacteria to determine whether they are capable of producing carcinogens, (2) To develop the host-mediated assay for mutagenesis as a screening technique for carcinogens, and (3) To test other procedures for rapid screening of carcinogens.

Methods Employed:

Bacterial species, isolated from individuals with bowel tumors, are obtained from the Anaerobe Laboratory, VPI and State University. The various strains are studied in pure culture or in mixed cultures in the presence of such components as bile acids or tryptophane. The products of bacterial activity are isolated and tested by chemical procedures. Culture filtrates and compounds isolated from them, as well as fecal extracts, are tested by the host mediated assay.

Major Findings:

(1) The project was initiated in July with the establishment of the Frederick Cancer Research Center at Frederick, Maryland. Cultures, representing seven genera, were received in November and were characterized. The staff was trained in anaerobic techniques, chemical procedures were developed, and experience with the host-mediated assay was obtained.

(2) Studies on tryptophane metabolism with several species were initiated. Preliminary results suggest that Bifidobacterium adolescentis B and Bacteroides fragilis (substrain thetaiotaomicron III) possess a decarboxglase capable of converting tryptophane to tryptamine.

(3) Nutritional studies of the microorganisms were also initiated to develop defined media for metabolic studies. A medium consisting of casamino acids (an acid hydrolysate of casein), a carbohydrate, and, for some organisms, vitamin K and hemin supports slow and relatively poor growth. Yeast extract enhances growth, but, since its composition is variable and unknown, a substitute is being sought.

(4) In preliminary studies for training purposes, N-methyl-N^1-nitro-N-nitrosoguanidine was used in the host-mediated assay. The test organism, a histidine requiring auxotroph of Salmonella typhimurium, showed enhanced mutation frequency when compared to the controls in which saline was substituted for the mutagen. The in vivo mutation frequency was comparable to the in vitro frequency in the presence of nitrosoguanidine.

Significance to Biomedical Research and the Program of the Institute:

The information concerning the ability of the intestinal bacteria to produce carcinogenic substances will help in explaining the etiology of colonic cancer. Knowledge of the substrates used and the pathways of metabolism may aid in control. The usefulness of the host-mediated assays for the rapid screening of carcinogens will be determined; other in vitro assay techniques will be assessed.

Proposed Course of Project:

With the program now firmly underway, information concerning tryptophane and steroid metabolism of intestinal organisms should accumulate. As studies with pure cultures proceed, mixed cultures will be investigated to determine the interactions of the more prominent members of the microflora. The host-mediated assay will continue to be studied and purified bile acid and cholesterol derivatives, as well as fecal extracts, will be studied for their mutagenic activity.

Serial No. NCI-4549
1. Office of the Associate Scientific Director for Carcinogenesis, DCCP
2. Lung Cancer Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Organ Culture Studies on Tracheobronchial Epithelium

Previous Serial Number: None

Principal Investigators: Gerald Clamon, M.D., Michael B. Sporn, M.D., Curtis C. Harris, M.D., Mary Baker, M.S., and Joseph M. Smith

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	1.7
Professional:	0.7
Other:	1.0

Project Description

Objectives:

The principal objective is to study the direct effects of carcinogens and anti-carcinogens on the cell population which is of immediate relevance for development of the most common forms of human lung cancer, namely respiratory epithelium.

Methods Employed:

Classical methods of organ culture are being used. Tracheas are being obtained from either normal animals, from vitamin A-deficient animals, or from animals treated with respiratory carcinogens.

Major Findings:

The optimal conditions for long-term organ culture of hamster tracheobronchial epithelium are being defined. Explants of trachea survive well for one week in an atmosphere of 50% oxygen, 5% CO_2, and 45% nitrogen, when kept on a rocker platform and bathed in medium CMRL-1066. Other media are being evaluated. Tracheas from hamsters treated with carcinogenic

doses of benzpyrene-ferric oxide are also being cultured. The effects of vitamin A on these cultures are being evaluated. Since the project is of less than 6 months duration, detailed findings cannot be reported at present.

Significance to Biomedical Research and the Program of the Institute:

This is a critical technique for direct evaluation of effects of carcinogens and anti-carcinogens on respiratory epithelium. The techniques being developed for animal tissue studies will be of direct application to studies on human material.

Proposed Course of Project:

To continue as outlined above. To begin studies on human material at the earliest possible time at which the techniques will allow.

Serial No. NCI-4550
1. Office of the Associate Scientific Director for Carcinogenesis, DCCP
2. Lung Cancer Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Studies of Effects of Carcinogens and Anti-Carcinogens on Isoenzymes in Respiratory Epithelium.

Previous Serial Number: None

Principal Investigators: Gerald Clamon, M.D., Michael B. Sporn, M.D., Mary Baker, M.S., and Joseph M. Smith

Other Investigators: None

Cooperating Units: Professor Elliot Vesell, Department of Pharmacology, Pennsylvania State University, School of Medicine, Hershey, Penna.

Man Years:

Total:	1.8
Professional:	0.8
Other:	1.0

Project Description

Objectives:

The main objective is to use isoenzyme ("isozyme") measurements in an attempt to quantitate morphological changes that occur in respiratory epithelium in response to carcinogens or anticarcinogens. Isozyme patterns have been reported by others to change in tissues other than respiratory epithelium during the process of carcinogenesis.

Methods Employed:

Techniques have been developed for measurement of the following four isozymes in samples of respiratory epithelium: lactic dehydrogenase, malic dehydrogenase, aldolase, and hexokinase. Isozymes are separated by acrylamide gel electrophoresis and stained by tetrazolium methods.

Major Findings:

The lactic dehydrogenase pattern in lung tumors induced by benzpyrene and nitrosomethylurea is very different from that found in normal respiratory epithelium. A shift in the isozyme pattern of aldolase was also found in these tumors. These studies are now being performed on respiratory epithelium during the process of carcinogenesis.

Significance to Biomedical Research and the Program of the Institute:

These are important new methods to assess the state of differentiation of respiratory epithelium. Since these isozyme measurements can be made on very tiny amounts of tissue, they offer some eventual promise of being of use in human lung cancer diagnosis.

Proposed Course of Project:

To continue as outlined above.

Serial No. NCI-4551
1. Office of the Associate Scientific Director for Carcinogenesis, DCCP
2. Lung Cancer Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Eukaryotic DNA Synthesis and Carcinogenesis

Previous Serial Number: None

Principal Investigators: David G. Kaufman, M.D., and Valerio Genta, M.D.

Other Investigator: Curtis C. Harris, M.D.

Cooperating Units: C.W. Dingman, M.D., Nucleic Acids Section, Chemistry Branch, NCI, and J.W. Grisham, M.D., Washington University School of Medicine, St. Louis, Mo.

Man Years:
Total: 0.7
Professional: 0.7
Other: 0.0

Project Description

Objectives:

The aim of this project is to determine features of eukaryotic DNA synthesis and the control of this process. Results of these studies will provide the framework against which the effects of carcinogens on this process can be analyzed.

Methods Employed:

Partial hepatic resection has been employed in order to provide a partially synchronized cellular population *in vivo*. Brief or prolonged periods after exposure to ^{3}H-thymidine or bromodeoxyuridine have been employed to preferentially label nascent or non-nascent DNA. Nuclei have been isolated from liver cells by gentle methods and have either been used *in vitro* DNA synthesis assays or subjected directly to disruption and fractionation.

Major Findings:

Nascent DNA associated with as yet unidentified materials has been isolated on the basis of the intrinsic density of the complex. The method used is readily reproducible and new in its application to nascent

DNA in eukaryotes. Liver nuclei have been prepared and assayed such that either DNA replication or DNA repair predominates. Differential influences of some substrates, inhibitors, and other co-factors on the different types of DNA synthesis in isolated liver nuclei, have been determined.

Significance to Biomedical Research and the Program of the Institute:

These studies contribute to the basic understanding of the processes of DNA replication and the repair of DNA damage. The control of DNA replication and cell proliferation, and the repair of carcinogen induced damage to DNA, are currently significant subjects for investigations directed toward elucidation of the basic mechanism of carcinogenesis. These studies have been conducted in tissues and systems in which they are most readily and easily investigated. These results and observations contribute to the part of the lung cancer program directed toward an understanding of metabolic processes in respiratory tract tissues. Comparable studies can then be designed for this target organ of high susceptibility. Thus, these basic experiments provide the basis for new studies to be conducted in respiratory carcinogenesis in this and future years.

Proposed Course of Project:

The present studies will be continued and the effects of carcinogens on these processes will be explored. Efforts will be made to apply fruitful or promising results or methods developed in the present experimental system to the study of carcinogenesis in the respiratory tract.

Publications

Kaufman, D.G., Grisham, J.W., and Stenstrom, M.L.: Unscheduled incorporation of [^{3}H]-TTP into DNA of isolated rat liver nuclei. Biochim. Biophys. Acta 272: 212-219, 1972.

Grisham, J.W., Kaufman, D.G., and Stenstrom, M.L.: ^{3}H-TTP incorporating activities in isolated rat liver nuclei. Biochem. Biophys. Res. Commun. 49: 420-427, 1972.

Serial No. NCI-4552
1. Office of the Associate Scientific Director for Carcinogenesis, DCCP
2. Lung Cancer Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Studies on the Mucin of Chemically-Induced Adenocarcinomas of the Rat Duodenum and Colon

Previous Serial Number: None

Principal Investigator: Luigi De Luca, Ph.D., and Jerome Ward, D.V.M.

Other Investigators: None

Cooperating Unit: Carcinogen Screening Section, Experimental Pathology Branch, CG, DCCP

Man Years:

Total:	0.3
Professional:	0.3
Other:	0.0

Project Description

Objectives:

(1) To compare the normal rat intestine goblet cell glycoprotein, isolated and characterized in our laboratory with the product of chemically-induced adenocarcinomas of the same tissue; and (2) To gain understanding of reasons for faulty mechanisms of biosynthesis of well-defined products of cell differentiation in chemically induced tumors.

Methods Employed:

Rat intestinal mucosa fucose-glycoprotein has been isolated and purified. Its synthesis has been shown to depend on vitamin A. By using the technique of indirect immunofluourescence, the fucose-glycoprotein was localized in goblet cells. Chemically-induced adenocarcinomas of the small and large intestine were obtained by Dr. Ward. Periodic acid shiff's staining clearly indicated the well-differentiated nature of the tumors and the presence of mucus in mucous cells. Indirect immunofluorescence was used to study crossreactivity between the anti-serum to fucose-glycopeptide and the adenocarcinoma mucin.

Major Findings:

The duodenal adenocarcinoma showed a very faint cross-reaction with the anti-serum to goblet cell glycoprotein. The colon adenocarcinoma mucin did not react at all. Thus the normal goblet cell mucin and the cancerous product may be different.

Significance to Biomedical Research and the Program of the Institute:

The significance of this project to biomedical research and the program of the Institute is threefold. First, it will allow a chemical definition of the biological product of differentiation in intestinal adenocarcinomas by using techniques already well established for the normal goblet cell mucin. Second, it will further understanding of faulty processes in cancer cells, that will cause production of faulty macromolecules. Third, this approach, if generalized for other tumors with different products of differentiation, will probably allow a better definition of cancers of different types on the basis of their main differentiation product. Detection of these products in serum may also be an early means of diagnosis.

Proposed Course of Project:

To continue studies as outlined above.

Serial No. NCI-4553
1. Office of the Associate Scientific Director for Carcinogenesis, DCCP
2. Lung Cancer Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Effects of Vitamin A and Analogs on Cell Cultures of Epidermis

Previous Serial Number: None

Principal Investigators: Michael B. Sporn, M.D., and Nancy Dunlop, M.S.

Other Investigators: None

Cooperating Units: Stuart Yuspa, M.D., Experimental Pathology Branch, CG, DCCP

Man Years:
Total: 0.8
Professional: 0.3
Other: 0.5

Project Description

Objectives:

The aim of the project is to use epidermal cell cultures as a primary screen for evaluation of biological activity of new synthetic vitamin A analogs, as well as for further elucidation of the mechanism of action of vitamin A.

Methods Employed:

The new methods developed by Yuspa and collaborators for culture of pure populations of mouse epidermal cells are being used. These have been modified in the Lung Cancer Unit so that it is now possible to work in serum-free media.

Major Findings:

Quantitative measurements of the amount of RNA, DNA, and protein are being made on the cultures, as influenced by vitamin A and analogs. Although this project was only recently begun, it is already clear that vitamin A and analogs can cause rapid major increases in the amount of RNA in these cultured cells.

Significance to Biomedical Research and the Program of the Institute:

Since the eventual use of vitamin A analogs for cancer prevention in man is currently under consideration, it is of utmost importance to have the most efficient primary screen for assessment of activity. This system will provide such a screen.

Proposed Course of Project:

To continue as outlined above.

Serial No. NCI-4697
1. Office of the Associate Scientific Director for Carcinogenesis, DCCP
2. Lung Cancer Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Experimental Respiratory Carcinogenesis

Previous Serial Number: Same

Principal Investigator: David G. Kaufman, M.D.

Other Investigators: Umberto Saffiotti, M.D., Joseph M. Smith, Curtis C. Harris, M.D., Valerio Genta, M.D., and Michael B. Sporn, M.D.

Cooperating Units: Dr. Russell Madison, Microbiological Associates, Inc., and Dr. Arthur R. Sellakumar, The Eppley Institute for Research on Cancer.

Man Years:
Total: 0.9
Professional: 0.5
Other: 0.4

Project Description

Objectives:

The aim of this project is to elucidate causative factors and pathogenetic mechanisms of respiratory tract cancer and to define biological models for the experimental study of bronchogenic carcinoma.

Methods Employed:

Carcinogens are administered to Syrian hamsters by intratracheal instillations: (a) Benzo[a]pyrene(BP) as a particulate suspension attached to particles of ferric oxide (Fe_2O_3); and (b) Nitrosomethylurea (NMU) dissolved in buffered saline. In addition to control groups, experimental groups are designed to consider the following points: (a) tumor yield when BP is given at high doses in a short-time period; (b) dose-response studies using single doses of NMU or multiple doses of BP plus Fe_2O_3; (c) influence of vitamin A deficiency or vitamin A supplementation on tumor yield where NMU is the respiratory carcinogen; and (d) influence of states of altered susceptibility. Results are to be derived from analysis of the gross and microscopic lesions observed at death in experimental animals.

Major Findings:

Dose-response relationships for carcinogenic regimens involving multiple doses of BP plus Fe_2O_3 have been evaluated and reported. Efforts directed toward evaluation of this data have provoked a more detailed study of methods of data analysis for comparing experimental groups. Among experiments in progress a number of observations have been made. There is substantial excess mortality in hamsters treated with biweekly doses of 0.5 mg or 2.0 mg of retinyl acetate after, respectively, 50 or 25 weeks of treatment. Multiple intratracheal instillations of BP plus Fe_2O_3 followed by multiple doses of NMU results in a synergistic increase in tumor response when compared with the sum of tumors with BP plus Fe_2O_3 alone plus NMU alone. However, multiple doses of NMU followed by or alternating with multiple doses of BP results in nearly complete rapid mortality. Single or multiple intratracheal doses of NMU results in appreciable numbers of colonic tumors. In addition, hamsters treated with acute large doses of NMU followed by small chronic doses of chloroform get liver tumors, whereas neither NMU or $CHCl_3$ are appreciable liver carcinogens in hamsters.

Significance to Biomedical Research and the Program of the Institute:

These studies are part of the efforts aimed at defining factors leading to the development of bronchogenic carcinoma in animal systems under conditions that resemble those of human lung cancer development. Studies in progress have the following as objectives: (a) development of a single-dose carcinogenesis system, to permit improved methods for study of tumor promotion and cofactor relationship in respiratory carcinogenesis; (b) to attempt to produce an animal model of bronchogenic small cell anaplastic carcinoma; and (c) to attempt to demonstrate an anticarcinogenic effect of vitamin A in long-term carcinogenesis studies where NMU is the carcinogen. These studies coordinate with, or compliment studies in progress both intramurally and in the extramural contract program of the Lung Cancer Segment.

Proposed Course of Project:

It is proposed to continue long-term carcinogenesis studies in progress. In addition, new studies will be initiated to examine elements of co-carcinogenicity or altered susceptibility in experimental animals. These studies will coordinate with short-term *in vitro* experiments conducted in the Lung Cancer Unit and life-time carcinogenesis experiments in the contract program of the Lung Cancer Segment.

Publications

Saffiotti, U., Montesano, R., Sellakumar, A.R., Cefis, F., and Kaufman, D.G.: Respiratory tract carcinogenesis in hamster induced by different numbers of administrations of benzo[a]pyrene and ferric oxide. Cancer Res.32: 1073-1081, 1972.

Saffiotti, U., Montesano, R., Sellakumar, A.R., and Kaufman, D.G.: Respiratory tract carcinogenesis induced in hamsters by different dose levels of benzo[a]pyrene and ferric oxide. J. Natl. Cancer Inst. 49: 1199-1204, 1972.

Sellakumar, A.R., Montesano, R., Saffiotti, U., and Kaufman, D.G.: Hamster respiratory tract carcinogenesis induced by benzo[a]pyrene and different dose levels of ferric oxide. J. Natl. Cancer Inst. 50: 507-510, 1973.

Serial No. NCI-4763
1. Office of the Associate Scientific Director for Carcinogenesis, DCCP
2. Lung Cancer Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Studies on DNA Polymerases and Nuclear Exoribonucleases from Normal and Tumor Cells

Previous Serial Number: Same

Principal Investigators: Michael B. Sporn, M.D., Anthony Schrecker, Ph.D., DR&D, DCT, NCI, and Robert Gallo, M.D., LTCB, DCT, NCI

Other Investigator: Mary S. Baker, M.S.

Cooperating Units: None

Man Years:
Total: 0.2
Professional: 0.2
Other: 0.0

Project Description

Objectives:

To characterize the enzymes of the normal and tumor cell nucleus which are responsible for the in situ destruction of the newly synthesized, nascent messenger RNA of the nucleus. To characterize enzymes of DNA synthesis in normal and tumor cells. To define the role of these enzymes in the cell nucleus, particularly with respect to control of gene action.

Methods Employed:

Highly purified nuclei have been isolated from many cell types, including tracheal epithelial cells, HeLa cells, and Ehrlich ascites tumor cells. The exoribonuclease which degrades messenger RNA in these nuclei has been highly purified. Inhibitors have been tested by classical enzymological methods for their ability to irreversibly inactivate exoribonuclease, without inactivating enzymes of RNA synthesis. Inhibitors of exoribonuclease have also been assayed for inhibition of the RNA-dependent-DNA polymerase (reverse transcriptase) of oncogenic viruses and leukemia cells, as well as for inhibition of DNA polymerase from normal cells.

Major Findings:

Several new agents have been tested during the past year and have been found to be potent inhibitors of both nuclear exoribonuclease and reverse transcriptase. The reverse transcriptase assays have been performed by Dr. Anthony Schrecker and Dr. Robert Gallo. A tetranucleotide bearing an alkylating group has been synthesized for the first time under contract with Ash Stevens and been found to be a very potent inhibitor of reverse transcriptase.

Significance to Biomedical Research and the Program of the Institute:

The role of the gene action system is a central problem in all studies of carcinogenesis, whether chemical or viral. Research on the factors which control gene transcription and translation is among the most basic in all of cancer research. Since messenger RNA is the primary product of gene transcription, it is of utmost importance to know the mechanisms which control both its synthesis and degradation. The present project is yielding data on the enzymes and mechanism of messenger RNA destruction in both normal and cancer cells. The testing of potential inhibitors of reverse transcriptase is an area of high priority and relevance in other aspects of the NCI total program.

Proposed Course of Project:

To continue studies on the molecular mechanisms whereby messenger RNA is destroyed in normal cells and cancer cells. To evaluate the role of enzymes of messenger RNA destruction in controlling the growth and differentiation of both normal and cancer cells, and in particular to focus on the effect of carcinogens on the activity of these enzymes. To continue with inhibitor studies on exoribonuclease and reverse transcriptase.

Publications

Schrecker, A.W., Sporn, M.B., and Gallo, R.C.: Inhibition of RNA-dependent DNA polymerase by thymidylate derivatives. Cancer Res. 32: 1547-1553, 1972.

Serial No. NCI-4777
1. Office of the Associate Scientific Director for Carcinogenesis, DCCP
2. Lung Cancer Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Effects of Carcinogens on Methylation of Nuclear RNA

Previous Serial Number: Same

Principal Investigators: Adhid Al-Arif, Ph.D., Miriam Poirier, M.S., and Michael B. Sporn, M.D.

Other Investigators: None

Cooperating Units: None

Man Years

Total:	0.4
Professional:	0.2
Other:	0.2

Project Description

Objectives:

The nucleolus is known to methylate RNA on the 2'-oxygen of ribose. Since nucleolar lesions are known to be a prominent feature of cells treated with chemical or viral carcinogens, it is important to evaluate whether carcinogens affect 2'-O-methylation of RNA. Failure to methylate RNA would lead to excessive destruction of RNA within the nucleus, and failure to transport RNA to the cytoplasm.

Methods Employed:

Methylation of RNA is being studied in two different systems: (1) isolated hamster tracheal organ fragment, and (2) isolated rat liver nuclei. New chromatographic methods have been developed to separate methylated nucleosides from normal nucleosides. RNA on acrylamide gels can be quantitatively converted to nucleosides and the extent of methylation measured. A new system for assay of methylation of RNA in vitro by isolated nuclei has also been developed.

Major Findings:

A new paper chromatographic method for the separation of 2'-O-methylated ribonucleosides from non-methylated and base-methylated ribonucleosides, using paper impregnated with ammonium borate and a butanol-borate developing solvent has been perfected. In this procedure, the four commonly occurring sugar-methylated ribonucleosides, namely 2'-O-methyl adenosine, 2'-O-methyl cytidine, 2'-O-methyl guanosine, and 2'-O-methyl uridine are separated from each other. The method can be applied to either *in vivo* or *in vitro* studies of RNA metabolism, for the purpose of differentiating between sugar-methylation and base-methylation of RNA. Preliminary experiments with the livers from rats treated with aflatoxin B_1 suggest that this carcinogen affects the sugar-methylation of RNA.

Significance to Biomedical Research and the Program of the Institute:

The problem of stabilization of newly synthesized RNA is a fundamental problem in cell biology and carcinogenesis. The nucleolus is believed to be a key intracellular organelle for RNA stabilization, and 2'-O-methylation of RNA is believed to be a key molecular step to this process in the nucleolus. It is well known that both chemical and viral carcinogens affect nucleolar ultrastructure. The above studies are thus aimed at elucidating a potentially critical molecular lesion in carcinogenesis.

Proposed Course of Project:

The post-doctoral fellow who was the principal investigator on this project has completed a three-year Visiting Fellowship. It will be necessary to terminate this project.

Publications

Al-Arif, A., and Sporn, M.B.: An analytical method for the separation of sugar-methylated ribonucleosides from base-methylated and nonmethylated ribonucleosides. Anal. Biochem. 48: 483-490, 1972.

Al-Arif, A., and Sporn, M.B.: 2'-O-Methylation of Adenosine, Guanosine, Uridine, and Cytidine in RNA of Isolated Rat Liver Nuclei. Proc. Natl. Acad. Sci. U.S.A., 69: 1716-1719, 1972.

Serial No. NCI-4792
1. Office of the Associate Scientific Director for Carcinogenesis, DCCP
2. Lung Cancer Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Histogenesis of Squamous Cell Carcinoma of the Hamster Respiratory Tract Caused by Benzo[a]pyrene-Ferric Oxide

Previous Serial Number: Same

Principal Investigator: Curtis Harris, M.D.

Other Investigators: Joseph M. Smith, Frank E. Jackson, Maria Yamaguchi, Michael B. Sporn, M.D., David G. Kaufman, M.D., Umberto Saffiotti, M.D., and Hollis Boren, M.D.

Cooperating Units: Ultrastructure Unit, Experimental Branch, CG, NCI; Department of Medicine, University of South Florida, Tampa, Florida.

Man Years:
Total: 1.0
Professional: 0.4
Other: 0.6

Project Description

Objectives:

Previous investigations of the histogenesis of squamous cell carcinoma in man and animal models have shown that respiratory epithelium becomes hyperplastic, squamous metaplastic and finally neoplastic. These sequential changes in morphology may be better understood and defined by ultrastructural analysis.

Methods Employed

The method of Saffiotti and co-workers is used to administer intratracheal instillations of benzo[a]pyrene(BP) carried on ferric oxide particles in hamsters. This treatment produces squamous metaplasia and squamous cell carcinoma in the respiratory epithelium of the Syrian golden hamster. Following single or multiple intratracheal instillations of (1) BP-ferric oxide; (2) pyrene-ferric oxide; (3) ferric oxide; or (4) saline, hamsters are sacrificed serially up to the time of gross tumor development. Specific segments of the trachea and bronchi are processed for

high resolution light and electron microscopic study. Untreated and sham-treated animals are also examined.

Major Findings:

In last year's report, the morphogenesis of squamous metaplasia caused by BP-Fe_2O_3 was described in detail. Prior to squamous metaplasia, BP-Fe_2O_3 caused basal cell hyperplasia. Defects in the basement lamina, enlarged nuclei indented by cytoplasmic invaginations and pleomorphic nucleoli were found in squamous metaplasia induced by BP-Fe_2O_3. These atypical squamous metaplastic lesions can progress into squamous neoplasia. These findings have now been confirmed in second series of experiments.

Significance to Biomedical Research and the Program of the Institute:

The incidence of bronchogenic squamous cell carcinoma in man has shown a progressive increase during the past five decades. The pathogenesis of bronchogenic carcinoma is being studied in this animal model by several experimental approaches by members of the Lung Cancer Unit. This multifaceted approach allows meaningful correlation of morphological and biochemical changes occurring during carcinogenesis. Our intramural investigations are closely coordinated with the activities of the Lung Cancer Segment.

Proposed Course of Project:

A serial sacrifice study to determine the effect of hypervitaminosis A on the histogenesis of squamous cell carcinoma is in progress.

Publications

Harris, C., Kaufman, D., Sporn, M., and Saffiotti, U.: Histogenesis of squamous metaplasia and squamous cell carcinoma in an animal model. Cancer Chemother. Rep. (in press).

Serial No. NCI-4793

1. Office of the Associate Scientific Director for Carcinogenesis, DCCP
2. Lung Cancer Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: In Vitro Metabolic Studies in Isolated Hamster Respiratory Tract Tissues

Previous Serial Number: Same

Principal Investigators: David G. Kaufman, M.D., Valerio Genta, M.D., and Mary S. Baker, M.S.

Other Investigators: Curtis C. Harris, M.D., Joseph M. Smith, and Michael B. Sporn, M.D.

Cooperating Unit: C.W. Dingman, M.D., Nucleic Acids Section, Chemistry Branch, CG, NCI

Man Years:

Total:	0.8
Professional:	0.7
Other:	0.1

Project Description

Objectives:

The general aim of this project is to examine metabolic processes in a respiratory epithelium. The specific current objective of these studies is to determine whether a short-term in vitro assay can be established which will have a high rate of correlation with the results of long-term carcinogenesis studies. It is hoped that such an assay might indicate situations affecting the susceptibility of the hamster respiratory tract to tumor induction and provide experiments with more rapid turnover than life-time studies.

Methods Employed:

Techniques have been developed for maintaining isolated hamster tracheas in vitro as organ cultures for at least four hours. Short periods of maintainence were specifically chosen so that conditions in vitro reflect the in vivo state as well as possible. Previous studies have shown that radioisotopically labeled nucleic acid precursors added to the incubation medium could be recovered in well preserved high molecular weight RNA.

Major Findings:

In current studies, specific inhibitors of RNA metabolism have been employed to determine the nature of a previously reported alteration in the high molecular weight RNA (>45S) of tracheal epithelial cells in vitamin A-deficient hamsters. The alteration appears to be a relative decrease in the heterodisperse high molecular weight RNA in tracheas of vitamin A-deficient hamsters. Current studies have shown that tracheas incubated in vitro with ^{3}H-Benzo[a]pyrene (^{3}H-BP) have ^{3}H-BP bound to the purified DNA extracted from epithelial cells and banded in cesium chloride gradients. This binding is inhibited by incubation in the presence of 7,8-benzoflavone or by incubation at 0°. Prior intratracheal treatment of hamsters with BP plus ferric oxide in vivo results in increased in vitro binding of ^{3}H-BP to tracheal DNA. Binding is also increased in tracheas from vitamin A-deficient animals. In addition, there appears to be a particularly high level of binding in tracheas of the 15,16 inbred hamster strain as compared to other inbred strains. The question of whether these results are relevant to, or predictive of the results of long-term carcinogenesis studies, is being addressed by comparable long-term carcinogenesis studies in progress in the collaborative contract program and in the Lung Cancer Unit.

Significance to Biomedical Research and the Program of the Institute:

This project contributes to the part of the lung cancer program, designed to develop biologic models for the study of respiratory carcinogenesis, its causative factors and host control mechanisms. This assay system permits in vitro identification of critical biochemical and morphologic alterations which occur in the course of respiratory carcinogenesis induced in vivo. The methods developed permit examination of certain aspects of nuclear and cytoplasmic metabolism in viable respiratory epithelial cells. The effects of carcinogens administered in vitro can be examined biochemically. Furthermore, these methods will permit direct biochemical study of a respiratory epithelium during the course of in vivo carcinogenesis. This work coordinates with the in vivo carcinogenesis bioassays being conducted under contracts.

Proposed Course of the Project:

During the next year, it is proposed to continue to explore how a variety of in vivo co-carcinogenic or susceptibility factors influence the binding of ^{3}H-BP to tracheal epithelial cell DNA during in vitro incubation of isolated tracheas with medium containing ^{3}H-BP. These experiments will be designed to test the correlation of these in vitro studies with life-time carcinogenesis studies conducted in whole animals. In addition, it is proposed to explore the feasibility of detecting DNA repair in tracheal epithelial cells.

Publications

Kaufman, D.G., Baker, M.S., Harris, C.C., Smith, J.M., Boren, H., Sporn, M.B., and Saffiotti, U.: Coordinated biochemical and morphologic examination of hamster tracheal epithelium. J. Natl. Cancer Inst. 49: 783-792, 1972.

Kaufman, D.G., Baker, M.S., Smith, J.M., Henderson, W.R., Harris, C.C., Sporn, M.B., and Saffiotti, U: RNA metabolism in tracheal epithelium: alteration in hamsters deficient in vitamin A. Science 177: 1105-1108, 1972.

Serial No. NCI-4794
1. Office of the Associate Scientific Director for Carcinogenesis, DCCP
2. Lung Cancer Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Localization of Carcinogens and Anti-Carcinogens in Respiratory Epithelium by Autoradiography.

Previous Serial Number: Same

Principal Investigators: Curtis C. Harris, M.D., David G. Kaufman, M.D., and Hollis Boren, M.D.

Other Investigators: Mary S. Baker, M.S., Valerio Genta, M.D., Michael B. Sporn, M.D., Russell Madison, D.V.M., Frank E. Jackson, Maria Yamaguchi and Joseph M. Smith

Cooperating Units: Microbiological Associates, Bethesda, Maryland; Ultrastructure Unit, Experimental Pathology Branch, CG, NCI; and University of South Florida, Tampa, Florida.

Man Years:

Total:	1.2
Professional:	0.6
Other:	0.6

Project Description

Objectives:

The respiratory epithelium of the trachea is composed of a mixed cellular population. It is the aim of this new project to distinguish the metabolic activities of the various cell types and thus correlate biochemical and morphologic studies in the course of respiratory carcinogenesis. Changes in cell populations, as well as changes in metabolic activities following carcinogen administration, are examined concurrently.

Methods Employed:

Radioisotopes are incorporated into isolated hamster tracheas *in vitro* using methods developed in this laboratory. Following *in vitro* incubation with isotope, autoradiograms are prepared from semi-thin and ultra-thin sections for light and electron microscopy. Grain counts are then performed on the developed autoradiograms, which distinguish the different cell types and location of grains over either nuclei or cytoplasm. The

collaboration of Dr. Boren's group is involved in the experimental design, grain counting, statistical analysis and interpretation of results.

Major Findings:

The cellular populations of tracheal epithelium maintained in vitro in organ culture incorporate radioactive chemicals from the culture fluid at different rates. Basal cells incorporate lesser quantities of uridine-^{3}H than do ciliated or mucous cells. Ciliated cells rarely incorporate thymidine-^{3}H, whereas basal cells and mucous cells do incorporate it. Mucous cells incorporate greater quantities of leucine-^{3}H than do either ciliated or basal cells. Tracheas isolated from animals previously treated in vivo with benzo[a]pyrene(BP) plus Fe_2O_3 incorporated greater quantities of BP-^{3}H in vitro than tracheas from previously untreated animals. Parallel studies have shown a marked increase in the BP-^{3}H bound to DNA. In nuclei, electron microscopic autoradiography revealed that BP-^{3}H was preferentially localized in the heterochromatin. ^{3}H-retinoic acid has also been preferentially localized, within nuclei, in the heterochromatin. Tracheas from normal and vitamin A-deficient hamsters were incubated in vitro with either BP-^{3}H or ^{3}H-retinoic acid. An increase in binding of these compounds was found in areas of basal cells hyperplasia in the tracheas of vitamin A-deficient animals. The in vitro binding of BP-^{3}H to respiratory epithelial cells was inhibited by either incubation at 0° or the addition of 7,8-benzoflavone, an inhibitor of aryl hydrocarbon hydroxylase, to the incubation mixture.

Significance to Biomedical Research and the Program of the Institute:

This project contributes to the part of the lung cancer program designed to develop biologic models for the study of respiratory carcinogenesis, its causative factors and host control mechanisms. This assay system permits in vitro identification of critical biochemical and morphologic alterations which occur in the course of respiratory carcinogenesis induced in vivo. The methods which have been developed permit some correlation of biochemical observations with respect to constituent cellular populations of this target tissue. Such correlations should be of critical interest where biochemical changes reflect changes in cellular population, such as those known to occur during in vivo carcinogenesis.

Propose Course of the Project:

These methods will be utilized to study the metabolism and binding of labelled carcinogens and anti-carcinogens in the respiratory epithelium of man and experimental animals.

Serial No. NCI-4796
1. Office of the Associate Scientific Director for Carcinogenesis, DCCP
2. Lung Cancer Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Squamous Metaplasia of the Hamster Respiratory Epithelium Induced by Either Vitamin A Deficiency or Carcinogens

Previous Serial Number: Same

Principal Investigator: Curtis C. Harris, M.D.

Other Investigators: Michael B. Sporn, M.D., David G. Kaufman, M.D., Frank E. Jackson, Maria Yamaguchi, and Umberto Saffiotti, M.D.

Cooperating Units: Ultrastructure Unit, Experimental Pathology Branch, CG, NCI

Man Years:
Total: 0.9
Professional: 0.3
Other: 0.6

Project Description

Objectives:

Mucous and ciliated cells of the respiratory epithelium provide a defense against inhaled pollutants and carcinogens. This columnar epithelium can undergo squamous metaplasia, which may be a morphological stage in the histogenesis of squamous cell carcinoma. It is of interest to compare squamous metaplasia induced by carcinogens to squamous metaplasia found during vitamin A deficiency.

Methods Employed:

Hamsters were maintained on a vitamin A-deficient diet. Animals were examined during four stages of deficiency based upon their weight course and clinical signs. Pair-fed animals served as controls. A second group of animals received 10 intratracheal instillations of benzo[a]pyrene(BP)-ferric oxide. A third group of animals received 10 intratracheal instillations of N-methyl-N-nitrosourea (NMU). Specific tracheal and bronchial rings were processed for high resolution light and electron microscopic study.

Major Findings:

The sequential changes in the trachea during vitamin A deficiency appear to be (1) the ciliated and mucous cells flatten; (2) basal cells increase in number; (3) a widened intercellular space develops between the hyperplastic layer of basal cells and the luminal cells are sloughed; (4) basal cells differentiate into flat layers of squamous cells. Intratracheal instillations of either BP-ferric oxide or NMU cause many histological changes including squamous metaplasia with atypical squamous cells. These carcinogens also cause a regular squamous metaplasia which could not be distinguished from squamous metaplasia induced by Vitamin A at the light microscopic level, whereas abnormal nucleoli and defects in the basement membrane were apparent at the ultrastructural level. Defects in the basement membrane have been observed in preneoplastic and neoplastic lesions of the skin, mammary gland and larynx.

Significance to Biomedical Research and the Program of the Institute:

Vitamin A has a pivotal role in the control of epithelial differentiation in the respiratory epithelium. The differentiation of basal cells into mucous and ciliated cells is dependent on adequate levels of vitamin A. Vitamin A deficiency results in an alternate path of differentiation, i.e., squamous metaplasia, which also appears to be a preneoplastic stage in the histogenesis of squamous cell carcinoma of the bronchi. Hypervitaminosis A has been found to decrease the incidence of squamous metaplasia and squamous carcinoma in this animal model. Squamous metaplasia induced by carcinogens can be distinguished at the ultrastructural level from squamous metaplasia observed during vitamin A deficiency. This study is designed to contribute to the elucidation of the control mechanisms involved in epithelial differentiation and carcinogenesis.

Proposed Course of Project:

The initial objectives of this project have been completed. Other benign causes of squamous metaplasia will be compared to that caused by carcinogens.

Serial No. NCI-4797
1. Office of the Associate Scientific Director for Carcinogenesis, DCCP
2. Lung Cancer Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Acute and Chronic Effects of Carcinogenic N-Nitroso Compounds in the Respiratory Epithelium

Previous Serial Number: Same

Principal Investigator: Curtis C. Harris, M.D.

Other Investigators: Michael B. Sporn, M.D., David G. Kaufman, M.D., Joseph M. Smith, Frank E. Jackson, Maria Yamaguchi, and Umberto Saffiotti, M.D.

Cooperating Units: Ultrastructure Unit, Experimental Pathology Branch, CG, NCI

Man Years:
Total: 0.9
Professional: 0.3
Other: 0.6

Project Description

Objectives:

N-nitroso compounds, such as diethylnitrosamine (DEN), dimethylnitrosamine (DMN) and N-methyl-N-nitrosourea (NMU) are respiratory carcinogens. NMU is a proximate carcinogen, whereas DMN and DEN require metabolism into active form(s). Intratracheal instillations of NMU induces squamous cell carcinoma of the lung as shown by Herrold. The objectives of this project are (1) to study the histogenesis of squamous cell carcinoma caused by multiple instillations of NMU and compare these changes with those from a similar histogenesis study using a polynuclear hydrocarbon, benzo-[a]pyrene; and (2) relate the ultrastructural lesions induced by these compounds to squamous metaplasia found during vitamin A deficiency.

Methods Employed:

NMU was given by intratracheal instillation. One hour prior to sacrifice, the animals were injected with ^{3}H-thymidine. At selected times, animals were sacrificed and tracheal rings are processed for light and electron microscopic examination. The incorporation of ^{3}H-thymidine was measured by light microscopic autoradiography.

Major Findings:

Ten intratracheal instillations of NMU caused squamous metaplastic and neoplastic changes in the tracheobronchial epithelium. Abnormal squamous metaplastic cells contained enlarged nuclei deeply indented by cytoplasmic invaginations, pleomorphic nucleoli, filamentous granules and many cytoplasmic fibrils. Prior to the appearance of tumors, autoradiograms revealed cells preparing for division, first in basal and then in all layers of the abnormal squamous metaplastic epithelium. Defects in the basement membrane were found in squamous metaplastic lesions. The ultrastructural changes in the tracheobronchial epithelium were similar to those described in hamsters exposed to benzo[a]pyrene-ferric oxide as well as to those described in smoking dogs and in human bronchogenic carcinoma. The squamous metaplastic changes induced by NMU were clearly distinguishable from squamous metaplasia found in vitamin A deficiency.

Significance to Biomedical Research and the Program of the Institute:

This project contributes to the part of the lung cancer program designed to develop biologic models for the study of respiratory carcinogenesis, its causative factors and host control mechanisms. N-nitroso compounds are a group of systemic carcinogens, which have marked tissue specificity. Their role in respiratory carcinogenesis is being actively investigated in several projects of the lung cancer program. The morphological and biochemical effects of these agents directly on respiratory epithelia are being compared to those of the carcinogenic polynuclear hydrocarbons.

Proposed Course of Project:

This study has been completed.

Publications

Harris, C., Kaufman, D., Sporn, M., Smith, J., Jackson, F., Saffiotti, U.: Ultrastructural effects of N-Methyl-N-Nitrosourea on the tracheobronchial epithelium of the Syrian golden hamster. Int. J. Cancer (in press).

Serial No. NCI-4798
1. Office of the Associate Scientific Director for Carcinogenesis, DCCP
2. Lung Cancer Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Control of Epithelial Cell Differentiation and Carcinogenesis

Previous Serial Number: Same

Principal Investigators: Luigi De Luca, Ph.D., Robert Barr, Ph.D., Nancy E. Maestri, and Carol Silverman

Other Investigators: George Wolf, Ph.D., Gloria Rosso

Cooperating Unit: Massachusetts Institute of Technology

Man Years:

Total:	2.7
Professional:	1.7
Other:	1.0

Project Description

Objectives:

To study control mechanisms of mucous-squamous cell differentiation by vitamin A and chemical carcinogens in the respiratory tract and in other epithelial tissues.

Methods Employed;

Whole-cell and cell-free systems from epithelial tissues are prepared from vitamin A-normal and -deficient hamsters and rats. Free and membrane bound polyribosomes are prepared by a discontinuous sucrose density gradient. The membrane bound polysome fraction is used to study synthesis of vitamin A-glycolipids and glycoproteins. The glycolipids are used as donors of the sugar moiety in the synthesis of membrane and secreted glycoproteins.

Major Findings:

In vivo, and *in vitro* experiments demonstrated that epithelial cells from rodents' tracheas and intestinal mucosa and from liver are able to synthesize a doubly-labeled mannolipid from ^{3}H-retinol and ^{14}C-GDP-mannose. The purified compound contained retinol and mannose in the

molar ratio of 1:2, 1:1. The biosynthesis of the mannolipid depended on ATP, Mn++ and retinol, when the enzyme was prepared from vitamin A-deficient animals. When normal animals were the source of the enzyme, retinol was no longer required; the requirement for ATP was decreased, presumably because of endogenous retinyl-phosphate. Hydrolysis data indicated a retinyl-phosphate-mannose compound. Absence of a pyrophosphate type of linkage was demonstrated by synthesizing the mannolipid in the presence of β-^{32}P-GDP-^{14}C-mannose, without incorporation of ^{32}P. A triple-labeled mannolipid was obtained when ^{3}H-retinol, GDP-^{14}C-mannose and ^{32}P-ATP were used. Hydrogenolysis for 25 hours liberated 100% of the ^{3}H activity as the hydrocarbon but only 40% of the ^{14}C-mannose. This, with biphasic hydrolysis and silicic acid chromatography, demonstrated the presence of at least two types of mannolipid, one containing retinol and the other containing dolichol or another isoprenol. UDP-glucose-^{14}C, UDP-xylose-^{14}C, UDP-N-acetyl-glucosamine-^{14}C and UDP-glucuronic-^{14}C acid were incubated separately with ^{3}H-retinol. Only glucose and glucuronic acid formed doubly-labeled glycolipids.

Significance to Biomedical Research and the Program of the Institute:

This project contributes to the identification of key control mechanisms in epithelial cell differentiation; it is a part of the lung cancer program addressed to the development of mechanisms of inhibition and control of respiratory carcinogenesis. The control of mucus cell differentiation by vitamin A is also of great interest in helping to understand the mechanism of carcinogenesis in epithelial tissues in general. The vitamin controls goblet cell formation and its absence leads to hyperplasia of basal cells, squamous metaplasia and keratinization: a sequence of events similar to those taking place in the respiratory epithelium after exposure to benzopyrene. The fucose-glycopeptide, shown to be present in the mucous structures of most epithelia can be used as a specific marker for differentiation responses in epithelial tissues.

Proposed Course of Project:

Honors and Awards

Member, American Institute of Biological Chemistry

Chairman, Session on "Vitamins A and K," IXth International Congress of Nutrition, Mexico City, September 2-9, 1972

Visiting Lecturer, Department of Nutrition and Food Science, Massachusetts Institute of Technology, Cambridge, Massachusetts

Invited Lecturer, "First International Chalone Conference, Brook Lodge, Augusta, Michigan, June 5-7, 1972

Invited Lecturer, "Glycoprotein Group", Harvard Medical School, Cambridge, Massachusetts, June 21, 1972

Invited Lecturer, Mead Johnson Company, Evansville, Indiana, October 6, 1972

Invited Lecturer, Conference on "Lung as an Organ of Defense", sponsored by the National Cystic Fibrosis Research Foundation, Durham, North Carolina, November 2-4, 1972

Invited Lecturer, Main Speaker, "Glycosamino-glycan glycoprotein meeting", American National Red Cross, Blood Research Laboratory, Bethesda, Maryland, November 28, 1972

Invited Lecturer, Duke Medical Center, Durham, North Carolina, December 5, 1972

Publications

De Luca, L., and Wolf, G.: Mechanism of action of vitamin A in differentiation of mucus-secreting epithelia. J. Agric. Food Chem. 20: 474-477, 1972

De Luca, L., Maestri, N., Rosso, G., and Wolf, G.: Retinol glycolipids. J. Biol. Chem. 248: 641-648, 1973

De Luca, L., Maestri, N., Bonanni, F., and Nelson, D.: Maintenance of epithelial cell differentiation: The mode of action of vitamin A. Cancer 30: 1326-1331, 1972

De Luca, L.: Commentary on article by Mark, F., entitled: A tissue specific factor inhibiting DNA synthesis in mouse epidermis. In Forscher, B. K. and Houck, J. C. (Eds.): Chalones: Concepts and Current Research. National Cancer Institute Monograph No. 38 (In Press).

Bonanni, F., Levinson, S. S., Wolf, G., and De Luca, L.: Glycoproteins from the hamster respiratory tract and their response to vitamin A. Biochim. Biophys. Acta 297: 441-451, 1973

Serial No. NCI-4539

1. Office of the Associate Scientific Director for Carcinogenesis, DCCP
2. Program and Data Analysis Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 2, 1972 through June 30, 1973

Project Title: Information Dissemination for the Carcinogenesis Program

Previous Serial Number: Same

Principal Investigator: Sidney Siegel, Ph.D.

Other Investigators: Norbert P. Page, D.V.M.; T.D.C. Kuch; Elizabeth Weisburger, Ph.D.; Umberto Saffiotti, M.D.

Cooperating Units: Hilary Burton, National Agricultural Library (Data Systems Application Division)

Man Years:

Total:	2.0
Professional:	1.5
Other:	0.5

Project Description

Objectives:

To develop, implement and maintain systems which gather, catalogue and disseminate information relevant to the Carcinogenesis Program.

In so doing, create and/or search data bases which will enable scientists within the broad spectrum of disciplines comprising carcinogenesis research to be made aware of information relevant to their own efforts.

Methods Employed:

While much of the information gathering and dissemination is performed by Unit personnel, the needs exceed in-house capability, and contractors are employed to perform specific tasks. The main contract activities are in producing Carcinogenesis Abstracts and PHS #149, "Survey of Compounds Which Have Been Tested for Carcinogenic Activity."

In order to function as a focal point for information and to perform searches relevant to the Program, data bases needed to respond to these requests are maintained and/or supported. Such data stores include information on chemicals such as general toxicologic phenomena, carcinogenicity, mutagenicity, metabolism, and also the production, distribution and exposure of materials to segments of the human population. Interest profiles have been developed to automatically examine chemical data from commercially available data bases. This selective information is then disseminated to program scientists as per their individual needs and interests. On occasion, the biomedical literature has been critically analyzed for specific topics. It often becomes necessary to help define the problem, determine the available literature, screen, extract and evaluate the information to whatever depths requested.

Major Findings:

Literature extraction activity required of the contractor for Carcinogenesis Abstracts and PHS #149 indicates that there has been a significant and stable increase of information relevant to carcinogenesis research.

Significance to Biomedical Research and the Program of the Institute:

Development, implementation and monitoring of research programs requires a continual flow of information, which must be grouped or indexed in a manner that the data can be searched or manipulated in a logical and useful manner. Once data bases are established, criteria concerning the reliability of the data can be used as a qualification filter for inclusion or as a method for annotating what is retained. Information projects which are now on-going and those ready for implementation are designed to meet scientific and administrative needs to the Carcinogenesis Program.

Proposed Course of the Project:

The project will continue its formal information publications as in the past. Informal surveys indicate that there is a high level of demand by the scientific community for their continuation.

The project will continue to be sensitive to the general and specific information needs of the Carinogenesis Program. Information systems to be generated will be so structured that they will be able to respond to the ever-changing profile of research. The systems will be designed to store new and different data with more conventionally formatted data of the past while still having the ability to search and retrieve both kinds of information.

Honors and Awards

Member, Information and Resources Segment Core Working Group

Session Coordinator at the International Cancer Research Data Bank Planning Conference at Airlie House, Warrenton, Virginia, May 22-24, 1972

Member, Review Panel to Evaluate Proposals for the National Cancer Program Cancer Information System (NCP-CIS), February 1973

Serial No. NCI-4541
1. Office of the Associate Scientific Director for Carcinogenesis , DCCP
2. Program and Data Analysis Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Animal Resources

Previous Serial Number: NCI-4799

Principal Investigators: Thomas P. Cameron, D.V.M.

Other Investigator: Norbert P. Page, D.V.M.

Cooperating Units: Samuel Poiley, Mammalian Genetics and Animal Production Section, DCT; Carl Hansen, Ph.D., Genetics Unit, Rodent and Rabbit Production Section, VRB, DRS

Man Years:
Total: 1.5
Professional: 1.2
Other: 0.3

Project Description

Objectives:

(1) Plan and effect procurement of animals used in the Carcinogenesis intramural and contract programs; (2) Assure compliance with the Animal Welfare Laws and the HEW Guide for Care and Use of Laboratory Animals (3) Plan and support the development of animal models for cancer research as well as to collect and provide information on laboratory animals.

Methods Employed:

Animal requirements for the Carcinogenesis Program are determined as far in advance as possible. These are then presented to the Mammalian Genetics and Animal Production Section for procurement from NIH or contract animal suppliers. Carcinogenesis provides financial support for this operation by contract with the suppliers. In addition, VRB has supplied the breeding stock for the rodent colonies at Frederick Cancer

Research Center (FCRC). Eventually these colonies will supply all FCRC's needs and perhaps other program requirements. VRB is also supplying production animals to a contract operation for a specific evaluation.

An NCI ad hoc group on animal welfare has been established to assure compliance with the Animal Welfare Laws (PL-89-544 and PL-91-579) and HEW Guide for Care and Use of Laboratory Animals (DHEW Publication No. 73-23). At the recommendation of the Group, the contracting office has modified contract provisions to obtain written assurances of compliances with the Animal Welfare Laws and DHEW standards. Discussions are held with the Contracting Office and legal counsel as necessary regarding the legal requirements. Visits are made to current or proposed contractors as well as to intramural facilities to determine adequacy of animal care. Consultation service is provided as requested. Surveys of intramural facilities are made by the NCI ad hoc group and DRS to determine measures needed to achieve maximum efficiency and quality of animal care. The group meets periodically to consider animal welfare problems.

Characteristics and availability of animals are obtained to provide better information to Carcinogenesis' scientists for selection of appropriate animal models. Support and guidance for the laboratory adaptation and breeding of new species or strains which have potential usefulness as animal models for cancer research is an important aspect of this project. Discussions are held with the DRS Veterinary Resources Branch, and the National Academy of Science/National Research Council, Institute of Laboratory Animal Resources, as well as other scientists as needed.

Major Findings:

An expansion of the bioassay program resulted in a significant increase in animal requirements. Early in the year the animal suppliers under contract with DCT were unable to expand their operations rapidly enough to meet the increased demand. The situation, at this time, is improving although some difficulties are still being encountered.

Several meetings of the NCI ad hoc Group on Animal Welfare have been held to implement the provisions of PL-91-579 in the contract and intramural programs. In so doing, the Group has worked closely with DRG and DRS. A survey conducted by the DRS revealed a number of undesirable situations regarding animal care. Specific recommendations to correct the situation have been formulated and presented to the Director, NCI.

An important aspect of this project is to develop and provide new and better animal species as models for cancer research. Animals are selected for the Carcinogenesis Program on the basis of certain

desirable characteristics such as availability, ease of handling, size, etc. Often, however, information on normal survival, spontaneous incidence of tumors and insidious medical problems is not readily available from the animal supplier. Studies are being pursued to obtain more information on animals and disseminate this to Carcinogenesis scientists. An example of an animal model being developed under contract at the Medizinische Hochschule in Hannover, Germany, is the wild European hamster (Cricetus cricetus) which develops bronchogenic squamous cell carcinoma similar to that seen in man. Considerable success has been obtained in the capture, adaptation and breeding of this species in the laboratory.

Significance to Biomedical Research and the Program of the Institute:

The quality of animal research depends greatly upon the animal model selected and their health and care in the laboratory. Adequate disease control, diet, and freedom from stressful conditions are especially important for carcinogenesis research in which animals are maintained for their lifespan. Animals dying from non-experimental influences midway or later in a carcinogenesis experiment represent a significant loss in time and money.

Although the chairman and one member of the NCI ad hoc Group on Animal Welfare are in the Carcinogenesis Program, the Group functions for all programs within NCI and has served as an example for the other institutes in fulfilling their obligation to the Animal Welfare Laws. The information on species now available as well as the development of new models will be made available to all programs of NCI as well as to the scientific community at large.

Proposed Course of the Project:

Alternative methods to the current system for meeting the program needs for animals will be explored. It would appear that within this reporting period, FCRC may be able to produce sufficient animals for both FCRC and the NCI intramural programs. It is possible that some animals will be available for other NCI contractors. The adequacy of animal care and adherence to NIH and legal standards by both contractor and intramural programs will continue to be assessed during the next year and assistance will be given where needed. It is expected that considerably more attention will be given to the collection and dissemination of information on laboratory animals. Cooperation and advice will be given to the Institute of Laboratory Animal Resources (NAS) in establishing a computerized information storage and retrieval system for species and strain and stock information.

Honors and Awards

Member, NCI ad hoc Group on Animal Welfare

Chairman, Program Committee, Annual Seminar National Chapter Area Branch, American Association for Laboratory Animal Science

Publications

Mohr, U., Althoff, J., and Page, N.: Tumors of the respiratory system induced in the common European hamster by N-diethylnitrosamine. J. Nat. Cancer Inst. 49: 595-597, 1972.

Cooney, D. A., Homan, E. R., Cameron, T. P., and Schaeppi, U.: Measurement of 4-(^{14}C)-L-asparagine in body fluids and tissue: Methodology and Application. J. Lab. Clin. Med. (In Press).

Serial No. NCI-4546

1. Office of the Associate Scientific Director for Carcinogenesis, DCCP
2. Program and Data Analysis Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Radiation Carcinogenesis Program

Previous Serial Number: Same

Principal Investigator: Norbert P. Page, D.V.M.

Other Investigators: Douglas H. Janss, Ph.D., Experimental Pathology Branch, Carcinogenesis; D. Jane Taylor, Ph.D., Head, Experimental Biology Project Section, DCBD

Cooperating Units: Atomic Energy Commission; Bureau of Radiological Health, FDA, HEW; Environmental Protection Agency

Man Years:

Total:	.50
Professional:	.40
Other:	.10

Project Description

Objectives:

To develop a program directed toward the understanding of interactions and potential synergism of radiation with chemical agents.

Methods Employed:

The status of research in radiation carcinogenesis has been determined by a review of literature and research project reports of various laboratories and agencies that support radiation carcinogenesis research.

It is the intent of this program to study the interactions of radiation with other environmental agents. A considerable effort is made to avoid duplication of research underway by other agencies.

The Principal Investigator remains informed of research support by other agencies by participating in reviews or scientific evaluations of on-going or proposed research programs. As limited facilities are available to expand the intramural research program, this area is being developed mainly through the collaborative contract program.

Major Findings:

Summaries of on-going radiation carcinogenesis research have been obtained through the Science Information Exchange (SIE) of the Smithsonian Institute. Contacts have been made with other government agencies to receive annual progress reports as well as other information pertaining to radiation carcinogenesis research. A close liaison has been established with the Atomic Energy Commission in this regard.

While much research has been conducted on the hazards of radiation, the mechanisms by which radiation exposure can result in cancer and of the interactions of radiation with other agents remains largely unknown. The results from these studies indicate that radiation acts synergistically with chemicals in inducing breast and lung cancer in animals. From the uranium-miner studies a similar relationship appears to exist in man.

Significance to Biomedical Research in the Program of the Institute:

The National Cancer Institute is concerned with carcinogenesis regardless of the etiological agent. Within the past few years the Institute has not supported a large program in radiation carcinogenesis mainly due to the commitment of other agencies in this regard. The mechanisms by which environmental agents induce cancer may be similar for all etiological agents. Radiation represents a research tool which is easy to use compared with chemical or biological agents. Understanding radiation carcinogenesis may be important in developing an understanding of carcinogenesis induced by other etiological agents. From the hazards viewpoint, radionuclides have been demonstrated to be a potent class of carcinogens and represents a risk to mankind that must be adequately evaluated. Preliminary results of current programs of the Carcinogenesis Program would indicate that in certain cases radiation and chemical agents can act synergistically to induce cancer. The expertise available in the National Cancer Institute's intramural and contract programs can provide important direction in the assessment of radiation hazard. With the introduction of new materials, for example, the neutron-emitter Californium-252 as an isotope useful in bio-medical research and industrial applications, additional research is required to assess their potential hazards.

Proposed Course of the Project:

It is expected that the liaison activities and the exchange of information with other agencies can be further expanded during the coming year. It is not anticipated that there will be a great expansion in this program until the results of the current contracts indicate that such is desirable.

Honors and Awards

Director, Bioassay Operations Segment, Carcinogenesis, NCI

Chairman, NCI ad hoc Group on Animal Welfare

Member, Division of Cancer Cause and Prevention Interprogram Group, NCI

Member, Civil Defense Committee on Vulnerability of Food Crops and Livestock Production to Fallout Radiation

Member, Carcinogenesis Contract Program Management Group

Member, Collaborative Group on Environmental Carcinogenesis

Member, Information and Resources Advisory Group, Carcinogenesis, NCI

Member, ad hoc Review Group on Concepts and Techniques in Pathology Applicable to Assessment of Long-Term, Low-Dosage Toxicity Studies in Animals for National Center for Toxicological Research, Federation of American Societies for Experimental Biology

Serial No. NCI-4699
1. Office of the Associate Scientific Director for Carcinogenesis, DCCP
2. Program and Data Analysis Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Carcinogenesis Bioassay Data System

Previous Serial Number: Same

Principal Investigators: John A. Cooper II, Ph.D.; T.D.C. Kuch
Norbert P. Page, D.V.M.

Other Investigators: Sidney Siegel, Ph.D, Ursula Evans

Cooperating Units: Data Management Branch, Division of Computer Research and Technology, NIH; Wolf Research and Development Corporation

Man Years: (NCI)

Total:	3.2
Professional:	2.8
Other:	.4

Project Description

Objectives:

To develop, implement, and maintain a comprehensive information system for collection, storage, manipulation, retrieval, and analysis of data pertaining to carcinogenesis bioassay studies. In doing so, to create and maintain a file of data about (a) substances studied as part of the Chemical Carcinogenesis Program, (b) experiments and related experimental design in bioassay studies, (c) diagnoses of animal deaths from suspected carcinogenic agents on trial, and corresponding control groups.

Methods Employed:

Standard taxonomic, system-operational, systems design, computer programming, and statistical computing techniques, as well as the NIH/DCRT hardware and software environment have been used in the development of the system.

A chemical data subsystem has been merged into the CBDS. This contains these types of data on substances under investigation:

Chemical Abstracts Service (CAS) name
Synonyms (common and trade names)
CAS number
NCI chemical number
Chemotherapy (NCI) chemical number
Wiswesser Line Notation
Molecular formula
Molecular weight
Category and subcategory of use (e.g., pesticide:rodenticide)
Information on bioassay contractors using this substance

Organization of the CBDS: The Carcinogenesis Bioassay Data System comprises these subsystems:

(1) Bioassay Contractor Subsystem - The activities of contractor pathologists and clerical staff in documenting the design and results of experiments in testing possible carcinogenic agents in animal systems, communicating these designs and results in standard form to the NCI through the Operations Subsystem.

(2) Operations Subsystem - The activities of data transcription and systems operation personnel in obtaining completed forms from bioassay contractors; checking them for accuracy and completeness; logging their progress through the system; microfilming them; keypunching and key-verifying input documents corresponding to data forms; submission of computer jobs representing new or replacement data for entry into the CBDS data base; and related functions.

(3) Computer Subsystem, Phase I - The activities of computer systems designers and programmers in designing, implementing, and maintaining the CBDS data base and the CBDS system of computer programs to manage that data base; and generating preliminary management and scientific output reports.

(4) Computer Subsystem, Phase II - The activities of computer programmers and computational statisticians in analyzing chronic toxicity data of CBDS test animals, using standard statistical methods.

Major Findings:

The implementation stage of the Computer Subsystem (Phase I) has been successfully completed. Phase I maintenance has begun, and will continue throughout the life of the project, as continual adjustments must be made to ensure that bioassay contractor data remains fully

compatible with data already in the system, and to modify the data system such that new studies, which may have been initiated with divergent experimental designs, can furnish data to the CBDS. The data base, stored on magnetic media, has now grown to approximately 12,000 records (est. 6/73). This includes 2,100 animal groups, 3,300 individual necropsied animals, and 6,600 other records, such as observations and dosages. While some experiments are nearing completion, allowing data from them to be fully analyzed in the Computer Subsystem (Phase II), others are in their early stages. Phase I output, in the form of summary reports and simple graphs, have been produced during the past year.

Significance to Biomedical Research and the Program of the Institute:

Because of the size, diversity, and complexity of the Carcinogenesis Bioassay Program, it is essential that a method be available to manage and make optimal use of the information generated by its various activities. The CBDS will provide this and will in addition contribute to uniformity of reporting results of bioassay studies and to standardization of chemical and tumor nomenclature. It will also provide for routine rapid evaluation of bioassay studies, including comparison of results from multiple sources, and for ready access to summary data for statistical analysis.

Proposed Course of Project:

New bioassay studies will be entered into the system as they are initiated. During FY74, these activities will include, by subsystem:

(1) Bioassay Contractor Subsystem - Continue to integrate new and existing contractors into the CBDS.

(2) Operations Subsystem - Continued operational responsibilities for data conversion, data logging, inputting of data into the Computer Subsystems, and microfilming of documents for the source document file. The contractor for this subsystem has proved extremely responsive and has functioned in an acceptable manner when given limited opportunity to interact directly with the bioassay contractor personnel (including pathologists). It is anticipated that he will be given greater responsibilities in this area.

(3) Computer Subsystem, Phase I - System development has now ended, and continued modification and maintenance of this Subsystem will be continued in the future, using personnel from the DMB, DCRT.

(4) Computer Subsystem, Phase II - Preliminary development of statistical reports has been done, and the more obvious statistical analyses are being made on animal groups as experiments on them are being completed. During FY74 more sophisticated statistical work will be planned and executed, in collaboration with the DMB, DCRT.

Honors and Awards

Director, Information and Resources Segment

Participant, Working Group on the Evaluation of Carcinogenic Risk of Chemicals to Man, International Agency for Research on Cancer, World Health Organization

Member, Cause and Prevention Executive Staff, Division of Cancer Cause and Prevention, National Cancer Institute

Member, Carcinogenesis Contract Program Management Group, Division of Cancer Cause and Prevention, National Cancer Institute

Member, Carcinogenesis Intramural Program Group, Division of Cancer Cause and Prevention, National Cancer Institute

Member, Division of Cancer Cause and Prevention Inter-Program Group, National Cancer Institute

Serial No. NCI-4548
1. Office of the Associate Scientific Director for Carcinogenesis, DCCP
2. Registry of Experimental Cancers
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Registry of Experimental Cancers

Previous Serial Number: None

Principal Investigators: Dr. Harold L. Stewart, Consultant
Dr. Thelma B. Dunn, Consultant
Dr. Margaret D. Barrett (Dr. Margaret K. Deringer)

Other Investigators: None

Cooperating Units: None

Man Years:

Total	4.0
Professional	2.0
Other	2.0

Project Description

Objectives:

(1) The storage and retrieval of pathological material and data on cancers and other lesions of laboratory animals (primarily rodents), and (2) the use of such for research and educational purposes.

Methods Employed:

The methods employed in the work of the Registry involve the selection of protocols, pathologic material, including histologic slides, paraffin blocks, and gross specimens; and illustrations in the form of lantern slides, gross photographs and photomicrographs in black and white and in color. The work of the Registry also includes the collection of records of experiments, reprints and references on this material.

The Registry of Experimental Cancers possesses a large collection of spontaneous and induced cancers and other lesions. The pertinent information on the collection is being indexed. Many of the data have been prepared for and entered into the computer. Printouts have been supplied, upon request, to the International Agency for Research on Cancer in Lyon, France and to the Institute for Experimental Gerontology in Rijswijk, Holland.

The Registry accesses material from investigators at NCI, other Institutes of NIH, other Governmental Agencies, industrial laboratories, and universities here and abroad. A total of 126 single or group accessions from investigators outside of NCI have been processed since March 1971.

The Registry is preparing Study Sets of slides, with explanatory material, relating to particular areas of the pathology of rodents. These sets will be loaned to interested investigators in this country and abroad.

The Registry has 24 Study Sets of slides on "Hematopoietic and Lymphoreticular Neoplasms" (prepared for a workshop on the comparative pathology of these neoplasms at NCI in May 1971) which are loaned, with descriptive material, to investigators who request them. Thirty loans for periods up to two months have been made.

Three investigators have spent varying periods of time at the Registry studying some of the available material.

Investigators come to the Registry for consultation. There have been 46 such consultations since July 1972.

Major Findings:

The functions (outlined in Objectives) of the Registry in the wider field of cancer research and more particularly in the Carcinogenesis program are such that there are no major findings to report.

Significance to Biomedical Research and the Program of the Institute:

The availability of the wealth of material possessed by the Registry advances the knowledge of spontaneous and induced disease processes in animals.

The existence of the Registry will contribute to the standardization of nomenclature of cancers and other lesions in laboratory rodents.

Proposed Course of Project:

The Registry will continue and expand all of its activities (already set forth in this report).

The Registry has received a request for formal union with the Committee on Pathology Standardization of the European Late Effects Project Group (Secretariat, Radiobiology Department, C.E.N., MOL, Belgium). The possibility of establishing such a union is being explored.

SUMMARY REPORT

BIOLOGY BRANCH

July 1, 1972 through June 30, 1973

This year the Etiology Area became the Division of Cancer Cause and Prevention. While there is probably general agreement about what the term "Cancer Cause" means, it is not so clear what is meant by "Cancer Prevention". In a strict sense, the only way to prevent cancer is to eliminate the chance of contact between the host and environmental carcinogens such as chemicals, viruses and physical agents. All other methods that have been proposed to reduce the incidence, morbidity and mortality of human cancer require treatment of the host. Once contact with a carcinogen has been made a positive action (treatment) must be inflicted on the host to interfere with penetration, transformation of normal to malignant cells, multiplication of malignant cells to produce a palpable mass and metastatic spread. The activities of the Biology Branch, therefore, are directed toward achieving the following goals of the Carcinogenesis Program:

1. Identification of population groups exposed to known carcinogens in their environment.

2. Identification and selection of chemicals for bioassay of their carcinogenic potential.

3. Development of measures to inhibit or modify tumor growth.

4. Application of corrective measures in human subjects.

The Molecular Separations Unit provided the nucleus of a new section named Tumor Antigen Section. The Biology Branch, therefore, now consists of the following sections: Cytogenetics and Cytology, Cellular Immunity, Immunochemistry and Tumor Antigen.

The incidence of several forms of cancer has been reduced by identifying environmental carcinogens and removing them from the environment. This is why a large portion of the Biology Branch and the Biology and Immunology Segment is devoted to finding fast and reproducible assays to detect the potential carcinogenic activity of environmental agents that may be responsible for major forms of human cancer. The task of developing and standardizing a fast bioassay for carcinogens has been attacked with considerable success in the Cytogenetics and Cytology Section which has worked out several cell transformation models suitable for screening agents that should be tested for carcinogenic potential.

Among the other accomplishments of the Biology Branch has been the demonstration by members of the Cellular Immunity Section that transplanted, established guinea pig cancers can be eliminated by the intralesional injection of Mycobacterium bovis (strain BCG) and as a consequence of this treatment early metastases are prevented from spreading and the animal is cured. The process leading to cure is complex and involves reversal of established disease as well as prevention of progression. Future plans include attempts to use BCG treatment at earlier stages of the carcinogenic process. This approach may be successful because there is evidence that one of the early consequences of cell transformation by a carcinogen is the acquisition of tumor specific antigens. Immunological interference with the carcinogenic process may also be accomplished with reagents of the humoral immune system, i.e., antibody and complement. Studies of humoral tumor immunity are being pursued in the Immunochemistry Section, while the major mission of the Tumor Antigen Section requires isolation and characterization of tumor specific antigens.

Cytogenetics and Cytology Section - The Cytogenetics and Cytology Section studies the process of chemical carcinogenesis in order to develop in vitro systems for neoplastic transformation by chemical and physical agents. A major application of these systems is in bioassays for the detection of carcinogenic agents in man's environment. The immediate aim of the studies is to use in vitro techniques to determine conditions which influence the morphologic, immunologic and pathologic changes that characterize transformation of cells from the normal to the cancerous state. These studies have dealt with the effects of chemical carcinogens, non-carcinogens, viruses and irradiation on cells in culture. Hamster embryo cells have been used in most of these studies and experiments have demonstrated that neoplastic transformation in vitro by chemical carcinogens is inductive, that transformation and toxicity associated with the chemical agents are separate events and that in vitro neoplastic transformation is relevant to in vivo carcinogenesis.

Three reproducible quantitative in vitro systems for neoplastic transformation employing mammalian cells in culture have been developed for the identification of chemical agents present in the environment as potential carcinogens and for the investigation of chemical, chemical-chemical, chemical-physical and chemical-viral interactions involved in the transition to neoplasia at the cellular level. In two of the systems, one employing freshly isolated diploid fetal Syrian hamster cells and the other using aneuploid cloned mouse Balb/3T3 cell lines, both uncomplicated by spontaneous transformation, the cells are exposed to chemicals in culture. Not all known chemical carcinogens are active in these systems, presumably due to the requirement of those chemicals for metabolic activation. For this reason, a host mediated in vivo - in vitro assay has been developed in which hamster fetal cells are exposed to the chemical while in utero via the transplacental route and transformation is subsequently observed after establishment of the cells in culture. The transplacental system reduces the number of false negatives that occur in the other in vitro systems and as in the other systems is not associated with false positives. The establishment of this host mediated in vivo - in vitro method represents a major advance towards the development of rapid means for the identification of the potential carcinogenicity of the wide variety of chemical agents present in man's environment, which require metabolic activation by the host for their carcinogenic effect.

During the past year a new model for transformation utilizing freshly isolated diploid guinea pig cells exposed to carcinogenic chemicals in utero or after introduction into culture has been developed. Transformed guinea pig cells exhibit in vitro properties characteristic of transformed hamster and mouse cells. When transplanted back to guinea pigs (or hamsters), they grow as sarcomas. Development of this model offers an additional avenue for studying the process of carcinogenesis using a species with established tumor biology in which spontaneous transformation has not been seen and which possesses well-defined immunological parameters. Freshly isolated human cells from therapeutic abortions and a human strain of fibroblasts from a patient with xeroderma pigmentosum have been subjected to chemical carcinogens. It has been demonstrated that human cells capable of metabolizing carcinogens since a variety of chemicals produce cell lethality. The surviving populations exhibit altered morphology which in some cases parallels that observed in transformed animal cells. Cells insulted with chemicals have an increased life span relative to untreated controls. Treated human cells are now being prepared for injection into conditioned animals to determine whether or not the cells are neoplastic.

Investigation of the interaction of chemical and physical agents is extremely important to the study of the causes of carcinogenic effects on a cell. X-irradiation of hamster cells prior to exposure to chemical carcinogens results in an enhancement of transformation. Substitution of a radiomimetic monofunctional aklylating agent, methylmethanesulphonate (MMS), for x-irradiation produces a similar enhancement of transformation. Pre-x-irradiation of fetal Syrian hamster cells with 250r increases benzo[a]pyrene (0.25-5µg/ml medium) transformation above the level observed in cells exposed to carcinogen alone and is consistent with a one hit hypothesis. Maximum enhancement of up to eightfold is obtained when benzo[a]pyrene is added 48 hrs post-plating of the irradiated cells and does not appear to be due to alterations of cell cycle, alterations in chromosome number or to chromosomal breaks attributable to x-irradiation. Substitution of 11 or 27.5 µg MMS/ml medium for x-irradiation enhances benzo[a]pyrene, 7,12-dimethylbenz[a]anthracene, N-methyl-N'-nitro-N-nitrosoguanidine or N-acetoxy-2-fluorenylacetamide transformation three to ten-fold. Pulsing of the cells with 11 µg MMS/ml medium for one hr 24 hrs after the cells are plated followed by subsequent exposure to benzo[a]pyrene (2.5 µg/ml medium) at 24, 48 or 72 hrs leads to enhancement of transformation which like x-irradiation enhancement is maximal at approximately sixfold at 48 hrs. No transformation occurs with x-irradiation alone and MMS itself results in only rare transformation. Analysis for unscheduled DNA repair reveals that most of the damage caused by the MMS is repaired during the first six hrs. Synchronized cells treated with MMS show no difference in DNA synthesis from untreated cells over 48 hrs. These results suggest that the enhancement of transformation due to the radiomimetic monofunctional alkylating agent is not associated with detectable chromosomal breakage or to a specific stage in the cell cycle. Further investigations of the interactions of chemical carcinogens with physical and chemical agents in the process of carcinogenesis are being pursued. It is important to determine whether enhancing agents cause a somatic mutation which expresses itself as neoplastic transformation or whether the agents have an indirect effect which facilitates the process of carcinogenesis.

Chromosome studies in Syrian hamsters have been extensively conducted using tissue culture and cells transformed by oncogenic chemicals or viruses. By routine staining procedures or by autoradiographs, the pairing of certain chromosomes in Syrian hamster is difficult and only a few of the 22 pairs can be exactly identified. Application of the acetic-saline Giemsa and trypsin techniques has made it possible to successfully identify (by chromosome banding patterns) each pair of the normal and neoplastic transformed Syrian hamster chromosome complement. Cell lines derived from cells transformed by 4-nitro-quinoline-N-oxide, benzo[α]pyrene, aflatoxin B_1, β-propiolactone and 1,3-propane sultone had near diploid and in rare instances subtetraploid chromosome modes. Aneuploidy is associated with most of the lines although the banding pattern is sometimes consistent with the normal pattern. The increase in chromosome number does not involve a specific chromosome group. In some instances a similar abnormal chromosome banding pattern was associated with different carcinogens but not all transformed lines had the same marker even with the same chemical. In some rearrangements new heterochromatin was detected in the abnormal chromosomes. The significance of the changes to the primary events in transformation is debatable and most likely reflects secondary alterations.

Multiplication aspects of chemically transformed Balb/3T3 and the parent cloned nontransformed Balb/3T3 cell lines have been investigated. Although Balb/3T3 cells are aneuploid, they demonstrate density dependent regulation of multiplication (contact inhibition) under normal culture conditions. Chemically transformed Balb/3T3 cells have lost this particular characteristic and consequently grow to a higher cell density by piling up on top of one another. The chemically transformed but not the nontransformed cells will grow to graded degrees depending upon the specific transformed cell line in medium containing ten percent agamma newborn calf serum and unlike the nontransformed cells their multiplication is not significantly stimulated by the addition of rat serum fractions or bovine β-globulin fractions quite stimulatory to the muliplication of nontransformed Balb/3T3 cells. Those chemically transformed cell lines that multiply efficiently in medium with ten percent agamma newborn calf serum furthermore, produce a higher incidence of tumors in weanling x-irradiated mice than do the lines that exhibit slower growth. These observations suggest that chemically transformed cell lines resulting from a single treatment with a chemical carcinogen without the presence of an exogeneous virus have lost the requirement of growth factor(s) which is absent in agamma serum to a graded degree and that the loss is not an "all or none" phenomenon.

Cellular Immunity Section - The Cellular Immunity Section studies the role of the immune system in the control of malignant disease. Over the past 10 years several transplantable tumor lines in inbred guinea pigs have been developed. The biologic characteristics of each tumor line have been defined. Features studied include: antigenicity, transplantability, histology and tendency to form metastases. These tumors have been used to develop models for the prevention of metastasis from established tumors by manipulation of the immune system and to gain understanding of the cellular basis of immune tumor rejection.

(1) Reversal of Established Malignant Disease in Guinea Pigs by Immune Manipulations - Initial studies demonstrated the efficacy of specific immunotherapy in the control of established tumor implants. A syngeneic tumor line was selected that underwent immunologic rejection following intradermal injection, but that grew progressively when implanted intramuscularly. Immunization with intradermal injections of the tumor cured approximately one-half of the guinea pigs bearing intramuscular implants.

Subsequent studies demonstrated that tumor cells could also be killed as "innocent bystanders" during immune reactions directed toward unrelated antigens.

These experiments led to investigations of the tumor suppressive properties of agents that evoked potent immune reactions. The attenuated form of the bacteria that is the cause of tuberculosis in cows was the agent selected for intensive study (Mycobacterium bovis [strain BCG]). Injection of BCG into established palpable intradermal tumors caused tumor regression and prevented the development of lymph node metastases. The requirements for optimal therapy were defined. These requirements included: limited tumor size, close contact between BCG and tumor, an adequate number of living BCG, and a host with an intact immune system.

Recent work has shown a nonliving BCG preparation to have potent tumor suppressive properties. The preparation consists of the BCG cell walls attached to oil droplets. This preparation was as effective as living BCG in causing tumor regression. Cell walls alone, oil droplets alone, or cell walls mixed with, but not attached to oil droplets were devoid of tumor suppressive properties. It was essential for the BCG cell walls to be attached to the oil droplet for tumor suppression. The major advantage of this preparation is that it is not infectious; the use of an infectious agent in cancer treatment is circumvented. Several related bacteria were tested for tumor suppressive properties; Nocardia asteroides, Mycobacterium smegmatis, Corynebacterium parvum lacked tumor suppressive properties; Mycobacterium kanasii possessed tumor suppressive properties.

Investigations aimed at obtaining chemically defined substances from the BCG cell wall that possess tumor suppressive properties have begun. Success in this venture would provide materials with increased tumor suppressive potency per unit weight and would eliminate molecules that acted to antagonize the tumor suppressive molecules of BCG. Initial studies were conducted on BCG cell wall extracts prepared by Dr. Edgar Ribi, Rocky Mountain Laboratory, NIAID. Cell walls extracted with organic solvents and treated with dilute acid or alkali had reduced tumor suppressive properties. The only fraction with tumor suppressive action was obtained by extraction of the cell wall with a mixture of chloroform and methanol; the active material was insoluble in boiling acetone (Contractor: Rocky Mountain Laboratory).

Clinical reports suggest that BCG prepared by different manufacturers varies in tumor suppressive action. It is of utmost importance in clinical trials to use the BCG substrain with maximal tumor suppressive properties and to ensure that the method of preparation of the bacteria preserves tumor suppressive action. Accordingly the ability of four BCG substrains to cause tumor regression was compared (performed under contract at Trudeau Institute, Saranac Lake, N.Y.). BCG substrains were formulated under identical conditions and tested in the guinea pig immunotherapy model. The four substrains were equal in tumor suppressive action.

(2) Cellular and Molecular Basis for BCG Mediated Tumor Killing: (a) In Vivo Studies - Prior studies in inbred mice and guinea pigs have shown that an immune response to antigens of the tubercle bacillus is essential for tumor killing. Elimination of delayed hypersensitivity to tubercle bacillus antigens in mice was accomplished by thymectomy and sublethal irradiation of adult mice. Elimination of delayed hypersensitivity to tubercle bacillus antigens in guinea pigs was accomplished by intravenous injection of 10^8 to 10^9 BCG. These procedures abrogated BCG mediated tumor killing.

The immunosuppressive procedures listed above affect more than one cell type. To gain a better understanding of this reaction, it is desirable to choose agents that selectively eliminate one component of the immune response. Cortisone acetate and antithymocyte serum as probes of BCG mediated tumor regression in mice have been used. Under the conditions tested, cortisone acetate and antithymocyte serum impair lymphocyte function and spare macrophages. Both agents abrogate BCG mediated tumor killing in mice. This is evidence that a lymphocyte population is essential for the development of BCG mediated tumor regression.

Prior histologic studies demonstrated that cells of the monocyte-macrophage series were present at sites of BCG mediated tumor destruction. Current in vivo experiments have been designed to test whether macrophages are required for tumor killing. Silica, a potent inhibitor of macrophage function, did not abrogate BCG mediated tumor killing.

Experiments are in progress to determine what classes of lymphocytes are required for BCG mediated tumor killing.

(b) In Vitro Studies - A test tube model for BCG mediated tumor killing has been developed. This model may provide a more rational basis for immune therapy. Inflammatory cells from the peritoneal cavities of guinea pigs previously infected with BCG were collected and incubated in a test tube with tumor cells. BCG antigens were added to some tubes and omitted from others. Inflammatory cells from BCG infected animals were found in the presence of BCG antigens killed tumor cells.

Many experiments are based on knowledge that the immune response to certain bacteria of activated macrophages from mice infected with one bacterium have increased resistance to infection with another antigenically unrelated bacterium. This year an assay in guinea pigs for "activated" macrophages was developed (using Listeria as targets) and it was found that cells washed from the peritoneal cavities of BCG infected animals in the presence of BCG antigens killed Listeria. Killing is mediated by a factor liberated from macrophages into the culture fluid and is not primarily an intracellular process. Tests for "activated" macrophages are needed to test the concept that such cells are tumoricidal.

(3) Studies of Aryl Hydrocarbon Hydroxylase (AHH) in Inflammatory Cells from the Peritoneal Cavity - AHH is the enzyme responsible for the metabolism of polycyclic aromatic hydrocarbon carcinogens. A program was started to study possible interrelationships between AHH, carcinogens and the immune system. It was found that intraperitoneal administration of carcinogen in oil to guinea pigs leads to elevated levels of AHH in peritoneal inflammatory cells.

Immunochemistry Section - The Immunochemistry Section has developed over the years a program investigating the mechanism of the interaction of cell antigens with their corresponding antibodies and the interaction of the resulting complex with the complement system. Knowledge derived from this and through other program areas of the Branch is then applied to the implementation of a target oriented contract program. During the past year concentrated studies were in three major areas: the mechanism of tumor cell killing by antibody and complement, the measurement of cell bound immune reactions, and the nature and use of complement.

(1) Mechanism of Tumor Cell Killing by Antibody and Complement - The sensitivity of a cell to the lytic action of complement is the result of (a) the combination of an appropriate antibody with cell surface antigen, (b) the initiation of complement fixation and (c) the attack of cell surface areas susceptible to the lytic action of complement.

To study these factors the Forssman antigen and the specific tumor antigens of guinea pig tumor cells of line-1 and line-10 were chosen. Some additional studies have also been made with guinea pig leukemia cells (L_2C) and Chinese hamster lung cells.

The choice of the Forssman system model to study antibody-complement mediated lysis was based on the facts that one can identify the class of antibody, determine its concentration on a molecular basis, determine the number of complement fixing molecules accurately and identify and measure Forssman antigen activity.

The Forssman antigen content of cells of line-1 and line-10 guinea pig hepatomas was compared by measuring the capacity of these cells to absorb hemolytically active IgM from antisera containing Forssman antibody. No significant difference was found in the absorption capacity for Forssman antibody between cells of line-1 and line-10 tumors. In contrast line-1 and line-10 cells differed in their susceptibility to lysis by Forssman antibody and complement. The difference could not be ascribed to differences

in the antibody class of the hemolysin or differences in the concentration of Forssman antigen on the cell surface. Line-10 cells sensitized with IgM anti-Forssman antibody were more resistant to lysis by human C than line-1 cells and were not lysed by either guinea pig or selected rabbit C. Line-10 cells could be lysed efficiently with human complement when sensitized with a specific anti-line-10 antiserum. Increasing the number of line-1 cells to give a surface area equivalent to that of line-10 did not change the cytotoxic activity. Since line-10 cells could be lysed when sensitized with a specific rabbit anti-line-10 antibody, it was concluded that their resistance to lysis was not a general property of the cells.

The differences in susceptibility to lysis by complement could not be ascribed to differences in antigen concentration and distribution, lack of antibody binding and difference in immunoglobulin class. Most likely it was due to the distribution or presence of areas on the cell surface which are sensitive to complement action. Additional evidence in terms of this interpretation was offered from studies of the effect of neuraminidase treatment of tumor cells. Although this enzyme increased slightly the Forssman antigen expression of line-10 cells it renders them very sensitive to lysis. This phenomenon is now under study.

Assessing the effectiveness of nucleated cell killing is not simple. For this reason the killing of Chinese hamster lung cells by antibody and complement was studied by measuring the release of Cr, inhibition of uptake of tritiated thymidine, uptake of trypan blue and colony inhibition. Antisera and complement were obtained from three different species of animals. The results showed that the cytotoxic titer of a given antiserum and a given complement was the same regardless which method was used to determine cytotoxicity.

(2) Measurement of Cell Bound Immune Reactions - Part of the efforts in this area was done conjointly with the Tumor Antigen Section and concerned the isolation and measurement of tumor antigens. Progress in this area has been included in that Section's report. Tumor antigens are most likely to be a component of the cell membrane. Methods have been devised for detecting and quantitating antigens that are not part of the membrane per se but have become bound to it by various measures. For example complement components may bind to cell surfaces without being injurious to the cell. This type of binding may occur in vivo and the presence of bound complement may indicate an in vivo anti-cell reaction. A rapid and simple method for the detection of cell bound third and fourth components of complement has been developed. The test is sensitive and has detected as few as 100 of these molecules on a cell. With this test cell bound complement components have been found on guinea pig tumor cells removed from the peritoneal cavity of guinea pigs.

(3) The Nature and Use of Complement - In studying the structure and action of the first component of complement it was found that the internal activation steps in the first component of complement will not be initiated when serum containing the precursor form of the molecule is diluted prior to its interaction with antibody-antigen complexes. The loss of self-activation due to dilution was traced to the dissociation of a fragment of the molecule

necessary to start the internal activation sequence. This fragment can be substituted for by trypsin. The fully activated first component itself can be replaced by trypsin in the reaction between the first component and the second. An analysis of this reaction indicated that trypsin was as effective as the first component except for the lack of binding to antibody-antigen complexes. These observations raise the point that the active sites of the enzymes in the complement sequence may be similar if not identical to other more common types of esterases and proteases and that the peculiar substrate specificity of these enzymes is due to portions of the molecule that are concerned in binding and orienting the enzyme and its substrates.

The complement fixation and the first component activating reaction was used to analyze L-asparaginase preparations for the presence of contaminating substances. Canadian workers found that L-asparaginase preparations derived from E. coli activated complement and they proposed that some of the hypersensitivity reactions observed in patients under L-asparaginase treatment was caused by this mechanism. An investigation of this problem revealed that of 15 L-asparaginase preparations tested, 13 inhibited whole human serum complement; the inhibitory effect ranged from 12 to 45%. The anticomplementary activity was separable from the L-asparaginase activity. L-asparaginase and the anticomplementary factor are antigenically distinct, and the anticomplementary factor was shown to be antigenically related to Escherichia coli lipopolysaccharide (endotoxin).

Tumor Antigen Section - The work of the Tumor Antigen Section can be summarized under three main headings: tumor antigen, mechanisms of tumor cell killing and calcium transport globulin system.

(1) Tumor Antigen - It has been previously shown that guinea pigs immunized with line-1 soluble antigen in complete Freund's adjuvant rejected an intradermal challenge of line-1 tumor cells faster than control animals. Experiments with line-10 antigen are stringent tests of the capacity of antigen to protect animals against tumor challenge, since in contrast to line-1, intradermally administered line-10 cells multiply, metastasize and kill the host. Despite the fact that all guinea pigs immunized with line-10 antigen had strong cutaneous delayed hypersensitivity responses to line-10 antigen, their capacity to reject line-10 tumor varied. The results included no growth of tumor at all, appearance of palpable tumor after a delay of several weeks post challenge, growth of tumor in the skin, and then regression and complete healing, only to be followed in some cases by metastases in regional lymph nodes. A model similar in certain respects to human cancer in which the outcome of the disease cannot be predicted has been developed. Despite the presence of a positive skin test to the cancer antigen, this is not necessarily a good measure of the capacity of the host to mount an effective cellular immune response against a tumor and other tests for cellular immune capacity are being developed. Antitumor antibody has been found in the serum of some of the challenged animals; the titer, the time course of appearance, and the nature of the antibody (complement-fixing? cytotoxic?) are being studied in relation to the clinical outcome.

In the extraction and purification of tumor specific antigen, the assay for antigenic activity has been the capacity of antigen extracts to elicit positive skin tests in appropriately immunized guinea pigs. A new antigen assay has been devised using rabbit antibody specific for line-1 or line-10 antigen. This antigen assay is more precise than the skin test and is being used in studies on extraction and further purification of line-1 and line-10 antigen.

New information on tumor specific antigens of line-1 and line-10 cells was obtained in collaboration with the Immunochemistry Section. Analysis with rabbit antitumor antisera showed that line-1 and line-10 have not only tumor specific antigens but also a common embryonic antigen.

Rabbit anti-line-10 antibody induces movement of line-10 antigen within the plane of the tumor cell membrane to form antigen-antibody aggregates that appear to extrude from the cell. Aggregation involves movement of the whole antigen-antibody complex, so that in effect the antigen is stripped from the tumor cell surface. It reappears in its uniform surface distribution within 4-6 hrs. This system may be useful for study of the dynamics of macromolecules in the cell membrane. It raises the question whether antibody in vivo can lead to stripping of tumor surface antigen, so that the host would have the double disadvantage of antigen-deficient tumor cells and tumor antigen immune complexes in the circulation.

(2) Mechanisms of Tumor Cell Killing - Studies are being made with the conviction that the knowledge obtained will provide a more rational basis for elimination of tumor cells by immunological means.

It was shown that murine tumor cell monolayers could be destroyed by PPD-stimulated spleen cells from BCG-immune mice and by supernatant culture fluids from the cells. The damage to the tumor cells appeared to be due to the elaboration of soluble toxins by antigen-stimulated cells in the general category of "lymphotoxin" as reported in the literature for other systems. Of great interest was the fact that the tumor cell monolayers were much more sensitive to the toxic action of the supernatants than were mouse fibroblast monolayers. The isolation and purification of mouse lymphotoxin were begun. A quantitative assay for mouse lymphotoxin has been worked out. Production of lymphotoxin by PHA-treated spleen cells has been obtained in serum-free tissue culture media, which provides a cleaner starting material for purification.

Another example of tumor cell damage during a cellular immune reaction to antigens unrelated to the tumor is being studied. Peritoneal exudate cells from guinea pigs immunized with BCG placed in tissue culture media containing PPD were toxic to line-10 tumor cells. The toxicity is mediated in great part by soluble cytotoxins elaborated by the PE cells in response to the addition of PPD. Studies are being done to determine what cells in the PE mixture account for the toxicity.

Another possible mechanism of tumor cell killing, combining humoral and cellular arms of the immune response is toxicity to antibody-coated cells mediated by normal lymphocytes which has been reported in systems made up from several animal sources, such as chicken erythrocytes coated with rabbit antibody and mixed with human lymphocytes. To be more than a laboratory curiosity the effect should be demonstrable in syngeneic systems. A small but regularly observable toxicity has been shown when normal guinea pig spleen cells are added to guinea pig line-10 tumor cells coated with IgG isolated from rabbit anti-guinea pig serum. Studies with guinea pig spleen cells added to line-10 cells coated with specific guinea pig anti-line-10 IgG antibody are in progress.

One element in the effector side of the cellular immune response is the accumulation of mononuclear cells at the antigen recognition site. The accumulation is mediated by lymphokines such as MIF an mononuclear cell - chemotactic factor. As part of an effort to quantify elements of the effector arm of cellular immunity, responses of human peripheral blood monocytes to standard concentrations of both complement generated (C5a) and lymphocyte generated chemotactic factors are being measured. Dose-response curves have been worked out, the variation of mononuclear cell responses from multiple bleedings of the same individual have been measured, and a normal range or responses for a series of normal subjects have been established. Studies on a group of 31 patients with cancer showed depressed responses in approximately half. Some of the depressed responses occurred in patients with localized tumors who were otherwise in good health. Measurements of mononuclear cell chemotaxis in hospitalized patients without cancer are in progress. Chemotactic responses have been quantified by counting the number of mononuclear cells which migrate through the 5 micron holes of a Nucleopore membrane and spread out on the membrane surface. As a substitute for visual counting, the responding cells have been labeled with $^{51}C4$ and chemotactic responses have been measured by putting the Nucleopore membrane with its adherent cells into a gamma counter. This approach should be especially useful for measurement of chemotactic activity from various sources, using a standard preparation of ^{51}Cr-labeled responding cells.

(3) Calcium Transport Globulin System (CTGS) - This system of mammalian plasma proteins has been shown to transport calcium into isolated frog heart muscle, thereby increasing contractile force. The frog heart is used for bioassay of the system. The hypothesis to be tested is that CTGS regulates calcium entry into many types of tissue cells and may play a role in control of immune responses. This is suggested by the fact that in systemic lupus erythematosus, which is characterized by a variety of autoantibodies, serum CTGS activity is markedly depressed. By glycerol extraction of CTGS-treated frog hearts, and injection of the extracts with complete Freund's adjuvant into bullfrogs, antibody against the B component of system (CTG-B), as evidenced by specific inhibition of the effect of CTGS on the bioassay frog heart were obtained. Isolation and purification of the CTGS components has been hampered by lack of stability of the system. During the past few months a dramatic change has occurred which is attributable to a different method of

obtaining human serum. Blood is drawn into an iced plastic bag, and the cells are separated from the plasma before clotting occurs. The CTGS components of the resulting serum are remarkably stable. Purification of these components is in progress, with the main purpose of obtaining monospecific antibodies for tissue localization studies.

Serial No. NCI-4664
1. Biology Branch, OASDC, DCCP
2. Cellular Immunity Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Immunologic Approaches to the Prevention and Treatment of Cancer

Previous Serial Number: Same

Principal Investigators: Berton Zbar, M.D., and Herbert J. Rapp, Sc.D.

Other Investigators: Bruce H. Littman, M.D., Robert C. Bast, Jr., M.D., Adam Bekierkunst, Ph.D.

Cooperating Units: For supply of BCG substrains: Trudeau Institute, Saranac Lake, New York. For studies with nonliving mycobacterial preparations: Dr. Edgar Ribi, Rocky Mountain Laboratory, Hamilton, Montana. For studies of effect of x-irradiation on tumor growth: Dr. Frederic A. Gibbs, Radiation Branch, NCI.

Man Years:

Total:	2.2
Professional:	1.7
Other:	0.5

Project Description

Objectives:

The primary objective of this project is to establish conditions and methods suited to the prevention and treatment of cancer. This objective is based on the finding that tumors contain unique antigens capable of eliciting the formation of tumor specific humoral antibodies and of causing the appearance of cells which react specifically with tumor antigens. Transplantable syngeneic guinea pig tumors will be used to investigate conditions for effective immunotherapy.

Methods Employed:

Tumors. Malignant hepatomas have been induced in strain-2 guinea pigs by feeding them diethylnitrosamine in their drinking water. Transplantable ascites tumors have been derived from primary hepatomas. Two of these ascites variants are being maintained by periodic intraperitoneal trans-

plantation. A strain-2 guinea pig leukemia is being maintained by periodic intradermal transplantation. Portions of these tumors are periodically frozen and stored in liquid nitrogen. The work in this report was carried out with cells of a transplantable hepatoma (line-10) which when injected into the skin produces a progressively growing papule and regional lymph node metastases within a few days.

Tumor suppressive properties of mycobacterial preparations. In one assay, tumor cells and mycobacterial antigens were mixed in vitro and inoculated intradermally into strain-2 guinea pigs. The size of the inflammatory reaction and/or tumor was measured twice a week. In a second assay, tumor cells were injected intradermally into unimmunized strain-2 guinea pigs. When established intradermal tumors and regional lymph node metastases were present (7 days after intradermal injection) mycobacterial antigens were injected into the tumor.

Nonliving mycobacterial preparations. Bacterial cell walls and cell wall extracts were prepared in the laboratory of Dr. Edgar Ribi. Cell walls or cell wall extracts were mixed with a small amount of mineral oil and this mixture was ground to a smooth paste. Saline containing tween 80 was added to the paste and grinding was continued until a well dispersed oil in water emulsion was obtained.

Inhibition of pulmonary carcinogenesis. Urethane, an agent causing the production of pulmonary adenomas, is injected intraperitoneally into outbred mice. Preparations suspected of possessing tumor suppressive properties are inoculated intravenously. Mice are killed 2 to 3 months after administration of urethane and the number of adenomas per lung is determined.

Major Findings:

Comparison of Tumor Suppressive Properties of BCG Substrains

1. Phipps, Tice, Glaxo and Pasteur BCG substrains were tested for ability to cause regression of established intradermal tumors. BCG preparations were formulated under identical conditions. This procedure produced substrain preparations with similar concentrations of organisms and similar viability. The four substrains possessed equivalent tumor suppressive properties as measured by the number of animals "cured" by treatment. There was some suggestion that the Pasteur substrain possessed greater tumor suppressive properties than the other substrains as measured by prolongation of life.

Nonliving Mycobacterial Preparations

1. The minimum dose of BCG cell walls required for tumor regression was 30 µg. Three µg of BCG cell walls failed to cause tumor regression.

2. The quantity of oil admixed with BCG cell walls was critical. Thirty µg of BCG cell walls injected in admixture with 0.15 µl of oil was less effective in causing tumor regression than 30 µg of BCG cell walls injected in admixture with 1.4 µl of oil.

3. Analysis of tumor suppressive properties of all materials obtained during extraction of BCG cell walls was begun. Results indicate that considerable tumor suppressive activity is lost during the first extraction step. Cell walls after treatment with proteolytic enzymes have lost 90% of original tumor suppressive activity.

4. Cell walls obtained from several different bacteria were tested for tumor suppressive properties. Mycobacterium bovis and Mycobacterium kansasii possessed tumor suppressive properties. Mycobacterium smegmatis, Nocardia asteroides, and Corynebacterium parvum lacked tumor suppressive properties.

5. Intravenous administration of BCG cell walls to guinea pigs and mice (300-1500 µg) was not associated with significant morbidity or mortality.

Significance to Biomedical Research and the Program of the Institute:

BCG preparations are potent inhibitors of tumor cell growth. Although this observation has been repeatedly made, it has been difficult to harness the therapeutic potential of BCG. The studies in this laboratory are aimed at determining effective ways for using mycobacterial preparations in man. Our studies have shown some of the requirements for effective BCG treatment and some limitations of this type of treatment. Of particular importance is the demonstration that nonliving mycobacterial preparations are potent inhibitors of tumor growth. Such preparations avoid the risks inherent in the use of living bacteria in patients with cancer. This demonstration may lead to the identification of the molecules of mycobacteria essential for tumor inhibition.

Identification of the BCG substrain with maximal tumor suppressive activity is of great importance to the clinician. Identification of methods of preparation of BCG substrains which preserve tumor suppressive activity is also of great importance to the clinician.

Proposed Course of Project:

We will continue to perform experiments designed to determine the BCG substrain with maximal tumor suppressive activity and to determine conditions for preservation of antitumor activity. We will pursue attempts at isolating the molecules responsible for the tumor suppressive properties of BCG cell walls.

Experiments on impairment of pulmonary carcinogenesis by bacteria and bacterial products will continue. Animal models for study of metastatic disease will be developed.

Publications

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Serial No. NCI-4665
1. Biology Branch, OASDC, DCCP
2. Cellular Immunity Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Mechanism of Delayed Hypersensitivity and Tumor Graft Rejection

Previous Serial Number: Same

Principal Investigators: Berton Zbar, M.D., and Herbert J. Rapp, Sc.D.

Other Investigators: Robert C. Bast, Jr., M.D., Ronald P. Cleveland, B.A., Bruce S. Zwilling, Ph.D.

Cooperating Units: None

Man Years:

Total:	2.5
Professional:	1.5
Other:	1.0

Project Description

Objectives:

The main objectives of this project are: 1) to define the mechanism of the immunological rejection of antigenic tumors, and 2) to ascertain possible immunologic defects in tumor-bearing host to explain the progressive growth of an antigenic tumor.

Methods Employed:

Tumors. Malignant hepatomas have been induced in strain-2 guinea pigs by feeding them diethylnitrosamine in their drinking water. Transplantable ascites and intramuscular variants of these tumors currently maintained in this laboratory are used.

Bacteria Killing Assay. Cells are washed from the peritoneal cavities of guinea pigs immunized to BCG, and of nonimmune guinea pigs. Peritoneal cells are cultured with or without the purified protein derivative of tuberculin (PPD) for 18 hours. At the end of this incubation period, cells not adherent to the culture dish are removed, and media containing Listeria monocytogenes is added to the culture dish containing adherent cells. The bacteria are

incubated with the adherent cell monolayer for one hour. At the end of this incubation period the supernatant fluid is removed and the number of viable bacteria is determined. In some experiments, bacteria are grown in medium containing radiolabeled thymidine. Listeria monocytogenes labeled in this manner are added to adherent cell monolayers.

Listericidal Activity of Cell Free Supernatants. Supernatants from cultures of peritoneal cells incubated for 18 hours were poured off and the nonadherent cells removed by centrifugation. Adherent monolayers were washed six times and covered with 2 ml tissue culture medium. Following one hour of incubation the supernatant was poured off and the nonadherent cells removed by centrifugation. One ml samples of each supernatant and of normal medium incubated in dishes without cells were equilibrated with 5% CO_2 - 95% air and mixed with 0.1 ml aliquots containing 2.5 x 10^7 Listeria monocytogenes. After an additional hour of incubation, Listeria were diluted, plated and counted.

Major Findings:

1. Cells washed from the peritoneal cavities of immunized or nonimmunized guinea pigs did not kill Listeria monocytogenes.

2. Cells washed from the peritoneal cavities of immunized guinea pigs killed Listeria monocytogenes if the peritoneal cells were cultured in the presence of PPD.

3. In this system, 80% of the bacteria were killed in the extracellular fluid.

4. Bacterial killing was mediated by a substance secreted by the adherent cells into the extracellular fluid.

5. The bactericidal substance was not secreted by tumor cells in culture of strain-2 fibroblasts in culture.

6. The bactericidal substance was heat labile, nondialyzable and was inactive when diluted one hundred fold.

7. Fluids containing the bactericidal substance did not kill strain-2 tumor cells in culture.

8. Cells washed from the peritoneal cavities of guinea pigs previously injected with mineral oil did kill Listeria monocytogenes. Bacterial killing was mediated by a substance secreted by adherent cells into the extracellular fluid.

9. Peritoneal cells with augmented ability to kill Listeria were not tumoricidal.

Significance to Biomedical Research and the Program of the Institute:

There has been considerable interest in the tumoricidal properties of "activated" macrophages. One problem is precisely what constitutes macrophage activation. Are there degrees of macrophage activation? The present results have defined an *in vitro* assay for Listeria killing. Peritoneal cells from BCG immunized animals when cultured in the presence of PPD had augmented ability to kill Listeria. Peritoneal cells with augmented ability to kill Listeria would be considered to be "activated" by many workers. However, peritoneal cells with augmented ability to kill Listeria were not tumoricidal. These results suggest that the broad biologic concept that "activated" macrophages kill tumor cells is imprecise and incorrect.

Proposed Course of the Project:

We will turn to murine systems where "activated" macrophages have been shown to kill tumor cells *in vitro*. Immunization methods will be modified in hopes of obtaining macrophages with maximal tumor suppressive properties. Substances such as poly I - poly C, and pyran copolymer will be tested for ability to produce macrophages with tumor suppressive properties.

Serial No. NCI-4666
1. Biology Branch, OASDC, DCCP
2. Cellular Immunity Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Tumor Specific Immune Reactions in Mice

Previous Serial Number: Same

Principal Investigators: Herbert J. Rapp, Sc.D., and Berton Zbar, M.D.

Other Investigators: Ed B. Chung, M.D.

Cooperating Units: For supply of BCG: Trudeau Institute, Saranac Lake, New York. For radiation facilities: Radiation Branch, NCI.

Man Years:

Total:	0.6
Professional:	0.5
Other:	0.1

Project Description

Objectives:

A long-range goal of this project is to improve our understanding and control of the processes by which a cancerous individual, animal or human, may be able to protect himself from his tumor. An important feature of such processes is the ability of the individual to specifically interfere with tumor growth by means of immune mechanisms. Such tumor specific immune reactions tend to be relatively weak, even in experimental circumstances. The immediate objectives of the project are, 1) to study tumor specific immune reactions to ascertain those factors which impose limitations on the effectiveness of the immunity, 2) to study both inductively and deductively means of strengthening anti-tumor immune reactions and 3) to develop methods for the study of tumor specific immunity *in vivo* and *in vitro*.

One successful approach to augmenting tumor immunity in guinea pigs has been to immunize with mixtures of tumor and BCG (*Mycobacterium bovis*) rather than with tumor cells alone; the immunizing tumor inoculum failed to grow progressively when mixed with BCG and strong tumor immunity resulted. This project has adapted this technique to a mouse tumor system to facilitate certain manipulations of the immune response and to permit comparison of the response of several tumors.

The work was intended to determine what types of processes are required to prevent tumor growth at the site of a BCG injection, and how such an interaction affects the subsequent development of tumor immunity.

Methods Employed:

A variety of mouse sarcomas of the C_3H strain are available: four have been used thus far. Three were induced in subcutaneous tissue, by 3-methylcholanthrene; one developed, without carcinogen treatment, du ng prolonged culture of connective tissue in a diffusion chamber. One of the chemically induced tumors is highly antigenic; it grows with difficulty in normal syngeneic mice but grows readily in pre-irradiated mice. Another MCA sarcoma grows slowly and has been moderately antigenic in previous tests. The third chemically induced tumor grows very rapidly, metastasizes to regional lymph nodes, and has not effectively immunized syngeneic mice in previous tests. The "spontaneous" sarcoma has an average growth rate and has been very weakly antigenic. Portions of the tumors are frozen and stored in liquid nitrogen. They are maintained by serial, approximately biweekly transplantation in syngeneic mice which have been immunosuppressed by thymectomy and 450 r of x-irradiation.

The solid sarcomas are minced and digested with Pronase and DNAase. Appropriately diluted suspensions of the tumor cells are injected intradermally, either alone or after mixture and short (10-30 min.) incubation with living BCG. In other cases, living BCG is infiltrated into tumors which have been growing in syngeneic mice for several days. Tumors are measured biweekly. After growth of primary tumor grafts, tumors are excised. Evidence of tumor immunity is obtained by comparing the growth of a second tumor inoculum in pretreated mice with growth of a similar inoculum in mice receiving control treatments. Evidence for delayed hypersensitivity to BCG is obtained by measuring the degree of swelling of the footpad 24 hours after injection of 10 µg of PPD.

Living BCG organisms are killed *in vitro* by incubation at $65^{o}C$ for 60 minutes. Isonicotinic acid hydrazide is administered in the drinking water to achieve an *in vivo* mycobactericidal effect.

Cortisone acetate is injected subcutaneously on the day of injection of BCG-tumor cell mixture at a dose of 0.125 mg per gram body weight. Antithymocyte serum (ATS) is injected into the peritoneal cavity of mice one day before and 1, 3 and 5 days after injection of tumor cell-BCG mixtures.

Immunosuppression and reconstitution. Adult C3H/HeN male mice are thymectomized. Two to four weeks after thymectomy, mice are subjected to 450 r whole body x-irradiation. On the day of x-irradiation, mice receive an intravenous injection containing thymus, spleen or bone marrow cells, or combinations of these cell types. On the day following x-irradiation a mixture containing BCG plus tumor cells is injected intradermally.

Major Findings:

1. The effect of BCG on tumor suppression depended on the number of BCG organisms injected: 10^7 colony forming units (cfu) suppressed the growth of 5×10^5 tumor cells in 100 percent of mice; 10^6 cfu suppressed the growth of 5×10^5 tumor cells in 50 percent of mice.

2. Eliminating the ability of BCG organisms to multiply by heat treatment in vitro or isonicotinic acid hydrazide treatment in vivo did not influence the local tumor suppressive effect of BCG or interfere with the development of delayed sensitivity to purified protein derivative of tuberculin (PPD).

3. Cortisone acetate and antimouse thymocyte serum (ATS) treatment abolished BCG mediated tumor regression and temporarily impaired delayed hypersensitivity to PPD.

4. The time required for completion of BCG mediated tumor killing was greater than 7 days.

5. Foot pad tests were performed 7 days after x-irradiation in immunosuppressed mice reconstituted with various cell populations: thymus cells (50×10^6) or bone marrow cells (5×10^6) failed to reconstitute the ability to respond to BCG. Spleen cells (50×10^6) partially reconstituted the ability to respond to BCG. Mixtures of thymus cells and bone marrow cells, or mixtures of spleen cells and bone marrow cells were more effective in reconstitution of response to BCG than either cell population alone. Immunosuppressed mice reconstituted with thymus cells (50×10^6) and bone marrow cells (5×10^6) that developed delayed sensitivity to PPD failed to inhibit tumor growth at the site of BCG infection.

Significance to Biomedical Research and the Program of the Institute:

BCG has been shown to inhibit viral and chemical carcinogenesis, to inhibit the growth of transplanted experimental tumors and to cause regression of established intradermal tumors. The mechanism of this impressive antitumor effect remain unknown. The results of the present studies confirms other work indicating that BCG mediated tumor suppression requires the development of cellular immunity to mycobacterial antigens. Agents that temporarily suppress cellular immunity to mycobacterial antigens may have profound results in terms of potential tumor suppression. Prior studies have implicated the necessity of intact immune function but did not implicate any particular cell type. The present study demonstrates that elimination of lymphocytes of a particular class, eliminates BCG mediated tumor killing. Immunologically committed small lymphocytes appear essential for BCG mediated tumor killing. Several experiments have suggested that tumor suppression mediated by BCG requires a chronic inflammatory reaction. Present work supports this concept since an immunosuppressive agent administered 7 days after inoculation of BCG-tumor cell mixtures abrogated BCG mediated tumor killing.

Proposed Course of Project:

In Vivo Studies. Reconstitution studies will be performed in immunosuppressed adult mice to further delineate cells essential for tumor suppression. Thoracic duct cells will be tested for ability to reconstitute the response of immunosuppressed mice to BCG. Reconstitution studies will be performed with cells from donors immunized to BCG antigens. This strategy will separate the afferent and efferent arcs of the immune response. Reconstituted mice will be challenged with BCG-tumor cell mixtures at varying intervals following x-irradiation and reconstitution.

In Vitro Studies. We will try to reproduce the findings of Hibbs, et al, that macrophages from BCG treated mice are tumoricidal in vitro. The potential tumoricidal properties of this cell population will be tested in vivo by neutralization tests.

Serial No. NCI-4679
1. Biology Branch, OASDC, DCCP
2. Cellular Immunity Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Interaction of Carcinogenic Polycyclic Hydrocarbons with Mammalian Macrophages

Previous Serial Number: None

Principal Investigators: Robert C. Bast, Jr., M.D., Harry V. Gelboin, Ph.D., and Herbert J. Rapp, Sc.D.

Other Investigators: Barbara W. Shears, M.S.

Cooperating Units: Chemistry Branch, OASDC, DCCP, NCI.

Man Years:

Total:	0.6
Professional:	0.2
Other:	0.4

Project Description

Objectives:

The main objectives of this project are: 1) to determine whether macrophages are capable of uptake, metabolism and binding of carcinogenic polycyclic hydrocarbons; 2) to measure the effect of polycyclic hydrocarbons on the microsomal and lysosomal enzymes of macrophages; and 3) to determine whether interaction of polycyclic hydrocarbons with macrophages facilitates the induction and/or expression of contact hypersensitivity to these compounds.

Methods Employed:

Macrophages were obtained from the peritoneal cavities of guinea pigs 3 to 6 days following injection of sterile mineral oil.

Uptake and Binding of 7,12-Dimethylbenzanthracene (DMBA). Peritoneal macrophages were cultured with tritium labeled DMBA for two hours. Following incubation cells were washed, homogenized and the total amount of intracellular DMBA determined by scintillation counting. Protein in the macrophage homogenate was precipitated with trichloracetic acid (TCA) and the TCA precipitate

was extracted exhaustively to remove contaminating lipid and nucleic acid. The amount of DMBA, bound to protein was measured by scintillation counting.

Aryl Hydrocarbon Hydroxylase (AHH) Activity of Macrophages in Cell Culture. To study the effect of benz(a)anthracene (BA) on a microsomal enzyme of macrophages in culture, peritoneal exudate cells were cultured in the presence or absence of BA for 18 hours. Adherence to culture dishes permitted separation of a macrophage-rich subpopulation that was washed, homogenized and assayed for AHH activity and protein content.

AHH Activity of Macrophages and Lymphoid Tissues In Vivo. To study the effect of BA on AHH activity of macrophages in vivo, guinea pigs received intraperitoneal injections of sterile mineral oil with or without the addition of BA. On the third day following injection, cells were removed from the peritoneum, washed and homogenized. Aliquots of homogenate were assayed for AHH activity and protein content.

To study the effect of BA or 3-methylcholanthrene (MC) on lymphoid tissues, guinea pigs received corn oil intradermally either with or without the addition of BA or MC. Twenty-four hours following injection regional lymph nodes, spleen, thymus, bone marrow, and leukocyte rich plasma were removed. Each tissue was washed, homogenized and assayed for AHH activity and protein content.

Inhibition of AHH Activity by Benzoflavone (BF) In Vitro. Homogenates of BA treated lymphoid tissues were assayed for AHH activity in the absence or presence of different concentrations of BF.

Major Findings:

1. DMBA is taken up by peritoneal macrophages and bound covalently to cellular protein.

2. Treatment of peritoneal macrophages with BA in vivo or in cell culture increases levels of AHH.

3. Intradermal injection of BA or MC increases levels of AHH in lymph nodes and in spleen.

4. AHH, obtained from lymphoid tissues, is inhibited in vitro by BF.

Significance to Biomedical Research and the Program of the Institute:

1. The microsomal mixed function oxygenase complex of mammalian cells metabolizes a variety of carcinogenic polycyclic hydrocarbons and facilitates their binding to cellular protein and DNA. AHH, a part of this complex, detoxifies several polycyclic hydrocarbons, but can also catalyze their conversion to more carcinogenic forms. Detection of AHH in macrophages

and in lymphoid tissues, suggests that the reticuloendothelial system (RES) can participate in the metabolism of carcinogens. Macrophages contribute to inflammatory exudates throughout the body and could concentrate AHH at sites of chronic inflammation. Stimulation or depression of reticuloendothelial function alters the activity of a number of carcinogens. While these effects have generally been attributed to modulation of immune surveilance, the functional state of the RES might also influence the metabolism of carcinogens.

2. Cutaneous application of certain carcinogenic polycyclic hydrocarbons can produce contact hypersensitivity. Although these carcinogens are not highly reactive chemically they probably attain antigenicity through conjugation with host proteins. AHH might contribute to the formation of immunogenic conjugates by converting small haptenic molecules to reactive intermediates capable of covalent binding to macromolecules. Macrophages have been implicated in the processing of a variety of antigens; the uptake and/or binding of DMBA by these cells may be an important step in the development of contact hypersensitivity.

3. A major obstacle to the development of *in vitro* assays for hypersensitivity to carcinogens has been the production of antigenic carcinogen-protein conjugates. Polycyclic hydrocarbons associated with macrophages may provide these reagents.

Proposed Course of Project:

Additional studies are in progress:

1. To determine whether BF blocks the binding of DMBA to proteins of macrophages and lymphoid tissue.

2. To measure the AHH content of different macrophage and lymphocyte populations in the presence and absence of inflammatory stimuli.

3. To quantitate the effect of BA and other polycyclic hydrocarbons on the lysosomal enzymes of macrophages.

4. To observe the effect of BCG on the induction of contact sensitivity to polycyclic hydrocarbons and to unrelated compounds.

5. To determine the antigenic activity of polycyclic hydrocarbons associated with macrophages both *in vivo* and in cell culture.

Serial No. NCI-4629
1. Biology Branch, OASDC, DCCP.
2. Cytogenetics and Cytology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The Mechanism of Cell Transformation

Previous Serial Number: Same

Principal Investigator: Joseph A. DiPaolo, Ph.D.

Other Investigators: Paul J. Donovan, B.S., Charles H. Evans, M.D., Ph.D. and Nicolae C. Popescu, Ph.D.

Cooperating Units: None

Man Years:

Total:	3.9
Professional:	1.9
Other:	2.0

Project Description

Objectives:

The primary objective of this project is to establish conditions and methods for the quantitative study of chemical transformation. The immediate targets of study are:

1. Development of rapid assay systems for chemical carcinogens suitable for the screening of compounds and populations.

2. Determination of ways to increase the susceptibility of primary cell lines or cell strains to chemical transformation.

3. Development of assays to determine whether *in vitro* transformation is accompanied by the appearance of tumor specific antigens.

4. Determination of the role of non-oncogenic and oncogenic viruses in chemical transformation.

5. Determination of the ultrastructural changes of cells during the course of *in vitro* chemical transformation so that they may be contrasted with normal cells and with information pertaining to viral transformation.

6. Determination of the role of radiation and alkylating compounds in enhancing neoplastic transformation by chemicals.

7. The transformation of epithelial-like cells which result in the formation of carcinomas when the cells are transplanted into animals.

8. Analysis of variations in populations of somatic cells and their correlation with genetic changes.

Methods Employed:

All procedures performed are with the view of quantitating phenomena in vitro. Such procedures are required in order to determine whether or not the transformation observed is due to the direct or indirect effect of the carcinogen and in order to study the early events associated with in vitro transformation. Cells used come from freshly isolated cells from animals that as controls have many of the attributes of "normal" cells and from cell lines which are known to exhibit some of the properties associated with non-transformed cells. Discrete cells are grown in complete medium in the presence or absence of irradiated rat or hamster cells. The cells may be derived from whole animal embryos or may be from specific organs. The cells are exposed to chemical carcinogen either prior to or subsequent to seeding the cells in the petri dish.

Approximately one week subsequent to treatment the cells are examined under phase or under stained conditions for number of transformed colonies, toxicity, and spectrum of morphology of both and normal and transformed colonies. The frequency of transformation is expressed in a number of different ways. These take into consideration the observed rate of transformation on a per-cell basis or on a number of colonies obtained.

Major Findings:

Over the past few years we have learned that transformation of Syrian hamster cells obtained by chemicals is Poission in distribution and that the incidence of transformation may be influenced by different factors. One of our observations is that pretreatment of cells by X-irradiation results in an increase in the number of transformations by benzo(a)pyrene. A study was designed to establish whether methyl methanesulphonate (MMS), like X-irradiation, enhances transformation obtained with other chemical carcinogens. Secondary subcultures from fetal Syrian hamster cells were plated on irradiated feeder cells. Pre-X-irradiation of hamster cells with 250r increased BP (0.25-5μg/ml medium) transformation, consistent with a one hit hypothesis. Maximum enhancement obtained when BP was added 48hr post-plating of cells does not appear to be due to alteration of cell cycle, incidence of tetraploids or total number of chromosome breaks attributable to 250r. Substitution of 11 or 27.5μg MMS/ml for X-irradiation also enhanced transformation approximately 6X. Substitution of DMBA, MNNG or N-acetoxy-fluorenylacetamide for BP 48hr after seeding for colonies, also enhanced transformation 3-10X. In other experiments, cells were pulsed (1hr) with 11μg MMS/ml 24hr after plating, and BP (2.5μg/ml) was added 24, 48 or 72hr subsequently, enhancement at 48hr was again approximately 6X. No transformation occurs with X-irradiation only, but MMS

results in rare transformations. Analysis for unscheduled repair shows that most of the damage caused by 12.5μg MMS/ml is repaired during the first 6hr. Synchronized cells treated with MMS showed no difference in DNA synthesis over 48hr.

We reported a 3rd quantitative system that appears uncomplicated by spontaneous transformation. Cloned Balb/3T3 cell lines derived from a Balb/3T3 line, provided by Aaronson and Todaro, are sensitive to the toxicity of known chemical carcinogens and undergo chemically induced transformation in vitro. Sublines from a Balb/3T3 line were sensitive to a variety of carcinogens. A quantitative system of chemical transformation resulted in cell lines that caused fibrosarcomas when injected into mice (10^6 cell/mouse) ; no tumors developed from control lines (10^8). Transformation, indicated by criss-crossing of fibroblast-like cells not seen in controls, was scored in discrete colonies at 10 to 11 days or in foci after 3 weeks. Transformation was observed with carcinogenic polycyclic hydrocarbons, aflatoxin B_1, N-acetoxy-2-fluorenylacetamide, and N-methyl-N'-nitro-N-nitrosoguanidine but not with diethylnitrosoamine or noncarcinogens. Transformation rate increased (based on transformed colonies/total colonies or original cell inoculum used), and cloning efficiency decreased as concentration of carcinogen was increased. The dose-response relationship was consistent with a one-hit phenomenon. The Poission distribution of frequency of transformed colonies per dish indicates that transformation is due to induction. Transformed cell lines from carcinogen-transformed colonies or foci had decreased doubling time and increased saturation densities relative to control lines. Recloned, carcinogen-sensitive, Balb/3T3 cell lines present a reliable in vitro quantitative bioassay model for the study of chemical carcinogenesis.

A host mediated in vivo - in vitro combination bioassay for identification of potential carcinogenicity of chemicals for mammalian cells has been developed. The system differs from the established quantitative in vitro assays by in vivo exposure of fetal target cells following intraperitoneal injection of pregnant hamsters with chemical compounds as opposed to direct application of chemicals to cells in culture. The compound-utilized, both noncarcinogens and carcinogens, include some which have been previously tested by direct application to cells which have been plated to produce discrete colonies. The finding that the direct acting carcinogens are capable of producing transformation in vitro as a result of injection into animals and that a number of other compounds which may require metabolic activation to produce the proximate carcinogen are also capable of producing transformation makes it possible to consider the challenge of potential carcinogenicity posed by therapeutic agents as well as other chemicals in the environment. Although the results to be expected with other carcinogens cannot be predicted, it is apparent that reduction of the number of false negatives which may be due to the requirement of metabolic activation of the chemical is possible with this system. Finally the model may help to explain as well as to predict some of the postnatal cancers which result from prenatal insult by the variety of agents in the environment during pregnancy.

One characteristic of cancer cells is their uncontrolled multiplication that results in overwhelming pressure in the surrounding tissues in the case of benign tumors or in metastasis to other tissues in the case of malignant tumors. Therefore, it is important to study the control mechanisms of cell replication in order to control cancer cell multiplication. Balb/3T3 cells show density-dependent regulation of multiplication with the final cell density depending on serum concentration in the media. Chemically transformed Balb/3T3 cells (Balb/3T3-D) pile up on each other, multiply to a high cell density, but have decreased DNA synthesis at very high cell densities. Balb/3T3-D cells require less serum for multiplication compared with original Balb/3T3 cells. A rat serum fraction and a bovine β-globulin fraction stimulate the multiplication of Balb/3T3 cells but only slightly stimulate Balb/3T3-D cells indicating different serum factors stimulate growth of these two cell types. The multiplication properties of Balb/3T3-D cells are very similar to those of SV-40 transformed 3T3 cells, however, these properties were brought about by a single treatment by a chemical carcinogen, without an exogenous virus. The transformation altered the contact of cells to one another, indicating a permanent chemical change in the membrane structure.

Quantitative transformation has been induced by carcinogenic chemicals in cells plated to produce discrete colonies. The cell lines derived from the transformation, regardless of carcinogen used, had near-diploid and in rare instances subtetraploid chromosome modes; the tumor-derived cultures were similar to the transformed cell lines in chromosome mode. Because of the lack of common karyotypic changes, the alterations were considered trivial, representing a random occurrance independent of the transformation. Syrian hamster karyotype was established by use of known banding techniques with ASG and trypsin. Each pair of chromosomes was definitely identified on the basis of banding pattern. Differences in patterns between the two techniques were limited to resolution of fine bands in some chromosomes. The pairing of chromosomes on the basis of arm ratio is sometimes uncertain, such confusion does not occur when pairing by matching bands. The trypsin technique results in a banding pattern degree of resolution similar to the ASG technique and is simpler. We have used banding techniques to determine the karyotype of transformed and tumor cells of Syrian hamsters. The results obtained with cells transformed by 4-nitro-quinoline-N-oxide, benzo(a)pyrene, aflatoxin B_1, β-propolactone, and 1,3-propane sultone are reviewed. Although aneuploidy is associated with most of the lines studied, the banding pattern of the chromosomes was sometimes consistent with the normal pattern. Furthermore, the increase in chromosome number did not involve a specific chromosome group. In some instances, a similar abnormal chromosome banding pattern was associated with different carcinogens but not all transformed lines had the same marker even with the same chemical. In some rearrangements, new heterochromatin was detected in the abnormal chromosomes. The significance of the changes to the primary events in transformation is debatable but probably reflects secondary alterations.

The discovery of the extremely large number of carcinogenic agents belonging to diverse classes of chemicals as well as agents of physical and viral origin led to attempts to develop model systems that could be utilized to study the phenomena of carcinogenesis. Many studies have been made to determine the nature of the cell target-insult interaction, the chemical nature of the ultimate carcinogen, the degree to which any agent acts alone (be it viral, chemical or radiation) , and the extent to which one agent interacts with another agent from the same or from a different category of carcinogens. In view of the various hypotheses concerning viruses, chemicals, radiation and cancer, and due to the inherent difficulties in studies in the intact animal, it is advantageous to study the various interactions in a controlled in vitro system. A review was made of studies that concern the interactions of chemicals, viruses and radiation that have been carried out for the most part in vitro. It is concluded that one logical explanation for the enhancement observed when carcinogenic agents of different types interact may be due to interference with normal duplication of DNA. For example, the role of chemical carcinogens in promoting viral tumorigenesis may be related to the formation of additional sites for attachemnt of viral genetic material into cell DNA. These additional sites may be in the form of gaps in the cellular DNA that result from the cells own excision-repair system or from gaps that appear in newly synthesized daughter strands of cell DNA during scheduled DNA synthesis as a result of unrepaired lesions on the parental strand. In the absence of chemical treatment, attachemnt sites may be restricted to points along the newly forming complementary strand in cells undergoing DNA synthesis prior to cell division. Therefore, at any one time, cells susceptible to virus transformation may constitute only a small proportion of the total cell population. The formation of gaps or lesions in the DNA of most or all of the cells exposed to a chemical carcinogen would create additional regions for the incorporation of viral DNA. If viral DNA is available while the lesions are present, an increase in viral transformation would be expected; however, repair of these lesions either by excision-repair mechanisms or by recombinational processes, prior to the availability of viral DNA, would nullify any expected increase.

Significance to Biomedical Research and the Program of the Institute:

The need to study chemical carcinogenesis in vitro is obvious when one realized that carcinogenesis is really a problem in cell ecology. Consequently, in vitro carcinogenesis provides an opportunity to study chemical-target cell interaction and chemical-nontarget cell problems such as other effects of the chemical on cells without the complications of in vivo problems. Therefore, in vitro carcinogenesis has wide applications in terms of studying compounds in man's environment for the sake of control as well as for determining how they alter the physiological process of cells.

Proposed Course of Project:

It is planned to further study the conditions which are responsible for or can alter in vitro transformation. These will include the use of

x-rays, combinations of carcinogens of different classes, the combination of in vivo-in vitro systems, and the effect of stimulators and inhibitors of mixed function oxidases on toxicity and transformation. The mechanisms of early events of carcinogen-cell interaction will be studied. Investigation of different proximate carcinogens and metabolic inhibitors is planned. In addition, experiments are planned to test whether neoplastic transformation has occurred as a result of direct induction by chemicals, as the result of selection or of viral activation. The testing of the tumorigenicity of cells exposed for different time intervals will continue.

Publications

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DiPaolo, J. A.: Quantitative aspects of in vitro chemical carcinogenesis. In Farber, E. (Ed.): Proceedings of the World Symposium on Model Studies in Chemical Carcinogenesis. (In Press).

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DiPaolo, J. A., Takano, K. and Popescu, N. C.: Quantitation of chemically induced neoplastic transformation of Balb/3T3 cloned cell lines. Cancer Res. 32: 2686-2695, 1972.

Oshiro, Y. and DiPaolo, J. A.: Loss of density-dependent regulation of growth of Balb/3T3 cells chemically transformed in vitro. J. Cell Physiol., (In Press).

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Popescu, N. C. and DiPaolo, J. A.: Identification of Syrian hamster chromosomes by acetic-saline-Giemsa (ASG) and trypsin techniques. Cytogenetics 11: 500-508, 1973.

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Serial No. NCI-4671
1. Biology Branch, OASDC, DCCP
2. Cytogenetics and Cytology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Properties of Variant Cell Lines Derived from Chemically Transformed BALB-c/3T3 Cells

Previous Serial Number: None

Principal Investigator: Stephen W. Asher, M. D.

Other Investigators: Charles H. Evans, M. D., Ph.D. Corneliu D. Olinici, M. D., and Joseph A. DiPaolo, Ph.D.

Cooperating Units: None

Man Years:

Total:	1.2
Professional:	1.1
Other:	0.1

Project Description

Objectives:

The primary objective of this project is to examine the properties of variant cell lines which are from a homogeneous population of chemically obtained transformed BALB/3T3 cells. Areas currently being investigated include:

1. Establishment of stable, contact inhibited and non-tumorigenic variants of the DMBA-transformed 3T3 line (D-1 line).

2. Enumeration of similarities and differences between the variants and either the parent cell line (D-1) or the control 3T3 cell lines.

3. Characterization of surface antigens present on the variants and on the D-1 line.

Methods Employed:

Variants are obtained by treatment of transformed cells (D-1) with 5-fluoro-2-deoxyuridine, which selectively favors emergence of cells which are synthesizing DNA more slowly than the transformed cells. The morphologically distinct survivors are isolated and cultured until they appear to be homogeneous. They usually are cloned several times by ring

isolation to assure that the culture is homogeneous. To determine whether the cells exhibit density-dependent regulation of multiplication, they are allowed to become confluent; those cultures not growing in monolayer fashion are either discarded or recloned. Tumorigenicity is screened by inoculating high cell doses (10^8 cells) into sublethally irradiated syngeneic male weanling recipients. Tumor specific transplantation antigens and/or fetal antigens will be investigated with immunofluorescent staining and with the colony inhibition system.

Major Findings:

1. Variants have been induced from a cloned population of DMBA-transformed 3T3 cells. These variants have differing degrees of stability.

2. The variants have growth parameters (doubling time, cloning efficiency and saturation density) which are intermediate between the control 3T3 and the DMBA-transformed parent line.

3. Cytogenetic data indicate that the modal chromosomal number in two of three variants is identical to the hypotetraploid 3T3 and to D-1. One variant remains hypotetraploid but has fewer chromosomes than either D-1 or control 3T3; more importantly, it possesses four unique marker chromosomes.

4. Cell-mediated colony inhibition data tentatively indicate that the D-1 line carried more antigens recognized by lymph node cells from pregnant mice (e.g. fetal antigens) than do the variant cells.

5. Variants are either non-tumorigenic or are tumorigenic only with very large inocula (10^8).

Significance to Biomedical Research and the Program of the Institute:

This system extends those observations made on the control and transformed BALB-c/3T3 cell lines. More importantly, it allows for analysis of certain fundamental properties associated with the process of transformation.

Proposed Course of Project:

Once specific antisera is developed, immunofluorescent characterization can be completed. The cell-mediated inhibitor technique using syngeneic lymph node cells from either pregnant mice of from mice immunized with fetal tissue will be employed in order to quantitate the expression of fetal antigens in the variants.

Serial No. NCI-4673
1. Biology Branch, OASDC, DCCP
2. Cytogenetics and Cytology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Neoplastic Transformation of Guinea Pig Cells by Chemical Carcinogens In Vitro

Previous Serial Number: Same

Principal Investigator: Charles H. Evans, M. D., Ph.D.

Other Investigators: Joseph A. DiPaolo, Ph.D.

Cooperating Units: Cellular Immunity Section and
Office of the Chief, Biology Branch, NCI

Man Years:

Total:	1.6
Professional:	1.1
Other:	0.5

Project Description

Objectives:

The primary objective of this project is to establish conditions and methods for the quantitative study of in vitro chemical transformation of guinea pig cells. The investigation complements the previously developed and current in vitro chemical carcinogenesis system established within the Cytogenetics and Cytology Section employing Syrian hamster embryo cells and the tumor immunology program utilizing in vivo chemical carcinogen induced tumors developed in strain-2 guinea pigs within the Cellular Immunity Section.

The specific objectives of the project are twofold:

1. The development of a rapid assay system for chemical carcinogens suitable for the screening of compounds and populations. As an inbred strain with a low incidence of spontaneous malignancy strain-2 guinea pigs offer a mammalian system in addition to the Syrian hamster for the analysis of chemicals as potential carcinogens and investigation of the mechanisms of chemical carcinogenesis at the cellular level.

2. The development of assays to determine whether in vitro chemical transformation is accompanied by the appearance of tumor specific antigens. The Biology Branch has extensive experience with tumor immunology in

strain-2 guinea pigs. A variety of in vivo and in vitro immunological techniques are presently being utilized. The availability of these techniques and the syngeneic system offered by the inbred strain-2 guinea pig make this system a better choice for immunological study at this time than the Syrian hamster. The development of neoplastic cells transformed in vitro by chemical carcinogens will permit assessment of cell surface antigen alterations associated with chemical carcinogens as well as further understanding of in vivo tumor immunity in strain-2 guinea pigs as a model system for carcinogenesis and tumor immunology in general.

Methods Employed:

The quantitative in vitro chemical carcinogenesis system developed employing Syrian hamster embryo cells within the Cytogenetics and Cytology Section has been utilized throughout this study. Cells are freshly isolated from whole or specific organs of strain-2 guinea pig embryos or fetuses and are grown in the presence or absence of irradiated rat, hamster or strain-2 guinea pig feeder cell layers. The cells as a mass culture or as individual cells are exposed to chemical carcinogen subsequent to seeding the cells in the petri dish.

Approximately one week subsequent to treatment colonies are examined under phase contrast microscopy or light microscopy following fixation and straining of the cells for the number of transformed colonies, toxicity and spectrum of morphology of both the normal and transformed colonies.

Major Findings:

1. The successful mass cell culture of strain-2 guinea pig embryo and fetal cells in Dulbecco-Vogt and minimal essential medium as well as other media incorporating 10% fetal bovine serum.

2. The ability of primary and secondary mass culture cells to clone with a high degree of efficiency in a variety of media with and without feeder layers. Cloning efficiencies of untreated cells as high as 50% with a feeder layer and up to 30% in the absence of a feeder layer have been regularly observed.

3. The development of toxicity following the addition of chemical carcinogens in both mass and cloned cell cultures was similar to that observed in hamster cells exposed to the same chemicals. Carcinogens from several chemical classes have been employed including the polycyclic compounds benzo(a)pyrene, 3-methylcholanthrene and 7,12-dimethylbenz(a)anthracene as well as the nonpolycyclic chemicals such as N-acetoxy-N-2-fluorenylacetamide and N-methyl-N'-nitro-N-nitrosoguanidine. Fetal cells have also been exposed to chemical carcinogen while in utero via the transplacental route following intraperitoneal innoculation of pregnant guinea pigs. The procedure is the same as that developed for the transplacental host mediated in vivo - in vitro hamster bioassay with excision of the guinea pig fetuses 48 hrs subsequent to maternal innoculation and identification of transformation following introduction of the fetal cells into culture.

4. Morphological cellular changes of guinea pig cells exposed to chemical carcinogens and compatible with in vitro chemical neoplastic transformation as observed in Syrian hamster embryo cells have been seen with cells exposed to chemical carcinogen but not to non-carcinogens either while in utero or after introduction into culture. Transformed guinea pig cells exhibit in vitro properties characteristic of transformed hamster and mouse cells. The incidence of tumor formation following innoculation of transformed guinea pig cells, however, is less in irradiated syngeneic guinea pigs than in irradiated hamsters. Complement dependent microcytotoxicity assay with antisera prepared to cultured untreated fetal cells and to cell strains derived from cells transformed following exposure to chemical carcinogens of different chemical classes indicates cross reactivity between the transformed cells and the untreated cultured guinea pig fetal cells. There is some evidence in addition for antigenic differences in the different cell strains but the differences have yet to be defined.

Significance to Biomedical Research and the Program of the Institute:

This project extends the current system of in vitro chemical carcinogenesis within the Cytogenetics and Cytology Section to an additional mammalian system. It also broadens the in vivo chemical carcinogenesis investigations within the Cellular Immunity Section to include in vitro chemical neoplastic transformation of strain-2 guinea pig cells. Development of this system will permit further opportunity to study chemical-target cell interaction without the complications inherent in in vivo analysis. In vitro chemical neoplastic transformation of strain-2 guinea pig cells will provide another assay system in addition to the Syrian hamster for screening of chemicals as potential carcinogens and broaden our understanding of chemical carcinogenesis at the cellular level through inspection of intracellular and cell surface alterations accompanying neoplastic transformation. Development of this new model offers an additional avenue for studying the process of carcinogenesis using a species with established tumor biology in which spontaneous transformation has not been seen and which possesses well defined immunological parameters.

Proposed Course of Project:

Conditions and methods will continue to be refined toward attaining optimal development of in vitro chemical transformation of strain-2 guinea pig cells as a reproducible, quantitative and reliable assay for potential chemical carcinogens. Conditions promoting the neoplastic growth of the transformed cells in syngeneic guinea pigs will be investigated. These will include in addition to irradiation the employment of immunosuppressive agents such as anti-lymphocyte serum and corticosteroids to determine if the absence of tumor formation is the result of immunological rejection of the transformed cells by the syngeneic host. Cell surface antigens of the transformed cells will be further defined to aid in this study and as part of the investigation of the mechanism of carcinogenesis at the cellular level. The establishment of the in vivo and in vitro antigenicity of the cells will be accompanied by specific definition of cell surface antigens including

organ specific, Forrsman, fetal or embryonic and tumor specific antigens. Examination will in turn proceed with extraction, isolation, purification and characterization of cell surface antigens seeking to define their relationship, if any, to chemical carcinogenesis as well as to tumor immunity. The elucidation of these relationships will provide further understanding of the mechanisms of chemical carcinogenesis at the cellular level and ultimately at the more complex levels of tumor establishment and immunity in the host animal.

Serial No. NCI-4636
1. Biology Branch, OASDC, DCCP
2. Immunochemistry Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Physico-Chemical Characteristics of Complement Components

Previous Serial Number: Same

Principal Investigator: Tibor Borsos, Sc.D.

Other Investigators: G. Michael Loos, Ph.D. and Herbert J. Rapp, Sc.D.

Cooperating Units: Johannes Gutenberg University, Mainz, Germany

Man Years:

Total	:	0.8
Professional	:	0.4
Other	:	0.4

Project Description

Objectives:

To isolate chemically and functionally pure serum complement components and to characterize the physico-chemical properties and structure functional relationships of these biologically active molecules.

Methods Employed:

Complement components are purified by a number of techniques including gel filtration, preparative ultracentrifugation (including the B XIV zonal ultracentrifuge), ion exchange chromatography, and a new technique for the separation of molecules based on differences in isoelectric point. The effects on structure and function of isolated complement components of a number of physico-chemical parameters, such as changes in ionic strength, trace metal content, temperature and osmolarity are studied with immunochemical and physico-chemical techniques.

Major Findings:

We have continued to explore the structure and function of the first component of complement (C1). We have obtained evidence that the primary activation of the molecule is due to a hitherto unrecognized fraction in the molecule. This fraction is dissociable, is necessary for starting the internal activation sequence after binding the molecule by antibody-antigen complexes and is not

necessary for the binding of the molecule. Thus C1 in serum consists of two populations: one self-activable and the other non-self-activable. Both populations, however, can be activated by trypsin. These findings show simply that extraneous (non-complement) enzymes in the body may play an important role in the complement sequence of reactions.

Significance to Biomedical Research and the Program of the Institute:

Basic studies of chemical and functional relationships of complement components are directed toward an understanding of the mechanism of complement fixation in general, and have potential application to studies of mechanisms of host resistance to infectious disease and tumors. Specifically, it is necessary to determine the factors which inhibit or enhance the immunologic response to antigens. In the case of complement mediated cytotoxic reactions, isolation of cytotoxic antibody, cell antigens and complement in chemically or functionally pure form permits study of controlled reactions in which an estimate of the importance of a variety of chemical and physical conditions can be made.

Proposed Course of Project:

Further examination of the functional and structural properties of guinea pig and human C1 and C2 are in progress.

Publications

Loos, M., Borsos, T., and Rapp, H. J.: The first component of complement in serum: Evidence for a hitherto unrecognized factor in C1 necessary for internal activation. J. Immunol. 110: 205-212, 1973.

Sassano, F. G., Colten, H. R., Borsos, T., and Rapp, H. J.: Resolution of the first component of guinea pig complement into three subunits, C1q, C1r and C1s, and their hybridization with human C1 subunits. Immunochemistry 9: 405-412, 1972.

Serial No. NCI-4639
1. Biology Branch, OASDC, DCCP
2. Immunochemistry Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Mechanism of Complement Fixation and Action

Previous Serial Number: Same

Principal Investigator: Tibor Borsos, Sc.D.

Other Investigators: Herbert J. Rapp, Sc.D., G. Michael Loos, Ph.D., and Barbara D. Hooks, Ph.D.

Cooperating Units: Johannes Gutenberg University, Mainz, Germany

Man Years:

Total	:	1.4
Professional:		1.2
Other	:	0.2

Project Description

Objectives:

To apply rational complement fixation tests based on the fixation and transfer of the first component of complement to the analysis of antigen-antibody reactions, in particular in the search for cancer specific antigens.

Methods Employed:

The model for studying cytotoxic reactions mediated by antibody and complement consists of sheep erythrocytes, hemolytic antibody and guinea pig complement. Purification procedures for antibodies and the complement components include: preparative (large scale) gel filtration, ion exchange chromatography and preparative free electrophoresis. Other techniques used include precipitin and immunoelectrophoretic analysis, analytical, zonal and preparative ultra-centrifugation and other immuno- and physico-chemical methods.

Major Findings:

The ultimate goal of this work is the development of immunological tools that permit exploration of (1) immunological approaches to cancer diagnosis and (2) possible host immune defense mechanisms against cancer. During the last year we have compared various methods of measuring cytotoxicity of nucleated

cells. We have studied cell killing of Chinese hamster lung cells by antibody and complement by measuring the release of Cr, inhibition of uptake of tritiated thymidine, uptake of trypan blue and colony inhibition. Antisera and complement were obtained from three species. The results showed that the endpoint for a given antiserum and a given complement was the same regardless which method was used to determine cytotoxicity.

In another study we have explored the interaction of phospholipase C with complement. Several enzymes have been claimed to interact with complement directly. Phospholipase C preparations from Clostridium welchii were shown to interact with human and guinea pig complement. This interaction resulted in some sera in the activation of the first component; in others in its inactivation. The complement interacting principle in phospholipase C preparations was separable from the enzymatic activity by Sephadex G-200 chromatography.

Significance to Biomedical Research and the Program of the Institute:

Complement fixation is one of the most widely used diagnostic tools. The development and successful application of a radically new and very sensitive complement fixation test, the C1FT test, opened up new possibilities in determining antibody-antigen reactions on cell surfaces.

Furthermore, cytotoxic reactions due to antibody and complement are prime examples of body defense mechanisms. We believe that fundamental research into the nature and mechanism of complement fixation and action will contribute greatly to the development of diagnostic tools and to the understanding of the mechanism of immune body defenses.

Proposed Course of Project:

This is a long-range project, and we expect little change in the scope of the work during the next few years. The ultimate goals of these projects are the development of better diagnostic tools based on fixation of complement and the elucidation of molecular events associated with the action of complement and antibodies. It is hoped that as a result of our program of inquiry into the basic problem of the interaction of antibodies, antigens and components of complement, tools will be developed that are of practical significance in our search for cancer antigens.

Publications

Loos, G. M. and Borsos, T.: Action of a phospholipase C preparation on the first component of complement of guinea pig and human serum: Lack of correlation with enzyme activity. Infect. Immun. 6: 648-650, 1972.

Loos, M., Borsos, T., and Rapp, H. J.: Immune hemolysis and the functional properties of the second (C2) and fourth (C4) components of complement. J. Immunol. 109: 434-438, 1972.

Opferkuch, W., Snyderman, R., and Borsos, T.: Generation of chemotactic activity by immune complexes carrying clustered or nonclustered $C\bar{4}2$ sites. Eur. J. Immunol., (In Press).

Rice, F. A. H., Ciavarra, R., and Borsos, T.: Effect of leucogenenol on formation of 19S and 7S hemolysin in normal and splenectomized rats. Proc. Soc. Exp. Biol. Med. 140: 471-474, 1972.

Serial No. NCI-4662
1. Biology Branch, OASDC, DCCP
2. Immunochemistry Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Immune Cytolysis

Previous Serial Number: Same

Principal Investigator: Sarkis H. Ohanian, Ph.D.

Other Investigators: Tibor Borsos, Sc.D. and Berton Zbar, M.D.

Cooperating Units: None

Man Years:

Total	:	0.6
Professional	:	0.6
Other	:	0.0

Project Description

Objectives:

A study of the molecular aspects of antibody-complement lysis and antigen topography of nucleated cells.

Methods Employed:

Antibodies to normal and tumor antigens are detected and quantitated by immune cytolysis, colony inhibition, C1 fixation and transfer (C1FT) and by uptake of I^{125} labelled antibody.

Immunochemical methods including Sephadex and DEAE chromatography, electrophoresis, immunodiffusion and ultracentrifugation are employed to isolate antibody protein and complement components.

Major Findings:

The malignant guinea pig cells, L1, L10 and L_2C appear to express less Forssman antigen than their normal tissue counterparts. Both L1 and L10 appear to have the same amount of Forssman antigen. L10 sensitized with anti-Forssman antibody is resistant to lysis by complement while L1 and L_2C are not. By C1FT tests both L1 and L10 sensitized with anti-Forssman antibody have the same number of C1 fixing sites per cell. L10 cells are lysable, however, when sensitized with specific anti-L10 antibody.

Human complement gives a higher degree of lysis than rabbit and guinea pig complement when cells are sensitized with the appropriate antibody.

The resistance of L10 cells to lysis is not due to elution of antibody from the cell surface during the sensitization period or is there loss of C fixing activity of fluid phase and cell bound antibody.

Neuraminidase treatment of L10 cells increases the Forssman antigen expression as measured by quantitative absorption and C1FT tests. These cells are also rendered very susceptible to lysis by Forssman antibody and human complement.

L1 and L10 cells grown in culture show the same characteristics to antibody-complement lysis as in vivo passed L1 and L10. Skin tests and tumor growth studies using tissue culture tumor cells indicate they are not different from in vivo grown tumor cells.

L1 and L10 tumor cells passed in guinea pig have detectable guinea pig C4 on their surfaces. Removal of macrophages from the ascites preparation reduces the number of C4 molecules/L1 cell. Similar treatment of ascites L10 cells does not affect the number of C4 molecules/L10 cells. L10 cells passed on C4 deficient guinea pigs have no detectable C4 bound to their surface.

One method tested thus far for isolating tumor cells in specific growth phases from mass cultures is promising. The method employs velocity sedimentation of the cells through a 5-20% Ficol gradient. Preliminary experiments suggest a separation of G1 and S phase cells from log phase growth cultures may have been achieved. This separation was not achieved if early stationary phase cultures were applied to the gradient.

L_2C, a strain 2 guinea pig lymphoma, has been found to be very susceptible to lysis by Forssman or anti-strain 2 antibody and human C. Quantitation absorption and C1FT tests indicate these cells have a very low expression of Forssman antigen activity as compared to L1 and L10 cells (approximately 2800 C1 site/L_2C and 30,000 C1 sites/L1 and L10 at saturating levels of antibody). Human C gives a higher degree of lysis than guinea pig and rabbit C when the tumor cells are sensitized with either Forssman antibody or anti-strain 2 antibody. L_2C cells sensitized with anti-Forssman are more susceptible to lysis with guinea pig C than with rabbit C.

Significance to Biomedical Research and the Program of the Institute:

The elucidation at the molecular level, of the effects of antibody and complement on both normal and malignant cells may be important in understanding control of cell growth by immunologic mechanisms.

Control of tumor cell growth in vitro makes it possible to study the action of specific and nonspecific agents on the cells at different stages of their growth cycle. The knowledge obtained from in vitro experiments will be very helpful when applied to the study of more complicated in vivo conditions.

Proposed Course of Project:

More extensive work will be carried out on the effect of antibody and complement on lysis of nucleated cells. This will include class of antibody (both Forssman and antitumor) and binding of complement components.

Neuraminidase treatment of L1, L10 and L_2C will be carried out and its effect on antigen expression and sensitivity to C lysis in more detail.

Work will continue on the role of cell growth phase on antigen expression and susceptibility to complement lysis.

Publications

Ohanian, S. H., Borsos, T., and Rapp, H. J.: Lysis of tumor cells by antibody and complement. I. Lack of correlation between antigen content and lytic susceptibility. J. Natl. Cancer Inst., (In Press).

Serial No. NCI-4663
1. Biology Branch, OASDC, DCCP
2. Immunochemistry Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Interaction of Cell-bound Complement with Antigen-Antibody Systems

Previous Serial Number: Same

Principal Investigator: Virginia C. Dunkel, Ph.D.

Other Investigators: Tibor Borsos, Sc.D.

Cooperating Units: None

Man Years:

Total	:	1.4
Professional	:	1.2
Other	:	0.2

Project Description

Objectives:

To measure complement components binding to cell surfaces as a result of interaction of antigen-antibody complexes with complement. This is being done for several purposes: 1) as a sensitive detection system for very low concentrations of antibody; 2) to study the basis for the differences among cells in susceptibility to complement lysis, and 3) to measure binding of complement to human and animal tumor cells.

Methods Employed:

Sheep red cells, appropriate antisera, lytic tests, immunoelectrophoresis and the C1 fixation and transfer test were used. A variety of solid phase C4 and C3 intermediates were made. For studies on C3 inactivator, we used both normal serum and serum from a patient deficient in C3 inactivator.

Major Findings:

The third component of complement (C3) binds to cell surfaces due to the interaction of cell bound antibody with complement. The bound C3 in tissue can be detected by antibodies to C3. Cells with bound C3-anti-C3 antibody complexes can be lysed by the addition of complement. We have been exploring the mechanism of "passive" lysis with various cell bound antigens including

C3 bound by the C sequence and by chemical means. Albumin and IgG also served as antigen. We have found that C3 bound by the complement sequence leads to rapid but limited passive lysis as compared to all other antigens and methods of coupling.

For this study we have also explored the various chemical methods for coupling antigens to cell surfaces. $CrCl_3$ turned out to be the best agent for this purpose.

Significance to Biomedical Research and the Program of the Institute:

Since C3 is pivotal in many of the reactions due to complement (cytotoxic, chemotactic, immune adherence, phagocytosis), the finding that C3 bound to cells by different mechanisms may function differently may elucidate mechanism of passive lysis and of amplification of cell damage by complement.

Proposed Course of Project:

We plan to couple tumor antigens to red cell surfaces to permit the development of a rapid and accurate method for measuring tumor antigens.

Serial No. NCI-4669
1. Biology Branch, OASDC, DCCP
2. Immunochemistry Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Separation of L-Asparaginase from Complement Fixing Antigenic Impurities

Previous Serial Number: Same

Principal Investigator: Tibor Borsos, Sc.D.

Other Investigators: G. Michael Loos, Ph.D., S. Vadlamudi, Ph.D., and Sidney Shifrin, Ph.D.

Cooperating Units: Johannes Gutenberg University, Mainz, Germany, Microbiological Associates, Bethesda, Maryland, and the Macromolecular Biology Section, LCBGY, NCI

Man Years:

Total	: 0.4
Professional	: 0.4
Other	: 0.0

Project Description

Objectives:

To separate L-asparaginase (E. coli) from contaminating bacterial antigenic material. To design methods for monitoring presence of biologically active impurities.

Methods Employed:

Standard biochemical and immunochemical methods for separation of macromolecules from complex mixtures are employed. They include gel filtration, ultracentrifugation, chromatography, complement fixation.

Major Findings:

Of 15 L-asparaginase preparations tested, 13 inhibited whole human serum complement; the inhibitory effect ranged from 12 to 45 percent. We have partially characterized the anticomplementary activity in one of the L-asparaginase preparations. The anticomplementary activity was separable from the L-asparaginase activity on Sephadex G-200. Fractions containing L-asparaginase had no effect either on whole human serum complement or on

human serum C1, the first component of complement; these fractions were therapeutic in leukemic mice. Fractions containing the anticomplementary activity had no significant therapeutic effect in leukemic mice. L-asparaginase and the anticomplementary factor are antigenically distinct, and the anticomplementary factor was shown to be antigenically related to Escherichia coli lipopolysaccharide (endotoxin).

Significance to Biomedical Research and the Program of the Institute:

It has been known that patients often react to L-asparaginase by exhibiting severe anaphylactic type reactions. If these undesirable reactions are primarily due to a bacterial, endotoxin type contaminant, its elimination from the enzyme preparations should relieve the patients from severe side effects and may permit the administration of more effective doses of the enzyme.

Proposed Course of Project:

Project ended.

Publications

Loos, M. and Borsos, T.: Inactivation of complement by L-asparaginase preparations not correlated with enzyme content. Nature (New Biol.) 237: 55-56, 1972.

Loos, M., Vadlamudi, S., Meltzer, M., Shifrin, S., Borsos, T., and Goldin, A.: Detection of endotoxin in commercial L-asparaginase preparations by complement fixation and separation by chromatography. Cancer Res. 32: 2292-2296, 1972.

Serial No. NCI-4674
1. Biology Branch, OASDC, DCCP
2. Immunochemistry Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Antigenic Changes in Neoplastic Transformation of Guinea Pig Cells by Chemical Carcinogenesis *In Vitro*

Previous Serial Number: None

Principal Investigator: Sarkis H. Ohanian, Ph.D.

Other Investigators: Charles H. Evans, M.D., Ph.D.

Cooperating Units: None

Man Years:

Total	:	0.3
Professional	:	0.3
Other	:	0.0

Project Description

Objectives:

A search for changes in surface antigen expression which may be associated with *in vitro* transformation of guinea pig cells by selected carcinogens.

Methods Employed:

Antibodies to normal and chemically transformed cells are produced in guinea pigs and rabbits. Specificity of the antisera is obtained by absorption and tested by complement lysis of cells.

Tissue culture techniques and animal inoculation are employed for maintaining the cells and testing for tumorigenicity respectively.

Major Findings:

Guinea pig embryonic fibroblasts can be transformed by selected carcinogens. An untreated embryonic cell line and two cloned morphologically distinct transformed lines exposed to carcinogens of different classes were chosen for study. All three cell lines appear to share antigens. Preliminary absorption tests suggest the transformed cells also possess specific antigens(s).

Dulbeccos and 1040 tissue culture media appear to give optimal growth as measured by the rate of log growth. Cells for cytotoxicity testing were obtained from log growth cultures (S phase enriched). EDTA was found superior to trypsin in harvesting of the cells. In the cytotoxicity test a higher percentage of dead cells in the cell and complement controls were noted with the trypsin harvested cells.

Significance to Biomedical Research and the Program of the Institute:

It will be possible to answer 1) what specific or general antigenic changes occur with in vitro chemical carcinogenesis and 2) when do the changes occur in relation to the development of transformation and neoplastic growth.

The detection of cell surface antigen changes during the transformation process would be of great importance in the detection of the precancerous state.

Proposed Course of Project:

Studies will be performed in guinea pigs to determine the tumorigenic capacity of these cell lines. More extensive work in the preparation of specific antisera in volumes adequate for long-range studies will be carried out. This work will include the preparation and use of anti-embryonic antigen as well as Forssman antigen.

Additional techniques for the detection of antibody will be employed and will include fluorescence microscopy, complement fixation and transfer test, and colony inhibition tests.

Serial No. NCI-4676
1. Biology Branch, OASDC, DCCP
2. Immunochemistry Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Detection of Complement Components on Nucleated Cell Surfaces

Previous Serial Number: None

Principal Investigator: Sarkis H. Ohanian, Ph.D.

Other Investigators: Tibor Borsos, Sc.D. and Berton Zbar, M.D.

Cooperating Units: None

Man Years:

Total	: 0.3
Professional	: 0.3
Other	: 0.0

Project Description

Objectives:

1. To determine whether tumor cells of human and guinea pig origin have detectable C4 on their surfaces.

2. To develop techniques for the detection of other complement components bound to the cell surfaces.

Methods Employed:

A new method has been developed to detect human and guinea pig C4 bound to cell surfaces. Immunochemical methods including DEAE chromatography, electrophoresis and immunodiffusion are employed to isolate antibodies, protein and complement components. Quantitation of antibody and complement components are carried out using radioisotopic labelling and quantitative precipitin procedures.

Major Findings:

Human and guinea pig C4 can be bound to the surface of sheep RBC and remain bound for up to two weeks. These cells are employed in an inhibition of lysis test which can detect as little as 184 ng guinea pig C4 and 157 ng human C4 (approximately 6×10^{11} molecules C4).

Tumor cells obtained from guinea pigs have detectable C4 on their surfaces. Removal of macrophages from ascites tumor preparations reduces the number of C4 molecules/L1 cell but not L10 cells. L10 cells passed on C4 deficient guinea pigs have no detectable C4 bound to their surfaces.

Quantitative precipitin tests using radiolabelled antibody indicate that human and guinea pig serum contain 942 ± 260 µg human C4/ml and 1107 ± 106 µg guinea pig C4/ml.

Significance to Biomedical Research and the Program of the Institute:

Techniques for detecting bound complement components will be very useful in determining whether a humoral immune response has been produced to tumor cells in vivo. Methods used for the detection of humoral immunity to tumor cells do not have the sensitivity of this test procedure and therefore may yield misleading results.

The knowledge obtained from these studies will be helpful in determining the role of humoral immunity in the control of tumor growth.

Proposed Course of Project:

Normal tissue and primary explants of a variety of human and selected guinea pig tumors will be examined for cell bound C4. New techniques will be developed for detection of late-acting complement components.

Serial No. NCI-4667
1. Biology Branch, OASDC, DCCP
2. Tumor Antigen Section
3. Bethesda, Maryland

PHS-N''
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Isolation and Study of Tumor-Specific Antigens

Previous Serial Number: Same

Principal Investigator: Edward J. Leonard, M.D.

Other Investigators: Arleen K. Richardson, Ph.D., Monte S. Meltzer, M.D., Howard G. Smith, M.D., Eva Klein, M.D., Tibor Borsos, Sc.D. and Herbert J. Rapp, Sc.D.

Cooperating Units: Department of Tumor Biology, Karolinska Institute Stockholm, Sweden

Man Years:

Total	1.7
Professional	1.5
Other	0.2

Project Description

Objectives:

To obtain tumor-specific antigens in soluble and partially purified form. To use these antigens in inducing, establishing and measuring tumor-specific immunity in guinea pigs. Knowledge derived from the guinea pig model will be applied to the isolation, purification and characterization of human tumor-specific antigens. These antigens will be then used in diagnostic studies of human tumor epidemiology, in monitoring patient response to treatment and as an aid in treatment. Initial studies will be on the ascites cell form of diethylnitrosamine induced hepatoma of strain-2 guinea pigs.

Methods Employed:

Initial extraction: 3M KCl and other hypertonic salts. Further purification: ammonium sulfate precipitation, gel filtration, DEAE chromatography. Assay methods: skin testing; *in vitro* correlates of cellular immune responses: lymphocyte blastogenesis, inhibition of macrophage migration; immunization of guinea pigs with extracts and challenge of test animals with live tumor cells. Production and testing of heterologous antisera to tumor antigen: immunization of rabbits with whole tumor cells, absorption of sera with various cell suspensions, testing of absorbed sera by C1 fixation and transfer test and by immune fluorescence.

Major Findings:

1. Line-1 and line-10 tumor antigens. Rabbit antiserum obtained by immunization with intact line-10 cells, after absorption with normal guinea pig tissue, had residual antibody against line-1 cells. This could be removed by absorption with either line-1 cells or guinea pig embryo cells. Anti-line-1 antiserum showed the same absorption pattern. We concluded that line-1 and line-10 have not only tumor-specific antigens but also a common embryonic antigen. The latter is not sufficient to elicit cross-reactive responses when cells are tested intradermally in line-1 or line-10 immune guinea pigs, but can be detected by C1FT tests for antibody binding.

2. Additional assays for line-1 and line-10 antigen. Assay for antigen by skin tests in specifically immune guinea pigs is reliable but precision is limited. Therefore we have devised a new antigen assay, using rabbit antibody specific for line-1 or line-10 antigen. Antigen is added to a fixed amount of specific antibody and then the mixture is equilibrated with a suspension of tumor cells. Residual antibody binds to the tumor cells. Binding has been measured either by the C1FT test or by estimation of fluorescence intensity on the cell surface after addition of fluorescein-conjugated anti-rabbit globulin. This assay is more precise than the skin test and is being used in studies on extraction and further purification of line-1 and line-10 antigen.

3. Rabbit anti-line-10 antibody induced movement of line-10 antigen within the plane of the tumor cell membrane. Cells were equilibrated at $0^{o}C$, first with anti-line-10 antiserum and then with fluorescein conjugated anti-rabbit serum. Under the fluorescence microscope the cells appeared to be outlined by fluorescent rings of uniform intensity. When the cells were re-examined after two to three hours incubation at $37^{o}C$, the fluorescence was in discrete aggregates and in some views the cells appeared to be extruding the aggregated material. Aggregation was shown to involve movement of the whole antigen-antibody complex, so that in effect the antigen was stripped from the tumor cell surface. It reappeared in its uniform surface distribution within four to six hours.

4. After negative results in previous years, we found circulating antitumor antibody in tumor-bearing guinea pigs. Sera were tested by equilibrating with line-10 tumor cells in vitro and then using a fluorescein-conjugated anti-guinea pig IgG for detection of bound antibody. The bound antibody has also been detected by the C1FT test.

5. Tumor-specific antigen recognition responses were demonstrated in guinea pigs with line-10 tumor by inhibition of macrophage migration when peritoneal exudate cells were tested in vitro with line-10 antigen extracts.

6. Capacity of guinea pigs immunized with line-10 antigen to reject a challenge of line-10 tumor cells. Despite the fact that all guinea pigs immunized with line-10 antigen developed strong cutaneous delayed hypersensitivity reactions to line-10 antigen, their capacity to reject line-10 tumor varied. The results included no growth of tumor; appearance of palpable tumor after a delay of several weeks post challenge; growth of tumor in the skin and then regression and complete healing, only to be followed in some cases by metastases in regional lymph nodes.

Significance to Biomedical Research and the Program of the Institute:

The newly developed assays for tumor antigen will facilitate attempts to isolate antigen in highly purified form suitable for studies on its chemical nature and for induction of specific antibody. Purified antigen and monospecific antibody would provide opportunities to experiment with antitumor vaccines, to detect development of antigen in tissue culture carcinogenesis models and to study host responses against neoplastic cells. Some of these studies are already in progress, using the rabbit antitumor antibody. The line-10 protection experiments provide a model similar in certain respects to human cancer in which despite evidence for delayed cutaneous hypersensitivity to tumor antigen the outcome of the disease cannot be predicted. The work on surface antigen movement raises the question whether antibody in vivo can lead to stripping of tumor surface antigen so that the host would have the double disadvantage of antigen-deficient cells and tumor antigen immune complexes in the circulation.

Proposed Course of Project:

Further purification of line-1 and line-10 antigen; analysis of the immune responses occurring in the line-10 protection experiments; additional studies on antigen movement if the reaction can be induced by a single antibody.

Publications

Leonard, E. J.: Cell surface antigen movement: Induction in hepatoma cells by antitumor antibody. J. Immunol., in press.

Leonard, E. J., Meltzer, M. S., Borsos, T., and Rapp, H. J.: Properties of tumor-specific antigen solubilized by hypertonic potassium chloride. Conference on Immunology of Carcinogenesis. J. Natl. Cancer Inst. Monogr. 35. U.S. Dept. of Haalth, Education, and Welfare, Public Health Service, Wash., D. C., U.S. Govt. Print. Off., 1972, pp. 129-134.

Meltzer, M. S. and Leonard, E. J.: Enhanced tumor growth in animals pretreated with complete Freund's adjuvant. J. Natl. Cancer Inst. 50: 209-218, 1973.

Meltzer, M. S., Oppenheim, J. J., Littman, B. H., Leonard, E. J., and Rapp, H. J.: Cell-mediated tumor immunity measured in vitro and in vivo with soluble tumor-specific antigens. J. Natl. Cancer Inst. 49: 727-734, 1972.

Serial No. NCI-4668
1. Biology Branch, OASDC, DCCP
2. Tumor Antigen Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Calcium Transport Globulin System (Cardioglobulin)

Previous Serial Number: Same

Principal Investigators: Edward J. Leonard, M.D. and Stephen Hajdu, M.D.

Other Investigators: John Coe, M.D.

Cooperating Units: Laboratory of Kidney and Electrolyte Metabolism, NHLI, and Rocky Mountain Laboratory, NIAID, Hamilton, Montana

Man Years:

Total	0.2
Professional	0.2
Other	0.0

Project Description

Objectives:

To develop immunochemical assay methods for free and cell-bound calcium transport globulin system (CTGS); to apply these methods to the isolation and purification of CTGS components; and to look for CTGS alterations in disease.

Methods Employed:

Isolated frog hearts were used for bioassay for human CTGS components. Standard techniques of protein fractionation were used to separate CTGS components from other serum proteins. After CTG-B was bound to frog hearts, it was extracted with glycerol and the extracts were injected with Freund's adjuvant into bullfrogs for production of antibody to CTG-B.

Major Findings:

1. Production of antibody to CTG-B in bullfrogs. Previous work suggested that the first step in CTGS action on bioassay frog hearts was binding of CTG-B to the heart. The subsequent action of CTG-A and -C resulted in calcium transport into the heart muscle cells. On the assumption that CTG-B did bind to frog hearts, we exposed hearts to solutions containing CTG-B, then equilibrated with saline solutions to remove unbound serum protein, then homogenized the hearts and made alkaline glycerol extracts of the homogenate which were injected

with complete Freund's adjuvant into bullfrogs. The bullfrogs made antibody to CTG-B which inhibited the biological activity of bound CTG-B on the frog heart. Use of bullfrogs to produce antibody avoided production of autoantibody against the assay heart. The observed inhibition of biological activity provided unequivocal proof of the presence of antibody to CTG-B and also confirmed our hypothesis that binding of CTG-B occurred as the first step in the action of the CTGS on the frog heart.

2. Stabilization of the CTGS in serum. Purification of CTGS components has been hampered by their great lability. This problem has been solved by a change in the method of obtaining human serum. Venous blood is drawn into an iced plastic bag without anticoagulant. At 0°C there is sufficient time to centrifuge the blood and remove the supernatant plasma before clotting occurs. The plasma is then warmed to room temperature and poured into a glass beaker; clotting then occurs promptly, the clot retracts and expresses a clear serum. The CTGS components in this serum have been remarkably stable. CTG-A has survived sodium sulfate precipitation, CM and DEAE chromatography, sucrose density ultracentrifugation and isoelectric focusing. CTG-B has been fractionated by salt precipitation into two components. A twenty-fold purification of CTG-C has been obtained with sodium sulfate and then precipitation as an euglobulin.

Significance to Biomedical Research and the Program of the Institute:

Although there is much speculation in scientific literature about the possible role of calcium as a regulator of cell functions, including secretory activity, motility and contact inhibition, there is practically no solid evidence. We have shown that CTGS is a serum protein system that can affect transmembrane calcium movement in isolated frog hearts. Isolation and characterization of the components of this system and the development of antisera to the components should enable us to answer questions about its tissue localization and biological function.

Proposed Course of Project:

Further purification of CTGS components, production of monospecific antisera to these components; use of the antisera to determine tissue localization of CTGS and to measure free- and tissue-bound CTG components in human disease.

Serial No. NCI-4675
1. Biology Branch, OASDC, DCCP
2. Tumor Antigen Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Immunological Mechanisms of Tumor Rejection

Previous Serial Number: None

Principal Investigators: Edward J. Leonard, M.D. and Monte S. Meltzer, M.D.

Other Investigators: Arleen K. Richardson, Ph.D., Howard G. Smith, M.D., Bruce S. Zwilling, Ph.D., Glenn E. Trivers, Gerald L. Bartlett, M.D., Robert C. Bast, Jr., M.D., Berton Zbar, M.D., and Tibor Borsos, Sc.D.

Cooperating Units: None

Man Years:

Total	: 1.7
Professional	: 1.5
Other	: 0.2

Project Description

Objectives:

To determine what immunological mechanisms occur in tumor cell killing in order to provide a rational basis for elimination of tumor cells by immunological means.

Methods Employed:

Chemically induced murine and guinea pig tumors, maintained by passage in syngeneic hosts, were used. Antigen recognition (afferent limb) responses were monitored by specific antigen induced lymphoblastic transformation, inhibition of peritoneal exudate macrophage migration and delayed cutaneous hypersensitivity reactions. Cytotoxic responses (efferent limb) were measured by inhibition of cellular uptake of radioactive amino acids or by release of radioactive label from damaged cells.

Major Findings:

1. Murine tumor cell monolayers were destroyed in vitro by PPD-stimulated, BCG-immune spleen cells. This cytotoxicity was initiated by antigens unrelated to the murine tumor associated antigens and was mediated by soluble cytotoxins released from the stimulated spleen cells. Syngeneic tumor cells were more susceptible to this form of cytotoxic injury than were syngeneic normal cells.

2. We have begun isolation and purification of mouse cytotoxins produced by mouse spleen cells after stimulation by PHA. A quantitative assay for mouse lymphotoxin has been worked out based on inhibition of radioactive leucine uptake by lymphotoxin-treated mouse tumor cells. Production of lymphotoxin by PHA-treated spleen cells has been obtained in serum-free tissue culture media; this provides a cleaner starting material for purification.

3. We have produced another example of tumor cell damage during a cellular immune reaction to antigens unrelated to the tumor. PE cells from guinea pigs immunized with BCG were placed in tissue culture media containing PPD. The mixture was toxic to line-10 tumor cells. The toxicity was mediated in great part by soluble cytotoxins. Studies are in progress to determine what cells in the PE mixture account for the toxicity.

4. Toxicity to line-10 cells in vitro by PE from guinea pigs with cellular immunity to line-10 tumor. We have demonstrated specific antigen recognition responses in vitro by inhibition of macrophage migration when soluble line-10 antigen was added to PE from line-10 immune guinea pigs. In order to study the effector arm of this response line-10 cells have been labelled with tritiated thymidine and subsequent loss of nuclear label has been followed as an index of cell death. The results are preliminary, but it is probable that cytotoxicity by specifically immune cells can be demonstrated in this system.

5. A regularly observable toxicity occurred when normal guinea pig spleen cells or normal peripheral blood human mononuclear cells were added to guinea pig line-10 tumor cells sensitized with IgG isolated from rabbit anti-guinea pig serum.

Significance to Biomedical Research and the Program of the Institute:

Analysis of the effector immune events leading to tumor rejection may provide a rational basis for manipulation of host responses or tumor cell in an effort to eliminate or prevent progression of the tumor.

Proposed Course of Project:

Physicochemical characterization of soluble cytotoxins, determination of their mechanism of action, administration of cytotoxins in animal tumor models. Analysis of the cytotoxic action of PE cells, stimulated either by tumor antigens or by antigens unrelated to the tumor. Further characterization of antibody-mediated lymphocyte cytotoxicity to ascertain its role in vivo.

Publications

Meltzer, M. S. and Bartlett, G. L.: Cytotoxicity in vitro by products of specifically stimulated spleen cells: Susceptibility of tumor cells and normal cells. J. Natl. Cancer Inst. 49: 1439-1443, 1972.

Serial No. NCI-4677
1. Biology Branch, OASDC, DCCP
2. Tumor Antigen Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Mononuclear Cell Chemotaxis

Previous Serial Number: None

Principal Investigators: Edward J. Leonard, M.D. and David A. Boetcher, M.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total	: 1.2
Professional	: 1.2
Other	: 0.0

Project Description

Objectives:

To develop quantitative measures of reactions occurring in the effector limb of the immune response and to determine whether they are altered in tumor-bearing animals or patients.

Methods Employed:

Complement-derived (C5a) and lymphocyte-derived (LGCF) chemotactic factors were generated, purified and stored in aliquots so that samples of standard preparations would be available over a long period. Venous blood samples were obaained from cancer patients, hospitalized patients without cancer and normal controls. Peripheral blood mononuclear cells were isolated on Ficoll-Hypaque gradients. Responses to chemotactic factors were measured in modified Boyden chambers, the cell suspension being separated from the solution of chemotactic factor by a nucleopore membrane with 5 micron diameter pores.

Major Findings:

1. Dose-response data have been obtained for the response of normal peripheral blood mononuclear cells to standard preparations of C5a and LGCF. We have established the range of response of mononuclear cells obtained by serial bleedings of normals over a period of months.

2. The response to a standard concentration of LGCF is impaired in some but not all cancer patients. The mean response for a group of 31 cancer patients was 15.8 (s.d. 2.2) cells migrated/oil field compared to 24 (s.d. 2.5) for a group of 10 normal controls and 25.0 (s.d. 1.3) for a group of 8 patient controls.

3. As a substitute for visual counting of responding cells, we have labelled cells with ^{51}Cr and measured chemotactic responses by counting the amount of gamma radiation adhering to the Nucleopore membrane.

Significance to Biomedical Research and the Program of the Institute:

Methods to quantify the effector arm of the immune response are needed in the evaluation of host response to tumor, from the viewpoint of both early changes in carcinogenesis and late alterations when there is a large tumor burden.

Proposed Course of Project:

Completion of the study outlined in item 2 above. Longitudinal study of abnormal chemotaxis in cancer patients.

SUMMARY REPORT

CHEMISTRY BRANCH

July 1, 1972 through June 30, 1973

The goal of the Chemistry Branch is to understand the molecular events causing cancer and to elucidate those processes which require insight in order to develop inhibitory mechanisms capable of preventing cancer. The research program is designed to understand the molecular basis by which carcinogenic agents cause malignant transformation and those exogenous and endogenous factors which modify the course of carcinogenesis. The Branch seeks to clarify the interaction of exogenous agents and internal factors in the living organism at the molecular, cellular and host levels and seeks to understand the consequences of these interactions in terms of cell regulation and carcinogenesis.

Cancer is a disease in which the expression of genetic information is altered to give the tumor phenotype. This altered phenotypic expression may be due to genetic or epigenetic events induced by either xenobiotic or endogenous factors which result in altered patterns of gene control. The central aim of the Branch is to understand how exogenous carcinogens and endogenous factors are processed by enzymatic mechanisms, how the carcinogen is converted to active forms and the nature of the interaction between the latter and the gene action system. Understanding is sought in how this initial interaction results in the modifications which characterize malignant transformation. The aim of these studies is modification, elimination, or prophylaxis of the carcinogenic response in the human population.

The Office of the Chief and the Molecular Carcinogenesis Section study the molecular events of malignant transformation induced by chemical carcinogens, in particular, the polycyclic hydrocarbon type. The primary focus of these studies is to understand the enzymatic conversion of carcinogens to either detoxification forms or to the active carcinogenic metabolite. In parallel studies the interaction of the active metabolite with the relevant cellular receptor has been investigated. During man's evolution he has been constantly exposed to foreign compounds and carcinogens and has developed metabolic systems for their detoxification and elimination. These studies have shown that this system is primarily that of microsomal Cytochrome P-450 mixed function oxygenase. Thus the vast majority of foreign compounds are metabolized by this enzyme system. It has been found that this enzyme was influenced by a variety of environmental factors such as previous exposure to drugs, pesticides or carcinogens, nutritional and hormonal state, the age, sex and genetic makeup of the organism. Also it was found that this enzyme system is responsible for the conversion of procarcinogens to their ultimate carcinogenic form. In addition this enzyme was found to be responsible for the toxic effects of the polycyclic hydrocarbons. The latter important conclusions are derived from five lines of evidence developed in this laboratory. 1) The toxicity of benzopyrene (BP) parallels level of enzyme in the cell. 2) The enzyme system catalyzes the formation of BP-DNA complexes. 3) The discovery of an inhibitor of the enzyme system (7,8-benzoflavone) which prevents BP cytotoxicity. 4) The inhibitor of the enzyme prevents the binding of BP to DNA,

RNA and protein. 5) Inhibition of the enzyme system reduces dimethylbenz-anthracene (DMBA) tumorigenicity by 90%.

Although certain carcinogens seem to be activated by the enzyme complex, others may be primarily detoxified. Current studies seek to identify the nature of the chemical structures which are activated by the enzyme system and those structures which are detoxified.

The role of the enzyme system in human carcinogenesis by chemicals is being investigated. A major finding has been that the enzyme system can be identified and measured in a preparation of lymphocytes from human blood. This is the first report of the presence of this enzyme in an easily obtainable human tissue. This finding will be the starting point of an analysis of the enzyme level in a human population and its relevance to chemical carcinogenesis.

A number of different aspects of the P-450 carcinogen metabolizing enzyme systems is being studied in which progress has been made. 1) A highly sensitive and quantitatively reproducible method has been developed for the isolation and identification of eight metabolites of benzopyrene. Those include the previously unreported K-region diol which seems to be particularly related to carcinogenic activity. It was found that the ratio of this possibly ultimate carcinogen may be altered by different enzyme levels and is found in different proportions in different tissues and species. The goal of this project is to relate the metabolic profile of BP to carcinogenicity in different tissues and species. 2) Determination of the rate of metabolite formation for the different metabolites and the relationship of this to substrate and enzyme level. This information may enable an understanding as to why certain kinds of exposures to carcinogens are partially effective in transformation while other exposures fail to cause malignancy. 3) The regulation of this enzyme system is being intensively studied. A major finding has been that that this enzyme is present and highly inducible in a cloned cell line derived from rat liver. This will eventually enable a study of the genetics of this enzyme system. In the past year the kinetics of aryl hydrocarbon hydroxylase (AHH) induction, the nutritional requirements, and the requirement of macromolecule synthesis have been described. An important finding has been that temporary inhibition of protein synthesis is followed by a large rise in enzyme level even in the absence of the polycyclic hydrocarbon inducer. This finding indicates that the level of regulation of this enzyme is present at least at two different sites in the cell. Thus enzyme level is controlled by the amount of RNA transcription from the gene and secondly by the process regulating the translation of messenger RNA (mRNA) into protein at the level of the cytoplasm.

In other studies the regulation of this enzyme system was determined in somatic cell hybrids of parent cells that differ both in their basal levels as well as in the levels of inducible AHH. In certain sets of hybrids, the hybrid cell enzyme was considerably less than either parent while in other hybrids the enzyme level was greatly enhanced. Thus these studies indicate the multi-faceted regulation of this enzyme and more importantly demonstrate that cells can be constructed experimentally with unique levels of carcinogen metabolizing activity, either with high, low or intermediate levels. These

cells can then be used for studies in transformation or in an assay for carcinogen activity.

In another approach, a number of compounds have been sought and found which can greatly modify carcinogen metabolism by either inducing the enzyme to high levels or by inhibiting the enzyme system. In one case a compound was found which can enhance enzyme activity by what appears to be an allosteric rather than an induction mechanism. These compounds are being tested for their effect on tumorigenesis. Thus upon learning enough about the role of the enzyme in carcinogenesis it may be possible to modify the enzyme activity in a specific manner and hence modify the course of carcinogenesis.

In an entirely new development, in collaboration with the Biology Branch, it was found that the enzyme is present and highly inducible in tissues engaged in immunological activity. Thus lymphocytes and macrophages, and other lymphoid tissues contain the enzyme and are highly inducible. This research opens up an entirely new area of investigations of the role of the enzyme system in the processing of the carcinogen as an antigen and in investigations on the role of the immune system in carcinogenesis.

The Cell Growth Regulation Section has the primary goal to define biochemically the tumor cell and in particular the nature of those molecules responsible for converting a normal cell to its malignant form. One approach is to examine certain classes of molecules known to be involved in protein synthesis. The class of molecules called transfer RNAs (tRNA) are known to function in the assembly of the proper amino acids into the growing protein chain. Another role which has been indicated for these molecules is that of the regulation of protein synthesis in terms of the initiation, elongation and termination of the growing protein molecule. The majority of these studies in the past have been with biochemical systems derived from microorganisms. A recent finding has been that mammalian cells were found to contain tRNAs which recognize nucleotide sequences which are considered "nonsense" sequences. The latter result in the termination of the growing peptide chain. In preliminary studies it was found that mammalian cells contain tRNAs which can suppress this "nonsense" sequence and prevent chain termination. Thus it seems that mammalian cells can suppress mutations which may result in "nonsense" sequences and which may be involved in mutational changes resulting in carcinogen transformation. The role of these important tRNA molecules may be central in the continued repair or suppression of mutagenic and carcinogenic events occurring either at a DNA or at the translational level.

Viruses which transform cells into malignant forms have been used effectively to examine physiological processes involved in development of the malignant state. Cellular division, although necessary to the growth of a tumor, was shown to be unnecessary for the induction of metabolic changes accompanying malignancy, i.e., a cell can become potentially malignant without dividing.

Mutants of tumor viruses have been isolated, and cells infected with these mutants act as tumor cells at one temperature and as normal cells at a higher temperature. The morphological change characteristic of malignant transformation can be separated from several biochemical changes which occur as a result of transformation, and a search for the temperature sensitive

molecule responsible for the morphological change is underway. A series of preliminary results suggest that the molecule is a protein directly involved in the uptake of water and cations into cells.

Much attention has been given recently to the possibility that RNA-containing tumor viruses may be responsible for tumors in man. A program to determine the biochemical sequence in the reproductive cycle of these viruses has been in progress form many years. The finding that a new DNA was involved in virus reproduction led directly to the discovery of enzymes which synthesize DNA using viral RNA as complementary template. A search for the viral DNA within cells is in progress. Other experiments suggest that new viral RNA is synthesized within the nucleus and that factors affecting the synthesis of "messenger" RNA also affect viral RNA, in contrast to factors which affect synthesis of ribosomal RNA or other types of viral RNA.

The Nucleic Acids Section has been involved with three major areas of intramural research during the last year. The first of these involves a continuing study of the subcellular events that follow infection of susceptible cells in tissue culture with oncogenic RNA tumor viruses. Further work on this project during the past year has shown more conclusively that the initial reverse transcription of the RNA tumor virus genome into DNA takes place on the plasma membrane. Both the reverse transcriptase activity and the RNA genome template can be isolated in association with these membranes after infection of cells, whereas other subcellular particles show no such activity. Furthermore, these membranes, isolated from infected cells, will support *in vitro* synthesis of DNA complementary to the RNA genome of the infecting virus. This *in vitro* activity is not present in uninfected cells.

The second major area of research is to understand the role of DNA repair mechanisms in normal cells exposed to DNA damaging agents and relationship of this process to carcinogen transformation. A reproducible, host-cell reactivation assay, using UV-irradiated Adenovirus-2 has been developed. With this assay it has been shown for the first time that the cells from an unusual patient with xeroderma pigmentosum (XP), thought from previous data to have normal DNA repair, do show a modest deficiency in their ability to repair UV-irradiated Adenovirus-2. Other unusual cases of XP with apparently normal repair have been found and cells from these patients will soon be under investigation in our laboratory. Because the exposed skin of patients with XP shows an unusually high frequency of malignant transformation *in vivo*, it is considered important to understand the nature of the metabolic defects that exist in these cells. Such defects may well be an important clue to the biochemical events underlying malignant transformation in all cells. The ultimate goal of these studies is to understand how carcinogenic events such as radiation or specific chemical carcinogens interact with DNA, the cellular processes capable of repairing certain of the lesions induced by the interaction, and the relationship of this repair process or its failure in carcinogenesis. The third major area under investigation is DNA replication. These studies have been initiated by studying DNA replication in *E. coli* where there is already a large body of data relevant to DNA replication and the unsolved problems are well delineated. When relevant and interesting data are obtained in this bacterial system it is planned to shift attention to mammalian (probably human) cells growing in tissue culture. Current

studies are beginning to yield potentially useful data about the structure of DNA in the neighborhood of the replication point and about the kinetics of synthesis and joining of nascent DNA fragments. A structural model of the replication point has been proposed based on the current evidence for bidirectional replication of the DNA of many different species. One possible lesion which may be relevant to chemical carcinogenesis is that resulting in an aberrant DNA replicative process. If this is so, then understanding of the normal mode of DNA replication would enable an examination of this process during chemical carcinogenesis and in the tumor state.

The Protein Section has been chiefly concerned in the past year with the development of better methods for the characterization and separation of ribonucleic acids and proteins. As a basis for any insight into the carcinogenic process or into the malignant transformed state, it is necessary to understand the nature of the molecules regulating growth and specific phenotype. The molecules of regulation are generally accepted to be nucleic acids and proteins. In order to characterize their activity they need to be separated, isolated, and chemically defined. The goal of this Section is to establish the means by which the latter can be accomplished so that investigations into the nature of cancer can progress.

Continued studies on methods for isolation of RNA have revealed that very heavy (100S) RNAs are present when high concentration of salt are present in the extraction fluids. Characterization of this RNA is incomplete. An attempt is being made to find out whether the apparent high molecular weight represents an in vivo condition or an artifact of preparation. Although stable to many operations such as precipitation, electrophoresis, centrifugatio the RNA aggregates may be melted or degraded by nucleases under appropriate conditions. The aggregate is composed wholly of nuclear RNA, free of ribosomal RNA and its precursors.

Studies on the factors controlling synthesis of nuclear RNA and processing of ribosomal RNA are in progress. Models of the synthetic process have been simulated on the computer, but a detailed analysis has proven difficult because of uncertainty in the analytical measurements on which the model depends. Although this work proceeds slowly, an understanding of the factors involved in the control of RNA transcription may be presumed to be of significance in understanding ways in which control processes may be involved in carcinogenesis.

In previous reports recurrent difficulties with methods intended for use in determining base composition of minute amounts of rapidly labeled RNA were described. During the past year an unexpected internucleotide conversions betweent uridine and cytidine and between guanosine and adenine make this approach impossible.

Studies on the mammary system and the effect of prolactin on the synthetic properties of mammary tissue have continued. Primary interest in this tissue is to study control mechanisms that lead to differentiation and specialized synthesis, an understanding of which may provide insight to the possible mechanisms of carcinogenesis. The role of prolactin is particularly

important because it appears to be required for the development of malignancy. A study of proteins produced in mammary explants under the influence of prolactin initiated last year has been continued with the finding that the chief product differs significantly from the composition of milk. The factors responsible for this difference have not been identified and are the subject of continuing research. The indication that the carbocyanine dye, "Stainsall," might be useful as a phosphoprotein stain was further studied during this year and conditions for such specific use of this dye were developed. The addition of such a specific staining technique may be valuable in other studies where phosphoproteins are involved.

Studies on serum haptoglobin subtyping, which were commenced last year, were carried to a successful conclusion.

Serial No. NCI-4520
1. Chemistry Branch, OASDC, DCCP
2. Cell Growth Regulation Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Biochemistry of Transformation of Cells by Avian Sarcoma Viruses

Previous Serial Number: NCI-4750

Principal Investigators: John P. Bader, Ph.D. and Michael Lew, M.D.

Other Investigators: Artrice V. Bader, Ph.D.

Cooperating Units: Virus Studies Section, Viral Oncology

Man Years:

Total	:	3.1
Professional	:	2.3
Other	:	0.8

Project Description

Objectives:

To delineate the basic biochemical defect responsible for the change to malignancy in Rous sarcoma virus-infected cells.

Methods Employed:

Cell culture, microscopic identification of cellular transformation, radioactive tracer techniques, isolation and purification of hyaluronic acid, polyacrylamide and agarose gel electrophoresis, election microscopy.

Major Findings:

Mutants of Rous sarcoma virus have been isolated which transform cells at 37° but not at 41°, although full cycles of virus reproduction proceed at both temperatures. The molecule directly responsible for transformation, therefore, is neither a structural component of the virion nor a substance involved in the synthesis of virion components. Certain biochemical changes accompany transformation, including increased hexose (both glucose-like sugars and galactose) uptake and hyaluronic acid synthesis. In contrast, no increase in the uptake of RNA or DNA precursor nucleosides was observed in

transformed over nontransformed cells. The noted biochemical changes can be prevented by inhibitors of protein or RNA synthesis, conditons which have little effect on morphological transformation. It is possible, therefore, to dissociate secondary metabolic changes from those directly responsible for the morphological change.

Further characterization of the transformation occurring upon a shift of temperature of the mutant-infected cells showed that transformation could be prevented by incubation of cells at low pH, by addition of substances which enhance cyclic AMP levels, or by antagonism of glucose metabolism. Manipulation of ion transport systems had an enhancing effect on the morphological change. It was also shown that morphological transformation was accompanied by a change in cell density, and transformed cells could be separated from nontransformed cells in polysucrose or silica gradients. Coordinate with the density change was an increase in cell volume of transformed cells. These data were correlated with a pronounced vacuolization of the cytoplasm observed by phase-contrast microscopy. This cytoplasmic vacuolization was verified by electron microscopy and the nature of membranes bounding the vacuoles was examined.

Considered together the above observations suggest that the morphological change characteristic of transformation by Rous sarcoma virus is caused by an alteration in the permeability of cell surface membrane to water and ions, and that the change in intracellular ions may lead to induction of a variety of metabolic processes.

Significance to Biomedical Research and the Program of the Institute:

These findings encourage us to think that the delineation of the specific molecule responsible for malignancy induced by Rous sarcoma virus is within sight. Such a determination would be a major step in defining the nature of cancer, and may be useful in devising programs for the control of cancer in man.

Proposed Course of Project:

To characterize more fully the morphological change in transformed cells; to search for the temperature sensitive molecule initially responsible for transformation in an attempt to define the specific biochemical defect responsible for malignancy in this system.

Honors and Awards

Invited Lecture, "Transformation of cells by Rous sarcoma virus." Ontario Cancer Institute, Toronto, Canada, April 1972.

Invited Lecture, "Reproduction of RNA tumor viruses and malignant transformation." University of Maryland Medical School, Baltimore, Maryland 1972.

Invited Lecture, "Transformation of cells by Rous sarcoma virus." Albert Einstein College of Medicine, Bronx, New York, June 1972.

Invited Lecture, "Reproduction of RNA tumor viruses and malignant transformation." New York University Medical School, New York, June 1972.

Presentation of "Research on temperature-sensitive transformation by Rous sarcoma virus." Gordon Conference on Animal Cells and Viruses, Tilton, New Hampshire, August 1972.

Presentation of "Research on conditional transformation by Rous sarcoma virus." World Symposium on Model Studies in Chemical Carcinogenesis, Baltimore, Maryland, October 1972.

Presentation of Research at annual meeting of the Special Virus Cancer Program, Hershey, Pennsylvania, October 1972.

Consultant to Grants Committee, American Cancer Society, New York, New York, December 1972.

Publications

Bader, J.P., Ray, D.A. and Steck, T.L.: Electrophoretic determinations of hyaluronate produced by cells in culture. Biochim. Biophys. Acta 264: 73-84, 1972.

Bader, J.P.: Temperature-dependent transformation of cells infected with a mutant of Bryan Rous sarcoma virus. J. Virology 10: 267-276, 1972.

Bader, J.P.: Conditional transformation of cells infected with a mutant of Rous sarcoma virus. In Tso, P., and DiPalo, J. (Eds.): World Symposium on Model Studies in Chemical Carcinogenesis. (in press)

Serial No. NCI-4527
1. Chemistry Branch, OASDC, DCCP
2. Cell Growth Regulation Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Assay of Mammalian tRNA for Nonsense Suppressor Activity

Previous Serial Number: None

Principal Investigators: Minoru Ishizawa, Ph.D. and Dolph Hatfield, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total	1.0
Professional	1.0
Other	0.0

Project Description

Objectives:

To determine if mammalian liver contains tRNA species which can suppress nonsense mutations in an E. coli mutant which is capable of utilizing tRNA from the media.

Methods Employed:

Construction of strains of a tRNA permeable mutant (M. Yamamoto, M. Ishizawa and H. Endo, J. Mol. Biol. 58: 103-115, 1971) which contain an amber (UAG), an ochre (UAA) and an opal (UGA) mutation. Addition of purified mammalian tRNAs which recognize nonsense codons to the media of strains which contain amber, ochre and opal mutations.

Major Findings:

Work is in progress.

Significance to Biomedical Research and the Program of the Institute:

A major unresolved question in biology is whether tRNA may play a role in cellular regulation and carcinogenesis. One approach to elucidating these

problems is a study of the occurrence of suppressor tRNAs.

Proposed Course of the Project:

Continuation of the problem.

Serial No. NCI-4748
1. Chemistry Branch, OASDC, DCCP
2. Cell Growth Regulation Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The Synthesis and Characterization of the RNA of RNA-containing Tumor Viruses

Previous Serial Number: Same

Principal Investigators: Yoshiyuki Kitano, Ph.D., M.D., Paul Okano, Ph.D. and John P. Bader, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total	:	2.2
Professional	:	1.4
Other	:	0.8

Project Description

Objectives:

To determine the molecular structure of the RNA of RNA-containing tumor viruses and to elucidate the intracellular site and mechanism of synthesis of viral RNA.

Methods Employed:

Cell culture, radioactive tracer techniques, density-equilibrium and sedimentation rate centrifugations, isolation and purification of nucleic acids, polyacrylamide gel electrophoresis.

Major Findings:

Several recent publications have heralded the discovery of a variety of molecular species of RNA within the virions of RNA-containing tumor viruses. Our continued experimentations on the resolution of viral RNA by electrophoresis in polyacrylamide-agarose gels have demonstrated that aside from a single slowly migrating species (approx. 6-8 x 10^6 m.w.) which can be denatured to a more rapidly migrating form (approx. 3-4 x 10^6), all other

observed RNA's can be explained by inadvertant enzymatic degradation of the larger forms.

Earlier studies had shown that viral RNA appears in extracellular virions about 70 minutes after intracellular synthesis. Attempts are now being made to determine the intracellular location of synthesis of viral RNA. While other information suggests that viral RNA is synthesized intranuclearly, definitive proof is lacking, and such information is essential to an understanding of the mechanism of malignant transformation by viruses.

Significance to Biomedical Research and the Program of the Institute:

The synthesis of viral RNA, its interaction with cellular organelles, its translation into viral structural and nonstructural proteins, and its packaging into complete virions, are all features of the viral reproductive process which are relevant to malignancy, but about which little is known. Analysis of these processes will help to give us a clearer understanding of the nature of carcinogenesis.

Proposed Course of Project:

To define the site of viral RNA synthesis, to analyze the interaction of viral RNA with cellular organelles, and ultimately to describe the products of the viral genes responsible for tumorigenesis.

Serial No. NCI-4759
1. Chemistry Branch, OASDC, DCCP
2. Cell Growth Regulation Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Nonsense Codon Recognition in Mammalian Tissues

Previous Serial Number: Same

Principal Investigator: Dolph Hatfield, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total	2.0
Professional	1.0
Other	1.0

Project Description

Objectives:

Isolation and purification of tRNAs in mammalian liver which recognize nonsense codons (UAG, UAA and UGA) in order to demonstrate if these tRNAs (or other mammalian tRNAs) can suppress nonsense mutations in protein synthesis.

Methods Employed:

Calf liver tRNA is fractionated by reverse phase chromatography (Kelmers et al., J. Biol. Chem., 240: 3979-3983, 1965) and assayed for recognition of [^{3}H]UAG, [^{3}H]UAA and [^{3}H]UGA by the procedure of Nirenberg and Leder (Science, 145: 1399-1407, 1964). Fractions which recognize nonsense codons are aminoacylated with labeled amino acid and their codon responses determined. Transfer RNAs which recognize nonsense codons are purified by reverse phase chromatographic and B-D cellulose columns.

Major Findings:

These studies have demonstrated: 1) a Ser-tRNA and Arg-tRNA recognize UGA; slight responses of Cys-tRNA and Trp-tRNA to UGA; 2) a Lys-tRNA recognizes UAG; a slight response of GluN-tRNA to UAG; and 3) slight responses of

Lys- and Tyr-tRNA to UAA. The Ser-tRNA specifically recognizes UGA in calf, rabbit and chicken liver (Hatfield and Portugal, Proc. Nat. Acad. Sci. USA, 67: 1200-1206, 1970) and has been resolved into two species of seryl-tRNA (both recognizing UGA) from each animal. The other AA-tRNAs recognize the nonsense codon in addition to their respective assigned codons.

Significance to Biomedical Research and the Program of the Institute:

A major unresolved question in biology is whether tRNA may play a role in cellular regulation and carcinogenesis. One approach to elucidating these problems is a study of the occurrence of suppressor tRNAs.

Proposed Course of Project:

Further purification of the tRNAs which recognize nonsense codons to determine their role in protein synthesis.

Publications

Hatfield, D.: Recognition of Nonsense Codons in Mammalian Cells. Proc. Nat. Acad. Sci. USA 69: 3014-3018, 1972.

Serial No. NCI-4765
1. Chemistry Branch, OASDC, DCCP
2. Cell Growth Regulation Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Studies on the Intracellular Replicative Genome of RNA Containing Tumor Viruses

Previous Serial Number: Same

Principal Investigators: John P. Bader, Ph.D. and Yoshiyuki Kitano, Ph.D., M.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total	:	1.4
Professional	:	1.0
Other	:	0.4

Project Description

Objectives:

To define the biochemical events which establish the replicative form of RNA-containing viruses, to identify that replicative form, and to analyze its interaction with the cell leading to malignancy.

Methods Employed:

Cell culture, radioactive tracer techniques, density equilibrium and sedimentation rate centrifugations, isolation and purification of nucleic acids, molecular hybridization techniques, enzymological analyses, polyacrylamide gel electrophoresis.

Major Findings:

Earlier studies in this laboratory demonstrated a requirement for new DNA synthesis in the reproduction of RNA tumor viruses and in transformation by these viruses. Along with other data, this indicated that a DNA complementary to viral RNA is synthesized and this DNA is the intracellular replicative genome from which progeny viral RNA's are synthesized.

Synthesis of viral DNA was shown to commence within one hour after attachment of virus to cells, and analysis of data suggested that reduplication of viral DNA's occurred during the ensuing 7 hours. Recent experiments involving labeling of the putative viral DNA with deoxythymidine-^{3}H and selective molecular hybridization with viral RNA, have identified the intracellular viral DNA under conditions where cellular chromosomal DNA synthesis is suppressed.

Significance to Biomedical Research and the Program of the Institute:

A more complete description of the reproductive process of RNA tumor viruses is essential to an understanding of tumorigenesis by these viruses, and may be useful in devising chemotherapeutic agents for the treatment of virus-caused neoplasms. The unequivocal demonstration of intracellular viral DNA is essential to an understanding of the interaction of the viral genome with the cell which leads to malignancy.

Proposed Course of Project:

To further characterize the intracellular viral DNA, to determine unequivocally whether or not viral DNA reproduces intracellularly, and to analyze the nature of the interaction of the newly synthesized viral DNA with cellular organelles, including chromosomes.

Publications

Bader, J.P.: Metabolic requirements for infection by Rous sarcoma virus III. The synthesis of viral DNA. Virology 48: 485-493, 1972.

Bader, J.P.: Metabolic requirements for infection by Rous sarcoma virus IV. Virus reproduction and cellular transformation without cellular division. Virology 48: 494-501, 1972.

Bader, J.P.: Virus-induced transformation without cellular division. Science (in press).

Serial No. NCI-4522
1. Chemistry Branch, OASDC, DCCP
2. Molecular Carcinogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Molecular Mechanisms of Aryl Hydrocarbon Hydroxylase (AHH) Induction in Mammalian Cell Culture

Previous Serial Number: Same

Principal Investigator: James P. Whitlock, Jr., M.D.

Other Investigator: Harry V. Gelboin, Ph.D.

Cooperating Units: None

Man Years:

Total	1.0
Professional:	0.4
Other:	0.6

Project Description

Objectives:

To understand the biochemical mechanisms of regulation of the AHH system in cells cloned from rat liver.

Methods Employed:

Cell culture; spectrophotofluorometry; recording spectrophotometry; agarose-polyacrylamide gel electrophoresis; radioactivity measurements by scintillation counting.

Major Findings:

A cloned cell line, in which AHH is highly inducible by the polycyclic hydrocarbon benz(a)anthracene and whose basal AHH activity is low, has been used. The kinetics of AHH induction, the nutritional requirements for induction and the biochemical requirements for AHH activity have been measured. An important finding was that temporary inhibition of protein synthesis is followed by a large increase in AHH activity, even in the absence of a polycyclic hydrocarbon inducer; this effect was also observed in several other cell types. These results

suggest that at least two events are involved in AHH induction; the inducer benz(a)anthracene, acts at the level of transcription; and a labile protein(s) regulates translation of AHH-specific RNA. A metabolic change at either site results in altered AHH levels.

Significance to Biomedical Research and the Program of the Institute:

AHH, a microsomal mixed-function oxygenase, is found in most tissues of many species, including humans, and plays an important role in both the detoxification and activation of carcinogenic polycyclic hydrocarbons. An understanding of the control of this enzyme complex is relevant both to chemical carcinogenesis and drug metabolism and to the problem of biochemical events in the regulation of enzyme induction.

Proposed Course of Project:

Further investigation of the biochemical events relevant to AHH induction; comparison of the regulation of AHH with other inducible enzymes; attempted isolation or identification of induction-specific RNA; characterization of the mechanisms of enzyme induction. The relationship of AHH regulation to carcinogen metabolism and carcinogenic activity.

Serial No. NCI-4523
1. Chemistry Branch, OASDC, DCCP
2. Molecular Carcinogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Measurement of Aryl Hydrocarbon Hydroxylase (AHH) in Human Tissues

Previous Serial Number: Same

Principal Investigator: James P. Whitlock, Jr., M.D.

Other Investigators: Harry V. Gelboin, Ph.D., Herbert L. Cooper, M.D.

Cooperating Units: Laboratory of Biochemistry, NIDR

Man Years:

Total:	0.2
Professional:	0.1
Other:	0.1

Project Description

Objectives:

Examination of human tissue for AHH activity.

Methods Employed:

Cell culture; spectrophotofluorometry, blood fractionation.

Major Findings:

AHH is present in human lymphocytes and is inducible by phytohemagglutimin and pokeweed mitogen and by the polycyclic hydrocarbon benz(a)anthracene.

Significance to Biomedical Research and the Program of the Institute:

AHH has been implicated in the detoxification and activation of chemical carcinogens; AHH has been implicated in tumorigenesis in experimental animals. An easily available human tissue with AHH activity would contribute to the study of chemical

carcinogenesis and drug metabolism in humans. This would facilitate and made possible the analysis of the variability of this enzyme in humans and its role in chemical carcinogenesis in humans.

Proposed Course of Project:

Examination of peripheral leukocytes for AHH activity. Examination of human cells in culture for the metabolism of carcinogenic polycyclic hydrocarbons. Define the profile of PCH metabolism in human cells.

Publications

Whitlock, J.P., Jr., Cooper, H.L., and Gelboin, H.V.: Aryl Hydrocarbon (Benzopyrene) Hydroxylase is Stimulated in Human Lymphocytes by Mitogens and Benz(a)anthracene. Science 177: 618-619, 1972.

Serial No. NCI-4530
1. Chemistry Branch, OASDC, DCCP
2. Molecular Carcinogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The Relationship of the Metabolic Profile of Benz(a)pyrene and DMBA to Tumorigenicity in Mouse Skin

Previous Serial Number: None

Principal Investigators: Nadao Kinoshita, Ph.D., Harry V. Gelboin, Ph.D., James K. Selkirk, Ph.D.

Other Investigator: Barbara Shears

Cooperating Units: None

Man Years:

Total:	1.0
Professional:	0.5
Other:	0.5

Project Description

Objectives:

Determination of the profile of metabolic products formed from benzo(a)pyrene.

Methods Employed:

Thin layer chromatography, spectrofluorometry, isotope analysis.

Major Findings:

The metabolism of benzo(a)pyrene (BP) by liver microsomes from normal and 3-methylcholanthrene treated rats was quantitatively analyzed by a double label methods using BP-^{3}H and BP-^{14}C. Qualitatively the metabolism of benzo(a)pyrene by both microsomal preparations was similar. The identified metabolites were 7,8-dihydro-7,8-dihydroxy-BP, 4,5-dihydro-4,5-dihydroxy-BP, 9,10-dihydro-9,10-dihydroxy-BP, 3-hydroxy-BP, 9-hydroxy-BP, and BP-1, 6-quinone and BP-3, 6-quinone. The quantitative

analysis by the double labelling method showed the profile of BP metabolites produced by rat liver microsomes from normal and MC-induced rats. The ratio of metabolites from induced microsomes to that from normal microsomes was greatest for the 7,8-dihydro-7,8-dihydroxy and 9,10-dihydro-9,10-dihydroxy metabolites, less for the phenols and K-region metabolites and least for the quinones. Similar ratios were obtained when microsomes from MC-treated rats were diluted except that dilution reduced the relative amount of phenol formation. These results suggest that higher enzyme activity favors the formation of the non K-region diols and phenols relative to the K-region diol. The product profile in mouse skin from a few strains have the same major components as those found in mouse and rat liver. Major work is still in progress.

Significance to Biomedical Research and the Program of the Institute:

Since skin is the target tissue for benz(a)pyrene it is important to determine derivatives formed during metabolism. This pattern once known should indicate those portions of the molecule important in the activation of the hydrocarbon to its tumorigenic state. This could feasibly be used in the prophylaxis of polycyclic hydrocarbon carcinogenesis.

Proposed Course of Project:

Once the thin-layer chromatography has been sufficiently developed to separate all metabolic components, we intend to compare skins of animals refractory to benz(a)pyrene to mice. We hope to determine if species resistant to benz(a)pyrene tumorigenesis metabolize the carcinogen to different inactive intermediates and the character of metabolism inducing cancer.

Honors and Awards

Harry V. Gelboin, Ph.D.

Invited lecturer, Ben May Laboratory for Cancer Research, University of Chicago, Chicago, Illinois, February, 1972.

Invited lecturer, Argonne National Laboratory, University of Illinois, Chicago, Illinois, April 1972.

Invited lecturer, Gordon Research Conference on Toxicology and Safety Evaluations, Meriden, New Hampshire, July-August, 1972.

Principle lecturer, 19th University of Michigan Cancer Retreat, Ann Arbor, Michigan, October, 1972.

Invited lecturer, Departments of Pharmacology and Biological Chemistry, University of Michigan, Ann Arbor, Michigan, October, 1972.

Invited lecturer, Course on Biological Regulations, Thomas Jefferson University, Philadelphia, Pennsylvania, November, 1972.

Invited speaker, World Symposium on Model Studies in Chemical Carcinogenesis, Baltimore, Maryland, November, 1972.

Publications

Gelboin, H.V.: Studies on the mechanism of microsomal hydroxylast induction and its role in carcinogen action. Revue Canadienne de Biologie 31: 39-60, 1972.

Gelboin, H.V., Kinoshita, N. and Wiebel, F.J.: Microsomal hydroxylases: Their induction and role in polycyclic hydrocarbon carcinogenesis and toxicity. Fed. Proc. 31: 1298-1302, 1972.

Gelboin, H.V., Kinoshita, N. and Wiebel, F.J.: Microsomal hydroxylases: Studies on the mechanism of induction and their role in polycyclic hydrocarbon action. Symposium Monograph, Environment and Cancer, M.D. Anderson Hospital. (In press)

Kinoshita, N. and Gelboin, H.V.: Aryl hydrocarbon hydroxylase and polycyclic hydrocarbon tumorigenesis: Effect of the enzyme inhibitor 7,8-benzoflavone on tumorigenesis and macromolecule binding. Proc. Nat. Acad. Sci. USA 69: 824-828, 1972.

Kinoshita, N. and Gelboin, H.V.: The role of aryl hydrocarbon hydroxylase in 7,12-dimethylbenz(a)anthracene (DMBA) skin tumorigenesis: On the mechanism of 7,8-benzoflavone inhibition of tumorigenesis. Cancer Res. 32: 1329-1339, 1972.

Kinoshita, N., Shears, B., and Gelboin, H.V.: K-Region and Non-K Region Metabolism of Benz(a)pyrene by rat liver microsomes. Cancer Res. (In press)

Serial No. NCI-4531
1. Chemistry Branch, OASDC, DCCP
2. Molecular Carcinogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Characterization of a Liver Cell Culture System for the Study of Aryl Hydrocarbon Hydroxylase (AHH)

Previous Serial Number: None

Principle Investigator: James P. Whitlock, Jr., M.D.

Other Investigators: Harry V. Gelboin, Ph.D., Hayden G. Coon, Ph.D.

Cooperating Units: Laboratory of Cell Biology, NCI

Man Years:

Total:	0.8
Professional:	0.3
Other:	0.5

Project Description

Objectives:

Definition and description of the basic parameters and variables involved in the regulation of AHH in liver cell culture.

Methods Employed:

Cell culture, enzymology

Major Findings:

AHH induction as a function of culture conditions has been measured. The metabolism of benzo(a)pyrene in these cells is identical to its metabolism by rat liver microsomes. The requirement for AHH activity have been described. The cells have been subcloned, and individual variation in AHH inducibility has been measured.

Significance to Biomedical Research and the Program of the Institute:

AHH is important in both the detoxification and activation of carcinogenic polycyclic hydrocarbons. An *in vitro* system for studying this enzyme complex should contribute significantly to the understanding of chemical carcinogenesis.

Proposed Course of Project:

After the effect of the basic variables on AHH levels is established, this cell culture system will be a valuable contribution to the *in vitro* study of chemical carcinogenesis.

Serial No. NCI-4532
1. Chemistry Branch, OASDC, DCCP
2. Molecular Carcinogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The Role of Microsomal Metabolism in the Malignant Transformation of Hamster Embryo Cells in Tissue Culture

Previous Serial Number: None

Principal Investigator: James K. Selkirk, Ph.D.

Other Investigator: Harry V. Gelboin, Ph.D.

Man Years:

Total:	0.6
Professional:	0.1
Other:	0.5

Project Description

Objectives:

Establishment of system where one can obtain malignantly transformed clones of cells to study their metabolism of polycyclic hydrocarbons.

Methods Employed:

Cell culture; enzymology.

Major Findings:

Studies just begun and are still in progress.

Significance to Biomedical Research and the Program of the Institute:

Working with a homogeneous population as obtained in cell culture should magnify any differences one can find in a heterogeneous population as in skin or liver. This is with respect to relative amounts and molecular types of polycyclic hydrocarbon derivatives.

Proposed Course of Project:

Cells will be treated with a number of parent hydrocarbons and those derivatives (e.g. epoxides) that have proven to be more efficient transforming agents than the parent compound. This will be done on both Hamster embryo cells and diploid liver cell lines. The roles of microsomal enzymes in transformation will be determined.

Serial No. NCI-4533
1. Chemistry Branch, OASDC, DCCP
2. Molecular Carcinogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Kinetics of Product Formation During Polycyclic Hydrocarbon Metabolism

Previous Serial Number: None

Principal Investigator: James K. Selkirk, Ph.D.

Other Investigators: Harry V. Gelboin, Ph.D.

Man Years:

Total:	0.5
Professional:	0.2
Other:	0.3

Project Description

Objectives:

Determination of rate of formation of products. Determination of precursor-product relationship as well as the relationship between phenol and dihyrdodiol, i.e. hydration vs. dehydration of the various metabolites.

Methods Employed:

High-pressure liquid chromatography.

Major Findings:

This project is just beginning. However, we have determined by ultraviolet spectrophotometry that all synthetic benz(a)pyrene derivatives available can be chromatographed through these columns without their being degraded.

Significance to Biomedical Research and the Program of the Institute:

There are a number of similar products formed during metabolism of benzo(a)pyrene. It is not yet known which of these derive from the activated carcinogenic state and which ones are from

other intermediates. This new technique should allow rapid analysis during metabolism so molecular activation can be observed. These separations are necessary in order to determine the relationship between metabolism and carcinogenicity.

Proposed Course of Project:

Columns of different characteristics are being tested as well as various solvent systems to obtain optimum component separation.

Serial No. NCI-4534
1. Chemistry Branch, OASDC, DCCP
2. Molecular Carcinogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Separation of Polycyclic Hydrocarbon Metabolites by High-Pressure Liquid Chromatography

Previous Serial Number: None

Principal Investigator: James K. Selkirk, Ph.D.

Other Investigator: Harry V. Gelboin, Ph.D.

Man Years:

Total:	0.8
Professional:	0.4
Other:	0.4

Project Description

Objectives:

The rapid and quantitative separation of the known derivatives of carcinogenic polycyclic hydrocarbons. We are attempting to maximize the efficiency of separation by variance of column packing, solvent and temperature while maintaining molecular structure.

Methods Employed:

Dupont 830. High Pressure Liquid Chromatograph.

Major Findings:

All labile functional groups (e.g. epoxy, hydroxyl) must be protected and must be able to be volatilized in order to utilize gas chromatography. In the case of polycyclic hydrocarbons, volatilizing temperatures are usually too close to the decomposition point to adequately separate polycyclic hydrocarbon derivatives. These compounds when injected can pass through this liquid chromatography system without decomposition.

Significance to Biomedical Research and the Program of the Institute:

The product profile of polycyclic hydrocarbon metabolism indicates some relationship between the various products formed and activity as a carcinogen. A rapid analytical technique will allow us to determine which metabolic route is the most related to carcinogenicity and which to detoxification. Rapidity of analysis will lessen the chance of oxidation or other non-metabolic breakdown product formation and give a more accurate picture of the system in its *in vivo* state.

Serial No. NCI-4535
1. Chemistry Branch, OASDC, DCCP
2. Molecular Carcinogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Genetic Control of Aryl Hydrocarbon Hydroxylase

Previous Serial Number: None

Principal Investigators: Friedrich J. Wiebel, M.D., Janet Leutz, Harry V. Gelboin, Ph.D.

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	0.6
Professional:	0.3
Other:	0.3

Project Description

Objectives:

To determine the genetic factors that regulate the constitutive level and the inducibility of the microsomal monooxygenase which metabolizes carcinogenic polycyclic hydrocarbons (aryl hydrocarbon hydroxylase) in various strains of mice. To study the relationship between the susceptibility of various strains of mice to carcinogenic polycyclic hydrocarbons and their levels of aryl hydrocarbon hydroxylase activity.

Methods Employed:

Fluorospectrophotometry, recording spectrophotometry, test systems of chemical carcinogenesis in mouse skin.

Major Findings:

Two major groups of mouse strains can be distinguished by their inducibility of the hepatic aryl hydrocarbon hydroxylase. The hepatic enzyme of a number of mouse strains (group I) is inducible 3-5 fold after administration of a polycyclic hydrocarbon inducer, but is not inducible in a second group (II) of

mouse strains. Enzyme activities in extrahepatic tissues such as lung, kidney, small intestine and skin can be induced in both types of mice. The kinetics of induction in extrahepatic tissues from mice of group I and II were similar, however, the magnitude of induction, at least in skin and kidney, was considerably lower in strains of group II than in those of group I.

Significance to Biomedical Research and the Program of the Institute:

The enzyme system under study metabolizes a large variety of drugs, carcinogens and possibly a number of endogenous compounds. To understand the specificity of chemical carcinogens in various strains and species of mammals and their "target" tissues it is essential to understand the factors that regulate the enzyme activities in these specific tissues and strains. This knowledge will furthermore be essential in the development of *in vivo* model systems of chemical carcinogenesis.

Proposed Course of Project:

To continue the study of the genetic control of these tissue specific aryl hydrocarbon hydroxylases and to test the correlation of enzyme form and activity with the susceptibility to chemical carcinogens in selected mouse strains.

Publications

Wiebel, F.J., Leutz, J.C. and Gelboin, H.V.: Aryl hydrocarbon (benzo(a)pyrene) hydroxylase induction in extrahepatic tissues of mouse strains not inducible in liver. Arch. Biochem. Biophys. 154: 292-294, 1973.

Serial No. NCI-4536
1. Chemistry Branch, OASDC, DCCP
2. Molecular Carcinogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The Role of RNA in the Regulation of Aryl Hydrocarbon Hydroxylase (AHH) Induction

Previous Serial Number: NCI-4717

Principle Investigator: Ricardo Brentani, Ph.D.

Other Investigators: James P. Whitlock, Jr., M.D., Harry V. Gelboin, Ph.D.

Cooperating Units: University of Sao Paolo, Brazil

Man Years:

Total:	0.6
Professional:	0.4
Other:	0.2

Project Description

Objectives:

To understand the requirement for RNA synthesis in the induction of AHH by carcinogenic polycyclic hydrocarbons.

Methods Employed:

Cell culture, spectrophotofluorometry, spectrophotometry.

Major Findings:

3'-deoxyadenosine inhibits AHH induction, suggesting a requirement for the synthesis of poly-adenylic acid for AHH induction. Studies with α-amanitin, an inhibitor of RNA polymerase, have been inconclusive. Polycyclic hydrocarbons induce the microsomal enzyme aryl hydrocarbon (benzo(a)pyrene) hydroxylase in the livers of intact rats and in hamster embryo cells. In vivo, methylcholanthrene causes an increased incorporation of precursors into nuclear RNA and an increased RNA polymerase activity in hepatic nuclei. Gel electrophoresis of RNA synthesized in

vitro by isolated nuclei has shown that the synthesis of all sizes of RNA is enhanced by the methylcholanthrene treatment in vivo. Hypophysectomized or adrenalectomized rats previously treated with hydrocarbons fail to exhibit an increase in RNA polymerase activity, although they manifest an increase in the level of aryl hydrocarbon hydroxylase. Double labeling techniques and acrylamide gel electrophoresis showed no detectable change in the pattern of RNA synthesized during enzyme induction in cell culture. The latter studies and those carried out in vivo with adrenalectomized and hypophysectomized rats suggest that the methylcholanthrene-induced gross changes in liver nuclear RNA metabolism in vivo are not requirements for microsomal enzyme induction, and that the induction-specific RNA synthesis, indicated as a requirement by our other studies, is of small magnitude.

Significance to Biomedical Research and the Program of the Institute:

An understanding of the regulation of the induction of AHH, a microsomal mixed-function oxygenase, is relevant to chemical carcinogenesis and drug metabolism, as well as the control of enzyme induction in mammalian cells.

Proposed Course of Project:

Further studies on the characterization of the RNA required for AHH induction are in progress.

Publications

L.R. Younger, R. Salomon, R.W. Wilson, A.C. Peacock, and H.V. Gelboin: Effects of Polycyclic Hydrocarbons on Ribonucleic Acid Synthesis in Rat Liver Nuclei and Hamster Embryo Cells. Mol. Pharm. 8: 452-464, 1972.

Serial No. NCI-4556
1. Chemistry Branch, OASDC, DCCP
2. Molecular Carcinogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The Role of Sulfhydryl Groups in Polycyclic Hydrocarbon Metabolism and Carcinogenesis

Previous Serial Number: None

Principal Investigator: James K. Selkirk, Ph.D.

Other Investigator: Harry V. Gelboin, Ph.D.

Man Years:

Total:	0.8
Professional:	0.4
Other:	0.4

Project Description

Objectives:

To determine the relationship of sulfhydryl binding of carcinogens to the metabolism and carcinogenesis of polycyclic hydrocarbons. The binding of sulfhydryl compounds such as glutathione renders the conjugate water soluble. We plan to investigate the kinetics of binding of polycyclic hydrocarbons to glutathione in mouse skin.

Methods Employed:

Enzymology, spectrofluorometry, mouse skin tumorigenesis, and colorimetric analysis.

Major Findings:

Project just beginning, however, preliminary results indicate the sulfhydryl reagents tested reduce skin tumorigenesis in mice.

Significance to Biomedical Research and the Program of the Institute:

Conjugation of toxic substances to metabolites such as glutathione or glucuronic acid are common pathways for drugs and

other foreign organic chemicals taken into the living organism. If the lifetime of the active form of the carcinogen is determined by the rate of its conjugation to a nontoxic product, then acceleration of the reaction should reduce the half-life of the proximate carcinogen and consequently the formation of tumors.

Proposed Course of Project:

To develop an accurate assay for glutathione in skin. Investigation of its conjugating ability toward a series of polycyclic hydrocarbons, carcinogenic and non-carcinogenic with the aim of determining the differing chemical affinities for the detoxifying agent.

Serial No. NCI-4766
1. Chemistry Branch, OASDC, DCCP
2. Molecular Carcinogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Cell Regulatory Controls of Microsomal Hydroxylase Metabolizing Polycyclic Hydrocarbons

Previous Serial Number: Same

Prinicpal Investigators: Friedrich J. Wiebel, M.D., Harry V. Gelboin, Ph.D.

Other Investigators: Hayden G. Coon, Ph.D.

Cooperating Units: Laboratory of Cell Biology, DCBD, NCI

Man Years:

Total:	1.0
Professional:	0.4
Other:	0.6

Project Description

Objectives:

To study the control mechanisms regulating the synthesis and degradation of the microsomal enzyme system that metabolizes polycyclic hydrocarbons in a controlled environment such as tissue culture.

Methods Employed:

Cell culture techniques, fluorospectrophotometry, recording spectrophotometry, radioisotope techniques, and acrylamide gel electrophoresis.

Major Findings:

The mechanism by which the basal and the induced levels of aryl hydrocarbon hydroxylase (AHH) activity are regulated was studied in somatic hybrids of cells that differ in basal as well as inducible AHH. The level of enzyme activity in various parental and hybrid cells was found to depend on their state of growth. Basal and inducible AHH could be detected in some cell types derived from the CNS. Fusion of

cells of neural origin that do not exhibit detectable AHH activity with active fibroblasts yield hybrid cells containing basal and inducible enzyme levels above those of the active parent. Fusion of parental cell lines without detectable AHH does not give rise to hybrid cells containing AHH activity.

Significance to Biomedical Research and the Program of the Institute:

The enzyme system is a key factor in the response of the organism to potentially toxic and carcinogenic foreign compounds. Understanding its mechanism of regulation will aid in its manipulation in experimental systems of chemical carcinogenesis and may ultimately help in the modification of the body's defense against chemical carcinogens.

Proposed Course of Project:

To study the mechanisms that regulate the level of enzyme activity. To devise means to control the activity of the enzyme system and the direction of alternative metabolic pathways. To determine the role of the enzyme complex in chemical carcinogenesis in vitro.

Honors and Awards

Invited lecture, Colloquium on Enzyme Induction, Biochemical Society Meeting, Guildford, England, July 1972.

Publications

Gelboin, H.V., Wiebel, F.J. and Kinoshita, N.: Microsomal aryl hydrocarbon hydroxylases: On their role in polycyclic hydrocarbon carcinogenesis and toxicity and mechanism of enzyme induction. In Boyd, G.S. and Smellie, R.M.S. (Eds.): Biological Hydroxylation Mechanisms. London, Academic Press, 1972, pp. 103-133.

Wiebel, F.J., Gelboin, H.V. and Coon, H.G.: Regulation of aryl hydrocarbon hydroxylase in intraspecific hybrids of human, mouse, and hamster cells. Proc. Nat. Acad. Sci. USA 69: 3580-3584, 1972.

Wiebel, F.J., Matthews, E.J. and Gelboin, H.V.: On the relationship between transcription, translation, and ribosomal RNA synthesis during microsomal hydroxylase induction. In Kenney, F.T., Hamkalo, B.A., Favelukes, G. and August, J.T. (Eds.) Gene Expression and its Regulation. New York, New York, Plenum Press, 1973, pp. 459-472.

Wiebel, F.J., Matthews, E.J., and Gelboin, H.V.: RNA synthesis dependent induction of aryl hydrocarbon hydroxylase in the absence of ribosomal RNA synthesis and transfer. J. Biol. Chem. 247: 4711-4717, 1972.

Serial No. NCI-4779
1. Chemistry Branch, OASDC, DCCP
2. Molecular Carcinogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The Evaluation of Various Compounds for their Effects as Inducers and Inhibitors of Aryl Hydrocarbon Hydroxylase Activity and Tumorigenesis

Previous Serial Number: Same

Principal Investigator: Friedrich J. Wiebel, M.D.

Other Investigators: Harry V. Gelboin, Ph.D., M.G. Stout, Ph.D.+, W.S. Burnham, Ph.D.*, B. Stripp, Ph.D.*

Cooperating Units: ICN Nucleic Acid Research Institute, Irvine, California+, Laboratory of Chemical Pharmacology, Heart and Lung Institute, NIH *

Man Years:

Total:	2.1
Professional:	1.5
Other:	0.6

Project Description

Objectives:

To develop methods to determine the activity of various compounds as inducers or inhibitors of carcinogen hydroxylating enzymes and polycyclic hydrocarbon tumorigenesis.

Methods Employed:

Cell Culture techniques, spectrophotofluorometry, recording spectrophotometry, fluorescent microscopy and radioisotope studies and mouse skin tumorigenesis.

Major Findings:

At least two forms of aryl hydrocarbon hydroxylases can be distinguished by the inhibitory or stimulatory effect of

flavonoid compounds. Using a flavone as probe, the distribution of the different forms of hydroxylases and their postnatal development was investigated. Three major groups of flavone derivatives are observed in respect to their differential effect on the two enzyme types. There is a close relationship between the physicochemical properties of these derivatives and their biological effect indicating a different hydrophobicity of the active enzyme sites or their environment. The mode of stimulation of aryl hydrocarbon hydroxylase activity is found to be compatible with an allosteric mechanism.

Significance to Biomedical Research and the Program of the Institute:

Compounds of the type investigated can be used to explore the structure and the reaction mechanism of the aryl hydrocarbon hydroxylases. They serve furthermore, to study the role of the enzyme system in the toxicity and carcinogenicity of polycyclic hydrocarbons.

Proposed Course of Project:

To use the inhibitors and stimulators of the flavonoid type to explore the oxidation mechanism of carcinogenic polycyclic hydrocarbons, to establish its relationship to the oxidative metabolism of other exogenous and endogenous compounds, to alter the course of tumorigenesis.

Honors and Awards

Invited Lecture, World Symposium on Model Studies in Chemical Carcinogenesis, Baltimore, Maryland, November, 1972.

Publications

Wiebel, F.J., Buu-Hoi, N.P., Stout, M.G., Burnham, W.S. and Gelboin, H.V.: Flavones and Polycyclic Hydrocarbons as Modulators of Aryl Hydrocarbon (Benzo(a)pyrene) Hydroxylase. Proceedings of the World Symposium on "Model Studies in Chemical Carcinogenesis." In The Biochemistry of Disease, E. Farber, (Ed.).

Serial No. NCI-4528
1. Chemistry Branch, OASDC, DCCP
2. Nucleic Acids Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Mutagenesis of Escherichia coli by Carcinogenic Polycyclic Hydrocarbons

Previous Serial Number: None

Principal Investigator: Minoru Ishizawa, Ph.D.

Other Investigators: Chikayoshi Nagata, Ph.D. and C. Wesley Dingman, M.D.

Cooperating Units: Biophysics Division, National Cancer Center Research Institute, Tokyo, Japan

Man Years:

Total	:	0.5
Professional	:	0.4
Other	:	0.1

Project Description

Objectives:

The purpose of this project is to establish conditions and methods for a quantitative study of the mutagenic activity of a series of carcinogenic polycyclic hydrocarbons in Escherichia coli.

Methods Employed:

E. coli strains harboring nonsense or frameshift mutations are used as test organisms. The induction of reversion mutations by chemicals is analyzed under various treatment conditions in liquid or solid media. When positive effects in terms of lethality and mutagenicity are obtained, the study will be extended to include the use of E. coli strains lacking in DNA repair capability.

Major Findings:

This work is still in the early stages. Benzo(a)pyrene and its derivative 6-hydroxy-benzo(a)pyrene are presently employed as test chemicals and it was found that although both compounds did not affect the viability of E. coli cells under some treatment conditions, 6-hydroxy(a)benzopyrene (but not

benzo(a) pyrene) exhibited a marked cell killing effect in the presence of 10^{-5}M $CuCl_2$ in phosphate-buffered saline, pH 7.0. The mutagenic effect of this derivative in the presence of $CuCl_2$, using nonsense and frameshift mutants of E. coli, is currently under study. Additional studies will be designed to determine the effect of cupric ion on the binding of carcinogens to DNA in vivo.

Significance to Biomedical Research and the Program of the Institute:

It is currently hypothesized by some that metabolically activated carcinogenic hydrocarbons cause cell transformation by inducing mutations. This project is an attempt to design a reliable test system in which the mutagenicity of various hydrocarbons and their metabolic derivatives can be analyzed and compared. Data from such tests will be of great help in assessing the potential for causing genetic damage possessed by various hydrocarbons and their metabolites in higher organisms.

Proposed Course of Project:

To pursue the goals as outlined above in Objectives.

Serial No. NCI-4529
1. Chemistry Branch,
OASDC, DCCP
2. Nucleic Acids Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: DNA Replication in Prokaryotic and Eukaryotic Cells

Previous Serial Number: None

Principal Investigators: C. Wesley Dingman, M.D. and Minoru Ishizawa, Ph.D.

Other Investigators: M. Patricia Fisher, Tsuyoshi Kakefuda, M.D., Ph.D. and Tina Bak

Cooperating Units: None

Man Years:

Total	:	2.0
Professional	:	1.3
Other	:	0.7

Project Description

Objectives:

To learn more about the molecular mechanisms of DNA synthesis, in particular the role of short, nascent DNA fragments and the role of the secondary and tertiary structure of DNA in the region of the replication point.

Methods Employed:

This project was initiated using E. coli as the organism to be studied. Much more is known regarding the mechanisms of DNA synthesis in this organism, and the major problems left unanswered are best delineated with these organisms. If we are successful in advancing our knowledge regarding DNA synthesis in this system, we intend to apply the techniques and knowledge we have gained to studies of DNA synthesis in human cells in culture.

We are using both thymine requiring wild-type cells and those with mutations affecting DNA replication, DNA repair, and DNA recombination. Our chief technique is to prelabel parental DNA by overnight labeling with ^{14}C-thymine and then pulse label newly synthesized DNA with ^{3}H-thymidine for 5 to 180 sec at 20°C. The DNA is then isolated and the size distribution and labeling characteristics of the nascent, newly-synthesized, DNA fragments examined, usually by polyacrylamide gel electrophoresis. The molecular

conformation of the replication point is examined by means of its electrophoretic mobility in polyacrylamide gels before and after heat denaturation and before and after treatment with various specific nucleases. Zone sedimentation analysis in alkaline and neutral sucrose gradients is also employed. Results obtained with in vitro DNA synthesis using highly purified preparations of E. coli polymerase I are compared with those obtained in vivo.

Major Findings:

In confirmation of the results obtained by others studying DNA replication in bacteriophages, we find strong evidence for the existence of single-stranded regions in the neighborhood of the replication point in E. coli. We have found that polyacrylamide gel electrophoresis allows us to separate forked replication points, high molecular weight DNA containing single-stranded regions, high molecular weight double-stranded DNA, and the various size classes of low molecular weight single-stranded DNA fragments, each from the other. The precursor-product relationships that may occur among these various forms are currently under study.

Significance to Biomedical Research and the Program of the Institute:

The replication and repair of DNA are critical and vital steps in the economy and survival of all cells. The mechanisms by which they are accomplished have not yet been elucidated nor has the role that these processes play in malignant transformation been discovered, in spite of widespread belief that the regulation of DNA replication may be an important key to understanding malignancy. This project is an attempt to gain some further insights into the process of DNA replication, first in prokaryotes and later in eukaryotes. Understanding even prokaryotic DNA replication should be of value in studies on the mechanism of malignant transformation of eukaryotic cells by oncogenic viruses.

Proposed Course of Project:

To pursue the goals as outlined above in Objectives.

Serial No. NCI-4784
1. Chemistry Branch, OASDC, DCCP
2. Nucleic Acids Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The Role of Molecular Conformation in Determining the Electrophoretic Properties of Polynucleotides in Agarose-Acrylamide Gels

Previous Serial Number: Same

Principal Investigator: C. Wesley Dingman, M.D.

Other Investigators: M. Patricia Fisher, Tsuyoshi Kakefuda, M.D., Ph.D., and Tina Bak

Cooperating Units: None

Man Years:

Total	:	0.4
Professional	:	0.2
Other	:	0.2

Project Description

Objectives:

To determine whether polyacrylamide gel electrophoresis under controlled conditions would be a useful technique for defining the structure of newly isolated, synthesized, or discovered polynucleotides.

Methods Employed:

A number of different polynucleotides having known structures were isolated from viral, bacterial, and mammalian sources. Low molecular weight DNA fragments were isolated from E. coli by sonicating and centrifuging purified high molecular weight DNA. The molecular weight of these linear fragments was determined by both electron microscopy and zone sedimentation in sucrose gradients. These various polynucleotides were then examined for their electrophoretic behavior in composite agarose-acrylamide gels under different conditions of temperature, gel concentration, and voltage gradient.

Major Findings:

We have further explored the relationship between the retardation coefficient, K_R (where log mobility = log mobility at zero gel concentration minus K_R multiplied by the gel concentration) and molecular weight. In general, for single-stranded polynucleotides, we found that Mol. Wt. = $A.K_R^{1.5} + B$, a result that might have been expected on the basis of theoretical arguments developed by others regarding acrylamide gel electrophoresis. The data for low molecular weight, linear, single-stranded, polynucleotides, and low molecular weight, linear double-stranded, polynucleotides indicated that the relationship between log mol. wt. and mobility was not linear but showed upward and downward curvature at low and high mobilities, respectively, as would be predicted from the above mentioned relationship between K_R and Mol. Wt. At an optimal gel concentration and voltage gradient, polynucleotides having molecular weights between 2×10^4 and 2×10^6 appear to fall into two classes with respect to having relative mobilities which are consistent with their relative molecular weights: one class consists of single-stranded polynucleotides which have been derived by the dissociation of molecules that are normally double-stranded; the other class consists of linear double-stranded polynucleotides and naturally occurring single-stranded polynucleotides such as ribosomal RNA's. At molecular weights below about 4×10^5, both classes of polynucleotides have retardation coefficients which are proportional to molecular weight, while, above this size, the retardation coefficients of linear double-stranded molecules are independent of molecular weight.

Significance to Biomedical Research and the Program of the Institute:

The results of this project will enable many investigators in both virology and nucleic acid metabolism to ascertain, rather inexpensively, the major structural aspects of newly isolated polynucleotides of biomedical significance. Furthermore, the techniques developed under this project are particularly useful when the amount of material available for investigation is very small. Thus, it is hoped that these procedures will aid in defining the nature of the genome of newly isolated viruses of oncogenic significance, prove useful in defining the various products of "reverse transcriptase" activity in both *in vivo* and *in vitro* studies, and be of help in defining the nature of the nascent DNA fragments that are involved in the replication of apparently all DNA genomes.

Proposed Course of Project:

To publish these results.

Publications

Dingman, C.W., Fisher, M.P., and Kakefuda, T.: The role of molecular conformation in determining the electrophoretic properties of polynucleotides in agarose-acrylamide gels, II. *Biochemistry* 11: 1242-1250, 1972.

Dingman, C.W., Kakefuda, T. and Fisher, M.P.: Electrophoretic properties of low molecular weight DNA fragments in agarose-acrylamide gels. Analytical Biochemistry 50: 519-528, 1972.

Serial No. NCI-4785
1. Chemistry Branch
OASDC, DCCP
2. Nucleic Acids Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The Role of DNA Repair Mechanisms in the Etiology of Cancer

Previous Serial Number: Same

Prinicpal Investigators: Rufus Day, III, Ph.D. and C. Wesley Dingman, M.D.

Other Investigators: M. Patricia Fisher, Tsuyoshi Kakefuda, M.D., Ph.D. and Tina Bak

Cooperating Units: None

Man Years:

Total	:	1.5
Professional	:	1.2
Other	:	0.3

Project Description

Objectives:

To learn more about DNA repair mechanisms in mammalian cells and about their role in carcinogenesis. In particular, to determine the nature of the biochemical defects in human cell lines grown from biopsies taken from persons having extreme susceptibility to skin carcinogenesis. The majority of such people, whose genetically inherited malady is termed Xeroderma Pigmentosum (XP), have defects in the repair of ultraviolet damage to DNA. A particularly interesting patient we have been concerned with has been diagnosed as having XP but his cells show completely normal DNA repair as judged by the criteria of several other investigators.

Methods Employed:

During this past year we have developed for the first time a method permitting the visualization of growth of individual adenovirus particles on human fibroblast cells (our method also works for mouse adenovirus infecting mouse cells). Using this method, we have been able to quantitate the deleterious effects of ultraviolet light (UV) on the ability of the virus to initiate and sustain infection. The method involves establishing a monolayer cell culture which is then infected with Adenovirus which has been irradiated with different doses of UV. The infected cells are then

incubated for 17 days with feeding by means of periodic overlaying with nutrient agar. Viable virions or those irradiated ones which have been "reactivated" by host cell repair mechanisms form plaques of dead, lysed cells and their number can be counted. With this method the plating efficiency using unirradiated Adenovirus is the same on all of the human cell lines tested so far.

Major Findings:

Using the method described above we have assayed 10 normal human fibroblast cell lines, 4 XP cell lines of the repairless type, and the 1 XP cell line of the normal repair type with regard to the amount of UV required to inactivate adenovirus 2 to a given survival level, using these cell lines as viral hosts. We found that adenovirus 2 must be irradiated with an average of 2200 ergs/mm^2 of 254nm UV light to obtain 37% survival measured on normal cell lines, while only 90, 150, 600, and 700 ergs/mm^2 are required when the 4 XP cell lines of the repairless type are used as hosts, and 1600 ergs/mm^2 are required when the one XP line with "normal" repair is used as host. All of the normal cell lines infected with virus irradiated with 1600 ergs/mm^2 give rise to greater than 37% viral survival, so that there is a possibility that this XP cell line of the "normal" repair type may indeed lack some repair function.

Significance to Biomedical Research and the Program of the Institute:

An evaluation of the role of DNA repair and/or related mechanisms in conferring resistance or susceptibility to mutagenesis and carcinogenesis is an important facet in any overall program having as its goal the understanding of the molecular pathways which, when perturbed, give rise to carcinogenesis. Physical, chemical, and viral carcinogens are all known to alter the structural integrity of the cellular genetic apparatus. It is a long range goal of this project to determine whether or not the elucidation of genetic repair mechanisms is important to the understanding of carcinogenesis, but it is expected that an understanding of genetic repair mechanisms in general will benefit many areas of biomedical research.

Proposed Course of Project:

To pursue the goals outlined above in Objectives and publish these results.

Serial No. NCI-4787
1. Chemistry Branch, OASDC, DCCP
2. Nucleic Acid Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Mode of Replication of RNA Tumor Viruses

Previous Serial Number: Same

Principal Investigator: Tsuyoshi Kakefuda, M.D., Ph.D.

Other Investigators: C.W. Dingman, M.D., Tina M. Bak

Cooperating Units: None

Man Years:

Total	:	1.0
Professional	:	0.5
Other	:	0.5

Project Description

Objectives:

To observe the early events of gene replication of RNA tumor viruses in host cells.

Methods Employed:

Three different experimental approaches have been designed and tested: (1) In order to detect the viral RNA genome introduced into the cell by infection, subcellular organelles were isolated from the cells and incubated in vitro. The DNA synthesized in this in vitro system was extracted, denatured, and hybridized with the original viral RNA and analyzed by isopycnic centrifugation in Cs_2SO_4. (2) Electron microscopic autoradiography of virus infected cells, using ^3H-thymidine labeling, was used to reveal the intracytoplasmic localization of viral DNA. (3) Total nucleic acids extracted from isolated subcellular organelles were hybridized with the high molecular weight RNA of the virus. An aliquot of the hybrid molecules, isolated by banding in Cs_2SO_4, was denatured in alkalie and further analyzed by centrifugation in alkaline Cs_2SO_4 density gradients and in alkaline sucrose gradients to determine the nature of the DNA products.

Major Findings:

Electron microscopic autoradiography of 3T3/BALB and chicken embryo cells infected with murine sarcoma virus and Rous sarcoma virus, respectively, showed labeling from ^{3}H-thymidine incorporation in the region of the plasma membrane beginning one hour after infection. The photopositive grains dispersed diffusely in the cytoplasm as the post infection time was prolonged from 2.5 to 24 hours. The in vitro transcription system used for assaying for reverse transcriptase activity specific for the infecting virus indicated that the activity was localized in the plasma membrane. DNA hybridizable to viral RNA was also isolated from the plasma membranes of infected cells and characterized. The DNA synthesized in vivo in the plasma membrane a short time after infection was heterogeneous in size, ranging from 10^{5} to 10^{6} daltons.

Significance to Biomedical Research and the Program of the Institute:

Incorporation of viral genome into the genetic system of the host cell appears to be one of the steps in viral carcinogenesis. The mechanism of replication and the nature of the intermediate products generated by the viral RNA in susceptible cells is, however, largely unknown. The autoradiographic and biochemical approaches used in the present experiments were found to be useful tools for elucidating the sequential events which occur in cells infected with RNA tumor viruses. The involvement of the surface membrane in the early stage of transcription is of particular interest since some alteration of its metabolism or structure by drugs or immunological treatment might offer a means for aborting the malignant transformation of cells by RNA tumor viruses.

Proposed Course of Project:

To extend the observations described above, a histochemical method for determining the intracellular localization of the RNA-dependent DNA polymerase reaction has been designed. By using simple, rapid and accurate testing systems, we hope to investigate a variety of cell lines (including human cells), viruses and the effect of drugs on this process under appropriate experimental conditions.

Serial No. NCI-4525
1. Chemistry Branch, OASDC, DCCP
2. Protein Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Studies on Electrophoretic Techniques for Protein, RNA, and DNA

Previous Serial Numbers: NCI-4721 and NCI-4731

Principal Investigator: Andrew C. Peacock, Ph.D.

Other Investigators: Sylvia L. Bunting and Roswell A. Reed

Cooperating Unit: None

Man Years:

Total:	2.8
Professional:	2.8
Other:	0.0

Project Description

Objectives:

To devise analytical approaches for the studies of synthesis of protein, RNA, and DNA and to use these techniques to gain an understanding of control processes involved in the synthesis of these compounds.

Methods Employed:

Cultures of HeLa cells, assay of radioisotopes, isolation and electrophoresis of RNA and DNA, analysis of nucleotides and nucleosides by chromatography, optical scanning of stained and unstained gels.

Major Findings:

1. Analysis of nucleosides. The regular use of nucleoside chromatography has revealed frequent contamination of nucleosides used as precursors by foreign substances and thus has proven to be a valuable first step in the use of these materials as tracers.

2. Interconversion of nucleosides. Uridine and cytidine are interconverted in the nuclear nucleotide pool. Each of these

nucleosides acts as a competitive inhibitor for the permeation of the other. Similarly, guanosine and adenine antagonize the permeation of each other and are interconverted, but to a lesser extent than for the pyrimidines. These interconversions were not observed in the cytoplasmic pool. Thus, base composition analysis by the use of specific nucleosides which we had earlier undertaken is invalid. The interconversions establish the existence of separate cytoplasmic and nuclear nucleotide pools for HeLa cells. Similar findings have been reported by other workers of Novikoff hepatoma cells. The implication of these findings for quantitative estimation of RNA synthesis are being explored.

3. Studies of RNA extraction at high salt concentration. Ultracentrifugal studies have shown that some of the RNAs extracted at 0.5 M salt concentration are of high molecular weight (> 100S), consistent with their slow migration in gels. The aggregate state persists through alcohol precipitation and digestion by DNAase and pronase. On the other hand, this RNA is disaggregated by heat with a melting point of approximately 83° and by treatment with 70% DMSO. Studies of the enzymatic susceptibility of this form of RNA are incomplete. Because these aggregates can be extracted only from cells and cannot be produced *in vitro*, the possibility that they result from some natural conformation which is preserved during high salt extraction is being explored.

4. "Chase" studies. We have found that simple transfer of HeLa cells from labeled to unlabeled medium results in a cessation of incorporation of tracer nucleosides. Because non-destructive methods of interfering with cellular processes are of great value in studying precursor relationships, the significance of these findings is being further investigated.

5. Additional functions. In addition to the research findings defined above, this unit has participated in numerous "community services" as follows:

a) Instruction in our methodology and consultation with investigators throughout NIH has contributed to their better use of gel electrophoretic techniques and contributed to numerous other research projects in an unidentified way.

b) Computer programs originally used by this Section have been extended and improved so that they are available for use by all Sections of the Branch.

c) Liquid scintillation counters in use by all members of the Branch have been monitored and calibrated at monthly intervals to assist others in obtaining valid data.

Significance to Biomedical Research and the Program of the Institute:

The ability to conduct detailed studies on interrelationships between macromolecules, such as RNA, proteins, and DNA, is probably fundamental to understanding the alterations which occur in malignancy. The above studies are directed towards this goal.

Proposed Course of Project:

Most of the studies described above are currently incomplete, and will be continued. The mechanisms by which RNA synthesis is controlled will be studied by a comparison between experimental results and computer simulation of proposed models.

Publications

Zeiger, R. S., Salomon, R., Kinoshita, N., and Peacock, A. C.: The binding of 9,10-dimethyl-1,2-benzanthracene to mouse epidermal satellite DNA *in vivo*. Cancer Research 32: 643-647, 1972.

Zeiger, R. S., Salomon, R., Dingman, C. W., and Peacock, A. C.: Role of base composition in the electrophoresis of microbial and crab DNA in polyacrylamide gels. Nature New Biology 238: 65-69, 1972.

Serial No. NCI-4526
1. Chemistry Branch, OASDC, DCCP
2. Protein Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Characteristics of Mouse Mammary Tumor mRNA

Previous Serial Number: None

Principal Investigator: Robert J. Cates, M.D.

Other Investigators: Andrew C. Peacock, Ph.D. and Marie R. Green, Ph.D.

Cooperating Unit: None

Man Years:

Total:	1.1
Professional:	1.1
Other:	0.0

Project Description

Objectives:

Compare mRNA in normal mouse mammary tissue with mRNA in mammary tumor with subsequent quantitative and qualitative comparison.

Methods Employed:

Tissue culture, radioisotope labeling, ultra-centrifugation, gel electrophoresis.

Major Findings:

Messenger RNA labeled with ^{3}H-adenosine and ^{14}C-uridine has been harvested from HeLa cells utilizing the binding of mRNA's poly A fraction to poly U filters. Messenger RNA, subsequently removed from the filter by formamide, is being analyzed by electrophoresis in composite agarose-acrylamide gels.

Significance to Biomedical Research and the Program of the Institute:

Identification of amounts and characteristics of mRNA in malignant tissue and its normal counterpart may help to understand the kinds of processes which lead to malignancy.

Proposed Course of Project:

To quantitatively and qualitatively compare normal and tumorous mouse mammary mRNA and investigate possible hormonal effects on same.

Serial No. NCI-4718
1. Chemistry Branch, OASDC, DCCP
2. Protein Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Disc Electrophoresis - A Study of the Fractionation of Protein Mixtures on Cylindrical Columns of Polyacrylamide Gels

Previous Serial Number: Same

Principal Investigator: Arthur T. Ness, Ph.D.

Other Investigators: Jullia V. Pastewka and Andrew C. Peacock, Ph.D.

Cooperating Units: None

Man Years:

Total:	2.1
Professional:	2.1
Other:	0.0

Project Description

Objectives:

There are five objectives: (a) study of the variables involved in the gel electrophoretic fractionation of protein mixtures; (b) identification of the fractions and their biochemical significance; (c) determine and interpret the significance of the secondary and tertiary structures of proteins; (d) study of protein-protein interactions and related variables; (e) study of the effect of hormones and other factors on the induction and rate of biosynthesis of proteins by various cells and tissues.

Methods Employed:

1. The disc polyacrylamide gel electrophoresis technique as developed by Ornstein and Davis and as modified by others for particular systems.

2. The use of other fractionation techniques such as column chromatography, preparative gel electrophoresis, ultra-centrifugation, etc., to obtain homogeneous, unaltered protein entities to aid in the identification of the components obtained

in disc gel electrophoresis and in the interpretation of the results obtained in studies outlined in the broad objectives.

3. The technique of isoelectric focusing in disc acrylamide gel systems. This establishes a pre-selected pH gradient range (wide or narrow) and permits the separation of proteins of isoelectric point difference of 0.02 pH unit.

Major Findings:

1. A reliable disc polyacrylamide gel electrophoretic procedure for haptoglobin subtyping has been developed and published.

2. The dye referred to as "Stains-all" or SA (Eastman 2718) has been found to be very useful by showing differential color staining of RNA and DNA electrophoretically resolved on gels.

Application of this dye to proteins separated by gel electrophoresis indicated a differential color staining of normal proteins versus phosphoproteins. This property suggested an important use of "Stains-all" in the study of milk and mammary tissue proteins.

The development of a satisfactory procedure has presented difficulties, due to (1) the light sensitivity of the dye, (2) the interference by sodium dodecyl sulfate (SDS) used in the electrophoretic systems, and (3) the availability of only a few types of phosphoproteins and these not in pure form (including caseins).

3. Dyes, such as Alcian Blue and others, reputedly specific for carbohydrate materials, are being studied for use in the identification and interpretation of changes in the gel electrophoretic patterns of milk and other mammary tissue proteins.

Significance to Biomedical Research and the Program of the Institute:

The high resolution and sensitivity of the disc electrophoretic technique permit study of the occurrence and significance of genetic variants of protein entities and also the analysis of subcellular fractions to determine the site or sites of protein synthesis and a study of the biochemical factors in protein synthesis. The technique may also permit the detection and assessment of physiological changes associated with the development of malignancy.

Proposed Course of Project:

None.

Publications

Pastewka, J. V., Reed, R. A., Ness, A. T., and Peacock, A. C.: An improved haptoglobin subtyping procedure using polyacrylamide gel electrophoresis. Haptoglobin gene frequency distribution among a group of blood bank donors. Anal. Biochem. 51: 152-162, 1973.

Serial No. NCI-4782
1. Chemistry Branch, OASDC, DCCP
2. Protein Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Hormonal Effects on Normal and Malignant Breast Tissue in Culture

Previous Serial Number: Same

Principal Investigator: Marie R. Green, Ph.D.

Other Investigators: Andrew C. Peacock, Ph.D. and Julia V. Pastewka

Cooperating Unit: None

Man Years:

Total:	1.2
Professional:	1.2
Other:	0.0

Project Description

Objectives:

To study the response of normal and malignant breast tissue in culture to hormones in terms of RNA and DNA synthesis and the production of proteins (particularly milk proteins).

Methods Employed:

Culture of mammary explants, isolation of RNA and DNA, electrophoretic analysis of RNA, isolation of milk proteins from explants and milk, assay of radioactivity, microscopic examination of explants, autoradiography.

Major Findings:

Explants of mammary tissue of mice in midpregnancy cultured on a synthetic medium with insulin and hydrocortisone respond to the addition of prolactin by increased synthesis of proteins. Previous investigations had shown that the amounts of material secreted into the alveolar lumen (estimated from the appearance of histological sections) varied with the concentration of prolactin in the medium. We have studied the nature and amount of the proteins made by the explants in response to prolactin

by electrophoretic analysis of the proteins. One band in particular increased in proportion to the amount of prolactin present in the medium during a five day culture. As little as 5 ng/ml ovine prolactin was required to produce a measurable increase in the accumulation of this band. In order to relate this protein to the proteins of milk, mouse milk has been fractionated and electrophoresed at pH 2.7 and pH 7.2. The mouse milk has been fractionated by standard methods to separate caseins from whey proteins. The proteins from post-mitochondrial supernatants of mouse mammary explants have been partially characterized in terms of mouse milk. The band which accumulates in response to prolactin is thought by preliminary analysis to be a form of casein. Measurement of this band forms the basis for a sensitive bioassay for prolactin.

Electrophoresis of proteins from post-mitochondrial supernatants of explants at pH 2.7 separates milk proteins from other tissue proteins into distinct groups. We have compared the proteins in mammary explants derived from virgin, midpregnant and late-pregnant mice. Milk proteins may be detected in midpregnant and late-pregnant tissue. The effects of several RNA and DNA synthesis inhibitors are being studied to determine optimal concentration and timing for inhibition of the hormonal response to prolactin.

During the course of the work on electrophoresis of milk proteins we have studied the cationic carbocyanine dye 1-ethyl-2-[3-(1-ethylnaphtho[1,2d]thiazolin-2-ylidene)-2-methyl-propenyl]naphtho[1,2d]thiazolium bromide which seems to have special staining properties for proteins containing phosphorus. We have determined the pH, electrolyte, solvent and dye concentrations at which this stain may be used optimally. Mouse milk and human milk have been electrophoresed on gels and the phosphorus containing caseins have been distinguished from whey proteins by means of this stain.

The stain has been adapted for distinguishing phosphoproteins from other proteins in tissue sections by further adjustment of the pH, and the concentrations of dye, solvents and electrolytes.

Significance to Biomedical Research and the Program of the Institute:

The mouse mammary explant system as a bioassay for prolactin offers advantages over the pigeon crop assay in terms of sensitivity and availability and numbers of animals used. Assay of proteins by the method we describe offers more precise quantitation than examination of histologic sections. No radioactive isotopes are required. Samples may be frozen.

We have determined characteristic proteins in mouse mammary explants in varying physiological states. The techniques we have developed can now be easily adopted to determine protein synthesis in response to hormones from mammary tissues of other species including human.

A stain particularly sensitive to phosphorus containing proteins when used in conjunction with stains ordinarily used for staining proteins on gels following electrophoresis would be of great utility in localizing and distinguishing phosphoproteins from non-phosphorus containing proteins. A technique applicable to the study of phosphorylated proteins in general would find wide application in studies of the control of cellular metabolism.

A histochemical technique for detecting phosphoproteins in tissues will be of considerable value in studying normal and pathological tissue specimens. It will provide a quick method for detecting whether hormonal manipulations in vitro are producing a particular phosphorylated product.

Proposed Course of Project:

Because mammary tissue in culture responds to prolactin in terms of increased RNA synthesis and milk protein production, this system may be used as a sensitive bioassay for measuring amounts of prolactin present in blood. The assay may be of use in testing prolactin activity of various preparations obtained in the course of purification procedures for human prolactin.

Biopsy material of breast tissue from malignant tumor-bearing hosts (animal or human) may be grown and examined for responsiveness to hormones for retrospective and prognostic purposes. The effect of individual hormones on such tissue, whether the malignancy is initiated by viruses or chemicals may be determined in culture.

Several hormones are important in regulating mammary development and function. The way each hormone interacts individually and in concert with responsive elements of the cell will be studied.

SUMMARY REPORT

EXPERIMENTAL PATHOLOGY BRANCH

July 1, 1972 through June 30, 1973

At the beginning of this fiscal year, the Experimental Pathology Branch consisted of two Sections and a Unit. Personnel in each of these groups conducted research in their own laboratories and were also responsible for establishing and monitoring collaborative programs through contracts. The Carcinogen Screening Section was largely responsible for the contracts in the Bioassay Segment whereas the Bioassay Section and Histopathology Unit were responsible for contracts in the Biological Models Segment. The Branch was reorganized recently in order to establish a structure which is more compatible with its program responsibilities. In this reorganization, the Bioassay Section was abolished, and the Perinatal Carcinogenesis Section, Endocrine Carcinogenesis Section, Digestive System Cancer Section, In Vitro Pathogenesis Section, and Ultrastructure Unit were established. The Histopathology Unit remains in existence as a service group without direct research responsibility. The Carcinogen Screening Section has remained intact, pending a proposal to establish its function as a Carcinogen Metabolism and Toxicology Branch.

With this reorganization, the Experimental Pathology Branch now has an organizational structure which permits it to focus its studies on the etiology and pathogenesis of many of the major forms of human cancer and to seek ways to prevent these cancers. As space and personnel become available, carcinogenesis research can be undertaken within this Branch on cancer of the breast, prostate, uterus, ovary, esophagus, pancreas, liver, stomach, rectum, skin, and those special types of cancer occurring in children including various types of sarcomas, lymphomas, and tumors of the central nervous system, adrenal, and kidney. It should be emphasized that although the structure has been established for working on all these types of cancer, through intramural and contract-supported research, the Branch does not currently have sufficient personnel or laboratory-office space to devote attention to each of these forms of cancer. Those on which active research is underway are described below under the reports of the Sections and under the Biological Models Segment report. Earlier plans to include a group working on cancer of the urinary bladder and kidney within the Experimental Pathology Branch as a focus for such activities within the Carcinogenesis Program have been dropped because of personnel limitations. Earlier projections of personnel increases in the Carcinogenesis Program had indicated that it would be possible to staff each of the Sections with a "critical mass" of scientists who were personally engaged in the type of research which they were required to manage under the collaborative program so that they would be aware of its problems, opportunities, and developments and could effectively serve the function of examining the plans and research of contract-supported organizations to be sure these represented the best interests of the Government. Revised personnel ceilings indicate it will be some years at best before the already-established Sections reach this operating level. Thus, some of the original plans for which the Experimental Pathology Branch was established will have to be curtailed or abolished.

The functions of the Sections and Units within the Branch and their activities during the past year are summarized in the following paragraphs:

The Carcinogen Screening Section (1) Develops and monitors screening programs for the detection of carcinogenic hazards of environmental chemical agents; (2) investigates the toxic, pharmacologic, and carcinogenic activity of selected chemicals or mixtures of environmental significance; and (3) explores metabolic pathways of selected test chemicals in different animal species. The Carcinogen Screening Section has as its chief extramural activity the supervision of contracts related to screening of environmental chemicals for possible carcinogenic hazard. Most of the professional personnel of the Section are involved in this activity and probably half their man hours are spent supervising contracts and administering other matters related to the contract activity.

The in-house activities of the Section are devoted to several projects. One is the metabolism of various chemical carcinogens and their analogs, to gain an insight on the pharmacodynamics of carcinogens. Laboratory activities involve metabolism studies in animals, enzyme studies and the organic synthesis of model compounds for possible metabolites. During the past year it was determined that the N-glucuronide of 6-amino-chrysene is a biliary metabolite of the amine. However, *in vitro* synthesis of the N-glucuronide was not successful due to the chemical instability of the product. In conjunction with a study on inhibition of the carcinogenic activity of 2-fluorenylacetamide (FAA) or N-hydroxy-2-fluorenyl-acetamide (N-OHFAA) by p-hydroxyacetanilide (p-OHAA), it was found that prefeeding p-OHAA increased the excretion of FAA or N-OHFAA metabolites excreted in the urine as glucuronides. Simultaneously, the binding of either carcinogen to liver and blood proteins was lower after p-PHAA. These studies led to the finding that p-OHAA feeding increased threefold the glucuronyl transferase levels in rat liver microsomes. S-(5-Acetamido-2-hydroxyphenyl)mercapturic acid and S-(5-acetamidophenyl)-mercapturic acid were identified as water soluble metabolites of acetanilide. The former compound was also a metabolite of p-OHAA. Contrary to expectations, the carcinogen auramine was not metabolized through to a benzylic alcohol intermediate.

Another part of the Section's activity is spent in studying the mechanism of action of various chemical carcinogens *in vivo*. In addition to some small scale bioassays, other studies are done to determine what factors inhibit or enhance the potency of chemical carcinogens. These may be dietary factors such as fat, protein, carbohydrate, and vitamin levels and other drugs. During the past year, bioassays of two compounds which had been synthesized as cancer chemotherapeutic agents were completed. One of them, 1-(4-N-nitroso-N-methylaminobenzylidene)-indene (NSC-100983), caused many liver lesions (fibrosis, cirrhosis, carcinoma) and ear duct and breast tumors. The other, 4-(4-N-nitroso-N-methylaminostyryl)quinoline (NSC-101984), was a less potent carcinogen but was much more toxic than the indene compound.

Since chronic occupational exposure to benzene has been implicated as a cause of leukemia or lymphomas in humans, tests were done in newborn mice. Repeated injection of benzene in corn oil over a one-year period led not to tumors but an earlier appearance of amyloidosis. Treatment of mice with butylnitrosourea in the drinking water yielded thymic lymphomas in 50% of the mice in three to six months.

A diet high in ground cellulose decreased the small intestinal tumor incidence from injected azoxymethane in rats. However, the colon cancer incidence was not decreased. In addition, four different chemically-induced intestinal tumors (three small intestine, one colon) have been transplanted in weanling rats. Two of the lines are fast growing and kill the host animals in two months.

The Section is also concerned with using cell cultures as a means for studying the carcinogenic process. It developed a cell culture method of growing rat liver parenchyma which can be transformed by various chemical carcinogens. It is hoped that studies of the biochemical and other mechanisms involved in the growth and transformation of these cells will lead to understanding the processes involved. In addition a cell culture system might be used as a pre-screen or quick bioassay for suspected environmental hazards. During the past year a project on levels of arylhydrocarbon hydroxylase (AHH) in rat embryo cell cultures infected with Rauscher leukemia virus (RLV) was completed. AHH levels were generally increased in RLV infected cells.

Human patients with liver cancer have an abnormal globulin, alpha-fetoprotein (AFP) in their serum. More patients from Africa show high AFP titers than those in Europe or the United States. In any event, the presence of AFP may serve as a useful diagnostic tool for the presence of liver cancer. In model studies, it was shown that several hepatocarcinogens led to early AFP production in rats. In contrast to negative reports from other research teams, this Section, in cooperation with Drs. Wogan and Newberne of MIT, demonstrated the early appearance of AFP in the serum of rats fed aflatoxin. The effect was highly dose-dependent. Further studies are in progress to follow this discovery with other types of carcinogens and hepatotoxic agents.

The Perinatal Carcinogenesis Section (1) Investigates the induction of cancer in experimental animals before birth and during infancy, with the goal of identifying factors responsible for the initiation and growth of tumors of childhood and infancy in man; (2) utilizes techniques of chemical synthesis, biochemistry, histopathology, immunology, and endocrinology to identify carcinogens to which, both individually and in combination, the fetus and neonate are particularly vulnerable, and to identify host factors which qualitatively or quantitatively modify their biological effects; and (3) develops measures for prevention of cancer in children or in later life as a response to conditions of high susceptibility to carcinogens during fetal life or childhood.

The Perinatal Carcinogenesis Section was created in February 1973 from a nucleus within the former Bioassay Section of the Experimental Pathology Branch. Studies of the physiological and biochemical factors involved in the higher sensitivity of experimental animals to chemical carcinogens during intrauterine and early postnatal life constitute the experimental program of this Section. Current studies include approaches to the chemical induction of nephroblastoma (Wilms' tumor) in rats; the induction of local sarcomas in mice by injection of water-soluble polycations; and the immunological basis for the high sensitivity of mice to transplacental induction of pulmonary tumors. The report from another laboratory that nephroblastomas could be induced by DMBA in ovariectomized Sprague-Dawley rats has proved non-reproducible, and efforts to study the chemical induction of this tumor are now directed towards the use

of nitroso compounds and the cycasin analogues. Progress has been made in maintaining pregnant rats in a state of borderline vitamin A deficiency, in an effort to produce viable offspring with the spectrum of urogenital malformations associated with an excess incidence of Wilms' tumor in man. Further studies on oncogenic polycations have shown that only two injections of DEAE-dextran at the concentration proposed for incorporation in veterinary vaccines is sufficient for the induction of a high incidence of subcutaneous sarcomas in mice. However, the rat and the Syrian hamster have proved completely refractory to treatment schedules which are highly effective in mice, showing that there is a pronounced interspecies variation in susceptibility to oncogenesis by this class of compounds. Combined treatment with polycations, halogenated pyrimidine nucleosides, and ENU have shown a synergism between ENU and the thymidine analogues in the induction of reticulum-cell neoplasms, probably due to the ability of these compounds to induce the synthesis and release of infectious C-type virus. No synergism has been detected, however, in the induction of soft-tissue sarcomas. Cultured cell lines derived directly from DEAE-dextran-induced sarcomas have been shown to release C-type viruses of typical morphology (EM) and bouyant density, which when injected into neonatal mice give rise to lymphocytic and myeloid leukemias. Neither soft-tissue nor osteo-sarcomas have yet been observed. In vivo transplantation studies have established that transplantable pulmonary tumors in strain C3Hf mice are cross-reactive with each other and with normal lung tissue from susceptible strain A, but not with C3Hf sarcomas or with normal lung tissue from genetically resistant strain DBA/2. This is the first experimental system to correlate genetically determined susceptibility and resistance to specific types of tumors with immunological tolerance and responsiveness to antigens characteristic of an entire class of tumors. In the case of the one allogenic mouse strain (C3H) to which these tumors are readily transplantable, classical genetic analysis of susceptibility in F_1, F_2, and backcross generations has indicated that the ability of C3Hf lung tumors to grow better in allogenic than in syngeneic mice is due to the action of a single gene, and correlates with the presence in strain C3H lung tissue of an antigen cross-reactive with the pulmonary tumors. Studies are now underway to determine whether there is a differential sensitivity to chemical induction of autochthonous pulmonary tumors in these two strains, and additional pulmonary and non-pulmonary (mesenchymal) tumors have been established in other genetically resistant strains of mice to extend and confirm the generalizations drawn from study of the C3Hf tumors.

The Endocrine Carcinogenesis Section (1) Develops experimental models of cancer of endocrine target organs including prostate, breast, uterus, and ovary; (2) utilizes these models to conduct investigations on conditions affecting the sensitivity of endocrine target organs to endogenous and exogenous chemical and physical carcinogens; and (3) studies the etiology and pathogenesis of human cancer of endocrine target organs to develop measures which can be used to prevent the induction or development of the disease. Because of the present small staff, only two areas (prostate and breast cancer) of major emphasis have been started this year. Since relatively little definitive information regarding the histological and morphological development endocrinology and carcinogenesis of the prostate has been reported, this Section's first concern has been to initiate programs in these areas. Intramural research has compared the histological and morphological development of the

various prostate lobes and accessory sex glands of six species of rodents. To establish their progressive changes, these structures were removed from these species at prenatal, neonatal, prepuberal, adult and old age states for examination by high resolution light and electron microscopy. In addition, the rate of epithelial cell proliferation and DNA synthesis in these various prostate lobes of these species at the various ages has been monitored. Studies of the effect of hormones (estrogens, androgens, and prolactin) on the morphological development and cellular proliferation rates have been started. Based on present findings, studies have been initiated in an attempt to induce prostate adenocarcinoma in the rat.

To allow for easy examination during tumor experiments, a conditioning site prior to organ culture and a transplant site following organ or cell culture, a suitable subcutaneous site for prostate growth has been sought. The cleared mammary fat pad of an androgenized female has been developed for this purpose.

In addition to these intramural programs, a significant proportion of staff time has been utilized to develop, direct and coordinate a collaborative contract program on prostate carcinogenesis. This area has not been adequately investigated by the scientific community, although today the incidence of prostatic adenocarcinoma is great and steadily rising. Furthermore, no adequate animal model exists for the investigation of hormone-dependent prostatic carcinoma. Thus, a need was felt to develop programs which would be of interest and stimulate scientists toward increased activity in this vital area of concern. Contract programs developed in this field are described in the Summary Report of the Biological Models Segment.

Little attention has been paid to epidemiological data in the design of present animal model investigations of mammary cancer. Thus, initial goals in this Section's intramural research on breast cancer have been in the areas of mechanism of action and the development of systems in which the integration of animal and human findings could be tested. Techniques were developed to allow for the use of isolated mammary epithelium for cell culture. In addition, some success has been realized from our efforts to further separate the mammary epithelium into its ductal and alveolar elements and examine hormonal growth requirements of these cells. It was found that cells (after becoming confluent in hormone enriched media) when cultured for up to 60 days in the presence of insulin alone do not appear transformed and can be transplanted. This system for the examination of *in vitro* carcinogenesis is being pursued. The legal and logistical problems for the procurement of human breast tissue from local hospitals have been solved. Thus, an attempt is now being made to isolate and culture ductal and alveolar cells from human breast. This will allow for ultimate correlation of hormone and carcinogen mechanism of action in animal systems to that of the human. In addition, a sensitive system for the development of diagnostic and preventive measure would be available.

Following isolation procedures, the epithelial cells are still capable of carrying out metabolic functions without undergoing a period of diminished activity. DMBA incubated in the presence of an epithelial cell microsomal fraction and NADPH was covalently bound to rRNA. The omission of NADPH from the system resulted in no detectable binding. Attempts to isolate and identify

metabolites have not been very productive. Another project has been a study of the influence of age on the sensitivity of the mammary epithelium to hormones and carcinogens. Preliminary findings indicate an increased sensitivity to certain hormones with increased age in the rat model system.

Much of the Section's intramural program has slowed due to the increasing involvement in the collaborative contract research. Since the Endocrine Carcinogenesis Section has only been recently formed, it was felt that much emphasis must be given to the development and initiation of sound contract programs which could be integrated with present epidemidological findings. The rapid progress of the knowledge in the past few years indicates that an in vitro system may provide an effective tool for elucidation of the various facets of the problems of chemical carcinogenesis without the involvement of complexities of in vivo systems. A contract program involving the development of an in vitro system of chemical carcinogenesis using organ culture of whole mammary gland was initiated; it is described in the Biological Models Segment Report.

The Digestive System Carcinogenesis Section (1) Plans and conducts research on the etiology and pathogenesis of cancer of the human digestive system including pancreas, liver, esophagus, stomach, colon, and rectum; (2) develops experimental models for laboratory investigation of the induction and development of those forms of cancer; and (3) utilizes experimental models to develop techniques for prevention of cancer of the digestive system in humans by interrupting the initial response to chemical and physical carcinogens or by arresting or reversing subsequent progression of the disease process.

This Section has just been established and a small group of personnel transferred to it from other portions of the Branch. One member of the Section, Dr. Richard Yamamoto, is spending a major portion of the year at the National Cancer Research Institute, Tokyo, Japan, where he is performing research on carcinogenesis of the stomach and colon. In contrast to the situation in the United States in which there is a high incidence of cancer of the colon and rectum but a relatively low incidence of cancer of the stomach, the reverse is true in Japan. There is considerable interest among Japanese scientists in carcinogenesis of the digestive system and Dr. Yamamoto is fortunate in being able to work with several such scientists. It is hoped that his presence in Japan will lead to a continuing working relationship upon his return giving the Digestive System Cancer Section access to two human populations with remarkably different incidences of cancer of various portions of the digestive system. His research activities in Japan include studying the effect of a high sodium chloride intake on induction of cancer of the stomach by N-Methyl-N'-nitro-N-nitrosoguanidine and the effect of inhibitors of proteolytic enzymes on the induction and metastasis of cancer of the colon in rats.

Other activities within the Section include the investigation of repair of carcinogen-induced damage to DNA, especially in human cells. It has been found that human diploid fibroblasts repair damage induced by N-acetoxy-2-acetylaminofluorene and 7-bromomethylbenz(a)anthracene by the insertion of all four deoxyribonucleosides. Both A-T rich and G-C rich areas of the genome appear to be equally well repaired, though in neither case is the

carcinogen completely removed. Techniques are being developed to permit further fractionation of DNA to determine whether some types are repaired more effectively than others. As an example of such an approach, gradients of Cs_2SO_4-Ag^+ result in better separation of mouse satellite from main band DNA than is ordinarily achieved in CsCl gradients.

At the present time the collaborative research activities under this Section are devoted to a search for experimental models for studying carcinogenesis of the pancreas, a common site of human cancer but a rare site in experimental animals. No adequately documented models are currently available. Principal approaches utilize techniques to increase the concentration of carcinogens within the pancreas, to increase the responsiveness of exocrine pancreatic cells to these carcinogens, and to develop cell and organ culture techniques for investigating the effect of carcinogens on human and animal pancreas. (See Summary Report of Biological Models Segment.)

The In Vitro Pathogenesis Unit (1) Works in collaboration with other Sections of the Branch to develop in vitro models for studying the pathogenesis of major forms of human cancer using both human and animal tissues; (2) utilizes these culture systems in biochemical, cytologic and biologic investigations to develop approaches to the prevention of cancer in humans. Stages in the pathogenesis of cancer are studied to learn which are amenable to arresting or reversing the progression of the disease before it threatens life; (3) develops procedures for evaluating the biologic potentiality of lesions induced by chemical or physical carcinogens in human tissues in vitro.

The In Vitro Pathogenesis Unit has directed its efforts at developing in vitro model systems for use in a mechanistic approach to carcinogenesis. Much of this effort has been directed at developing a system of epidermal cell culture to use as an in vitro parallel of the in vivo skin carcinogenesis system. Toward this goal, this group has been able to isolate and grow in culture pure epidermal cells as well as pure populations of hair follicle cells and dermal fibroblasts. The epithelial skin cells keratinize in vitro and, when grafted back to prepared skin graft beds of syngeneic hosts, will produce intact donor skin. Preliminary evidence indicates that these cells can be transformed in vitro by chemical carcinogens and efforts are now being directed toward a model system whereby epidermis can be initiated in monolayer culture in vitro and promoted in recipient animals in vivo.

A major effort is also being directed at understanding the differentiation of epidermal cells and the role this process plays in malignant transformation. To this end epidermal cell differentiation has been successfully interrupted in vitro by the use of retinyl acetate. However, once differentiation proceeds past a certain point, the process cannot be reversed. Experiments are now in progress to elucidate the mechanism of action of retinyl acetate and to look at effects of other vitamin A analogues.

Organ specific in vitro systems are likewise under study in this Unit. Initial efforts are underway to develop a cell culture system for prostate epithelial cells with the overall goal being in vitro prostate carcinogenesis. Early results of the culture system show that epithelial cells can be isolated free of fibroblasts and will grow for periods of one to two weeks in vitro.

Future efforts will be directed at maintaining these cells by the use of hormones, special media and vitamins. Intramural efforts in specialized cell growth are augmented by studies on organ and cell culture of prostate, pancreas, and liver epithelium through the contract program.

Mechanistic studies in this Unit are being conducted using skin cell cultures. The repair of DNA damage caused by the skin carcinogen β-propiolactone (BPL) was studied using tritiated deoxyribonucleosides. Whereas all four deoxyribonucleosides were utilized for DNA replication, only deoxyguanosine was incorporated in repair. Since BPL interacts primarily with guanine in DNA, this result may indicate the insertion of a single base at the site of damage. DNA repair is currently being studied after treatment with other carcinogens and in cells from other species. Initial results suggest that low doses of carcinogen may be repaired by insertion of a single base while larger doses are repaired by insertion of several bases at each repair site.

Animal data suggest that DNA synthesis at, or soon after, the time of carcinogen treatment is important in subsequent tumor development. Results from this Unit show that a single application of 0.5% croton oil to mouse skin in vivo increases the rate of epidermal DNA synthesis several-fold beginning at about 9 hours, reaching a peak at 18-24 hours and returning to normal by 4 days. Croton oil treatment times of -24, -6, and +1 hours with respect to the time of urethane injection all resulted in a threefold increase in the number of papillomas developing in initiation-promotion experiments. Thus, if enhancement of urethane initiation by a croton oil treatment is related to croton oil induced DNA stimulation, DNA replication at times longer than 10 hours after urethane injection appears to be most important in the process of skin tumor initiation.

The Ultrastructure Unit (1) Conducts research in chemical and physical carcinogenesis through the use of electron microscopic techniques; (2) advises and assists staff of the Carcinogenesis Program on proper utilization of such techniques for solution of experimental problems; and (3) provides service through the collaboration of intramural or contractors facilities and staff to the Experimental Pathology Branch in performance of electron microscopic evaluation of appropriate tissues from experimental or clinical material.

This Unit serves as a resource to provide a service to the other Sections and Units. Research based on the output of this Unit is reported under the Sections and Units for which the services are provided.

The Histopathology Unit (1) Serves the Carcinogenesis Program by sectioning and staining tissues for histologic, pathologic, and histochemical evaluation; and (2) provides assistance and advice on the use of histologic and histochemical techniques for the solution of experimental problems in carcinogenesis. This Unit serves as a resource to provide a service to the other Sections and Units. Research based on the output of this Unit is reported under the Sections and Units for which the services are provided.

Serial No. NCI-4508
1. Experimental Pathology Branch
OASDC, DCCP
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Cellular Response to Carcinogens: Repair Mechanisms

Previous Serial Number: None

Principal Investigator: Michael W. Lieberman, M.D., Ph.D.

Other Investigators: Miriam C. Poirier and Sol del Ande Eaton

Cooperating Units: None

Man Years:

Total	:	1.0
Professional	:	0.5
Other	:	0.5

Project Description

Objectives:

Much ofthe recent work has been directed toward an analysis of the cell's response to carcinogenic chemicals. Major emphasis has been placed in this area since it is becoming increasingly apparent that the critical aspect of the neoplastic process may not be the damage done by carcinogens (e.g. alkylation), but rather the metabolic events set in motion by such damage. One aspect of this problem, the DNA repair process, has been studied in detail.

Methods Employed:

Human diploid fibroblasts are grown as confluent monolayers in 150 mm petri dishes. After damage with a number of proximate chemical carcinogens (N-acetoxy-2-acetylaminofluorene, 7-bromomethylbenz(a)anthracene, methylnitrosourea) or ultraviolet light, repair is studied either with the hydroxyurea method previously described by us or by density labeling with bromodeoxyuridine. Both methods involve centrifugation in CsCl and analysis of radioactive nucleoside incorporation into non-replicating DNA. Ancillary methods employed are paper chromatography of DNA digests for identification of products, enzyme digestion studies with snake venom phosphodiesterase to assay for terminal addition, and hydroxyapatite chromatography of sheared DNA to investigate genomic localization. A detailed investigation of Cs_2SO_4 - Ag^+ gradient separation of intragenomic molecular species of DNA is also underway. It is hoped that the latter approach

will allow localization of DNA repair within the genome.

Major Findings:

Human diploid fibroblasts incorporate all four deoxyribonucleosides during the repair of damage induced with N-acetoxy-2-acetylaminofluorene and 7-bromomethylbenz(a)anthracene. This incorporation is not due to a terminal addition. Thus, it has become clear that "repair" of damage induced with carcinogenic chemicals is a meaningful biological process and probably not an agonal artifact. More recent studies have focused on the localization of repair in the genome with the hope of determining if there are selected areas which are more or less repairable. It appears, based on data from hydroxyapatite chromatography, that A-T rich and G-C rich areas of the genome are equally well repaired.

A second area of emphasis has been the development of methods to examine binding and removal of carcinogens from various areas of the genome. The use of Cs_2SO_4 - Ag^+ gradients shows great promise in this regard. We have been able to achieve much better separation of mouse satellite from mainband DNA than is usually achieved in CsCl gradients. This technique may now be applied to studies of carcinogen binding, replication of selected DNA fractions, and repair. The technique will be of great use in the mouse and at present we are trying to work out its application to human DNA.

Significance to Biomedical Research and the Program of the Institute:

It appears, based on present and previous work, that human cells have an effective mechanism for removing carcinogenic chemicals from the genome and restoring the integrity of the damaged area. In principle this system offers an exploitable approach to cancer prevention, assuming, of course, that DNA is the critical cellular target of these chemicals.

Proposed Course of Project:

With the use of Cs_2SO_4 - Ag^+ gradients and re-annealing techniques, it should be possible to localize genomic sites which are more or less sensitive to alkylating agents and potentially more or less repairable. Such findings should help elucidate carcinogen-genome interactions.

Serial No. NCI-4502

1. Experimental Pathology Branch
   OASDC, DCCP
2. In Vitro Pathogenesis Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The Role of DNA Replication and Repair in Chemical Carcinogenesis

Previous Serial Number: Same

Principal Investigator: Henry Hennings, Ph.D.

Other Investigator: Delores Michael, B.S.

Cooperating Units: None

Man Years:

Total	: 2.4
Professional	: 1.0
Other	: 1.4

Project Description

Objectives:

To examine the repair of DNA after carcinogen treatment; to examine the importance of cell proliferation in skin carcinogenesis initiated by urethane.

Methods Employed:

Cell cultures of newborn mouse skin grown in petri dishes or roller bottles are used for extraction of nucleic acids by a standard phenol procedure. BUdR-labeled DNA is separated from DNA of normal density by centrifugation in caesium chloride gradients in a Beckman L2-65B ultracentrifuge. In some experiments, DNA was denatured at pH 12.3 and the heavy and light strands were separated by centrifugation in an alkaline caesium chloride-caesium sulfate gradient.

In tumor induction experiments in susceptible female mice, skin tumors were initiated by the i.p. injection of 25 mg of urethane and promoted by weekly applications of 0.5% croton oil in acetone.

Major Findings:

DNA repair in primary cultures of mouse skin cells was studied by labeling newly-synthesized DNA with both BUdR and a tritiated DNA precursor, separating

the BUdR-containing DNA from DNA of normal density, and determining the location of the tritium in DNA with respect to the two peaks of absorbance. Tritiated DNA synthesized by cells in S phase should be found in the heavy (BUdR-containing) peak, while tritium associated with the peak of normal (light) density may represent incorporation of the precursor during repair of carcinogen-induced damage to the DNA.

The major product of the reaction of the skin carcinogen β-propiolactone (BPL) with cellular DNA is 7-(2-carboxyethyl)-guanine, which is released from the DNA. The incorporation of 4 tritiated deoxyribonucleosides into DNA was studied after BPL treatment to detect possible specific repair of the BPL-induced lesion. After treatment of skin cells with 5×10^{-4}M BPL for 1 hour, essentially all of the tritium incorporated in the next 18 hours was found in the heavy peak with either thymidine, deoxycytidine or deoxyadenosine as precursor. However, with deoxyguanosine-^{3}H as precursor, tritium was found in the light peak, as well as in the BUdR-containing peak. No tritium was found in the light peak from untreated cells. This evidence for repair (incorporation of the tritiated precursor into non-replicating DNA) was found only with deoxyguanosine as precursor.

BPL-induced incorporation of deoxyguanosine-^{3}H into non-replicating DNA was verified by labeling parental DNA with BUdR, treating with BPL for 1 hour, and incubating with a tritiated precursor for 18 hours. Under these conditions, semi-conservative DNA replication will result in tritium incorporation into light DNA strands, while incorporation into non-replicating DNA will be seen as tritium in heavy strands. When the DNA strands were separated in an alkaline density gradient, tritium was found in the heavy strand in DNA from BPL-treated cells with deoxyguanosine-^{3}H, but not with thymidine-^{3}H, as precursor.

Thus, the repair of the BPL-induced lesion in mouse skin cell DNA appears to be specific for the altered base since repair has been demonstrated with only one precursor, deoxyguanosine.

DNA synthesis at, or soon after, the time of carcinogen treatment has been reported to be important in subsequent tumor development. In initiation-promotion skin tumorigenesis experiments, a single application of 0.5% croton oil (which increases the rate of DNA synthesis several-fold beginning at 9-12 hours, reaching a peak at 18-24 hours and returning to normal by 3-4 days) 24 hours prior to an initiating injection of urethane has been reported to increase the tumor yield and shorten the latent period.

Croton oil treatment times of -24, -6, +1 and +24 hours were tested for their effect on initiation of skin tumor formation by urethane injected at zero time. In the controls treated with urethane alone, the papilloma yield was 1.7 per mouse after 30 weeks of tumor promotion. Tumors developed with a latent period (time until half the mice had developed at least one papilloma) of 21 weeks. Similar results were obtained in the group treated with croton oil at +24 hours (1.8 papillomas per mouse, 21 week latent period). In contrast, when croton oil was given at -24, -6 or +1 hour, tumor yields of 5.3, 5.3 and 4.8 papillomas per mouse were found, with a latent period of 16 weeks in all

3 groups. In the groups treated with croton oil at -6 and +1 hour, the earliest increases in rates of DNA synthesis occurred at about 3 and 10 hours after urethane injection. Thus, if the enhancement of urethane initiation by a croton oil treatment is related to the croton oil-induced stimulation of DNA synthesis in the skin, then DNA replication at times longer than 10 hours after urethane injection (when most of the urethane has been eliminated from the mouse) appears to be important in the process of skin tumor initiation. In mice treated with croton oil 24 hours before urethane injection, the effect on tumorigenesis of inhibition of DNA synthesis by hydroxyurea for about 9 hours at several time periods after the initiator is currently being determined.

Significance to Biomedical Research and the Program of the Institute:

If a carcinogen-induced alteration in DNA is necessary in the process of carcinogenesis, then the repair of the critical alteration should reduce subsequent tumor formation. Replication of the DNA prior to repair may result in a permanent non-repairable change in the base sequence of the newly-synthesized DNA. Conversely, errors in the repair process could also lead to DNA alterations. These experiments are designed to help define the roles of DNA replication and DNA repair in chemical carcinogenesis.

Proposed Course:

DNA repair in mouse skin cells will be examined after ultraviolet light treatment and after treatment with other types of chemical carcinogens. In addition, deoxyguanosine-specific repair after BPL treatment will be investigated in human, hamster and rat cells.

Publications

Hennings, H.: Commentary on article by Laurence, E. B. entitled: Experimental approaches to the epidermal chalone. In Forscher, B. K. and Houck, J. C. (Eds.): Chalones: Concepts and Current Research. National Cancer Institute Monograph No. 38 (In Press).

Houck, J. C. and Hennings, H.: Chalones: Tissue specific inhibitors of cell proliferation. Fed. Eur. Biochem. Soc. Lett. (In Press).

Serial No. NCI-4504
1. Experimental Pathology Branch
OASDC, DCCP
2. In vitro Pathogenesis Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Model Systems for the Study of Chemical Carcinogenesis at the Cellular Level

Previous Serial Number: None

Principal Investigator: Stuart H. Yuspa, M.D.

Other Investigators: Paul E. Dermer, M.D. and
David L. Morgan, B.S.

Cooperating Units:

Man Years:

Total	:	3.0
Professional	:	3.0
Other	:	0.0

Project Description

Objectives:

To develop model systems which can be utilized to study cellular events which occur during early stages of chemical carcinogenesis. Systems studied are directed to give both general information regarding malignant transformation within a mammalian cell and specific requirements of specialized cells which might shed light on events necessary for tumor development in a particular organ.

Methods Employed:

A method has been developed for cell culture of epithelial cells from mouse skin. This includes the technique of trypsin flotation for separation of epidermis from dermis and centrifugation of dissociated cells in ficoll gradients for isolation of hair follicles, dermal fibroblasts and basal cells. Cells grown for short periods of 8-10 days in monolayer culture can be transplanted back to syngeneic mice using a technique developed in this laboratory. Recipient mice are prepared prior to transplantation by dissecting an area on the back down to the panniculus carnosus and inserting a protective silicone dome and wire screen. At the time of transplant, cells taken from culture are placed in the graft bed on filter paper discs and the domes and screens

replaced. After two weeks the filter paper is removed and intact donor skin is present at the graft site. Epidermal cells treated with chemical carcinogens during their brief in vitro lifespan can also be used for skin grafts In addition, epidermal cultures can be chemically treated at an early stage and kept in vitro for long periods to observe for malignant transformation. Evaluation of transformation includes morphological alterations, electron microscopic changes, growth in agar, and tumor formation when injected back into animals. Skin tumors produced by painting animals with chemical carcinogens have been grown in cell culture using similar techniques and these have been studied in order to recognize specific characteristics of malignant epidermal cells in vitro. Viral techniques including XC testing have been used to evaluate these latter cells.

Because epidermal cells in vitro behave as a dynamic differentiating tissue as in vivo, this system lends itself to studying differentiation and its relationship to carcinogenesis. Methods were sought to delay the normal keratinization process and thus prolong the basal cell stage in epidermal cells in vitro. Techniques used are treatment of cells with Vitamin A compounds, autoradiography, electronmicroscopy, DNA turnover studies using tritiated precursors of DNA and standard DNA extraction procedures, and the skin graft system previously described.

In conjunction with an overall Branch interest in prostate carcinogenesis and a desire to study specialized cells in vitro with known hormone dependence but unclear relationships between hormones and carcinogenic chemicals, a cell culture system for prostate epithelium is being developed. Techniques include the use of an intermediate environment for growth of prostate cells prior to cultivation in vitro. Prostate pieces from mice are grown in cleared mammary fat pads under hormonal stimulation. Nodules are then removed and grown in vitro. Techniques used are trypsin dissociation, hormone manipulation, pH control and Vitamin A treatment. Eventual back transplantation to animals will be tried.

Interaction of chemical carcinogens with cells in culture are being studied using cytogenetic techniques. Chromosomes and subchromosomal fractions are isolated by standard techniques. Individual chromosomes or chromosomal groups will be isolated by zonal centrifugation.

Major Findings:

Epidermis can be separated from dermis and grown independently, free of fibroblasts. Epidermis in vitro will differentiate and keratinize just as in vivo. After 10-14 days epidermal cells appear to be non dividing keratinized squamous cells with tonofilaments and desmosomes seen on EM. Likewise, isolated hair follicle cells will form keratin. Epidermal cells grown in vitro 9 days will be accepted as a graft and will develop into normal or slightly hyperplastic skin. Epidermal cells treated in vitro with β propriolactone or dimethylbenzanthracene (DMBA) will be accepted as a graft at a somewhat lower frequency. Tumors have not yet developed. Malignant epidermal cells from a tumor induced by painting a mouse with DMBA will grow in vitro and keratinize. These cells will give epidermal carcinomas when transplanted back to mice.

Murine leukemia virus has been isolated from the malignant epidermal cells but not benign epidermal cells. Skin cells grown from a rhino mouse which is very susceptible to polycyclic hydrocarbon carcinogenesis, repair damage to UV light normally.

Retinyl acetate, a Vitamin A compound, allows epidermal cells in vitro to remain intact and growing for up to 30 days. Keratinization is inhibited. This effect is both dose dependent and vehicle (DMSO) dependent. Once cells begin keratinization they will not respond to retinyl acetate. Cells accumulate PAS positive granules as well as lipid bodies in the presence of retinyl acetate. Preliminary evidence fails to show the presence of mucous.

Dissociated prostate cells derived from glands transplanted previously to cleared mammary fat pads of mice grow well for brief periods (2 weeks) in vitro. Fibroblast contamination is not a problem. In the absence of hormone addition to the medium or other manipulation cells did not survive past 3 weeks.

Significance to Biomedical Research and the Program of the Institute:

It is essential to understand early changes at the cellular level which occur during initiation of malignant change in order to prevent this transformation from occurring or to revert cells from an initiated state back to normal. The use of the epidermal cell system developed in this laboratory has certain advantages in approaching this problem. It utilizes epithelial tissue which we have shown is normal in terms of differentiation and ability to perform properly when placed back in its environment in the whole animal. The development of an in vitro skin carcinogenesis model allows for the continued study at a cellular level of extensive in vivo data which could not be carried further due to limitations of the whole animal. The separation of skin components allows for the first time a model to determine which cell types (i.e hair follicle, basal cell, fibroblasts) are essential for the transformation to occur. With transplantation at an observable animal site performed at an early stage in the in vitro life of the cells, the longterm ambiguities of cell culture can be avoided. The use of retinyl acetate in this culture system allows a systematic approach to the study of differentiation and Vitamin A effects.

In addition there is a growing but ambigous literature on both Vitamin A effects on carcinogenesis and the relationship between differentiation and carcinogenesis. This should be amenable to study using this epidermal cell culture system.

The successful in vitro cultivation of prostatic epithelial cells is an essential step in an overall approach to prostatic carcinogenesis. It also will be a valuable tool in understanding prostatic physiology and in particular hormone dependence of these cells.

There is increasing evidence that chemical carcinogens alter genetic material. Chromosomes and subchromosomal units offer a way of studying intact genetic

information and the effects of chemical agents on those structures should be of great interest.

Proposed Course of Project:

Epidermal cells will be exposed to a variety of skin carcinogens at varying dosages to find the best conditions for malignant transformation. Certain parameters of cell growth such as stage of cell cycle, stage of differentiation, degree of mitotic activity will be correlated with sensitivity to carcinogens. The role of the RNA tumor virus found in malignant but not benign epidermal cells will be studied more fully.

Vitamin A effects on transformation and carcinogen-epidermal cell interaction will be studied. The prolongation of proliferation in vitro of mouse epidermis will be studied from a functional viewpoint utilizing transplantation techniques. Vitamin A analogues will be tested looking for more potent agents to affect differentiation.

Methods for evaluating prostatic epithelial cells such as enzyme assays, morphological and functional integrity will be developed to evaluate such factors as hormones, pH, Vitamins and metals in the in vitro environment. In addition carcinogens will be tested for ability to transform prostate cells.

Publications

Yuspa, S. H., Morgan, D. L., Levy, J. A.: In Vitro cultivation of a chemically-induced epidermal carcinoma: Establishment of three cell lines and isolation of murine leukemia virus. J. Natl. Cancer Inst. (In Press).

Serial No. NCI-4506

1. Experimental Pathology Branch
   OASDC, DCCP
2. Endocrine Carcinogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Further Developmental Models of Prostatic Carcinogenesis

Previous Serial Number: None

Principal Investigator: Winston D. Edwards, Ph.D.

Other Investigator: Lawrence Lanier

Cooperating Units: Bionetics Research Laboratories
Microbiological Associates

Man Years:

Total	:	1.5
Professional	:	1.0
Other	:	0.5

Project Description

Objectives:

These experiments are aimed at providing information about the morphology, mitotic activity, metabolic activity (DNA synthesis), endocrinology, and most important about the carcinogenesis of the prostate. These are designed to aid in the search for an experimental model for prostate carcinogenesis.

Major Findings:

As this is a long term study (2-3 years) a complete report on developments is being delayed. However, morphological and histological differentiation between the prostatic lobes is being established and the initial problems of fixation and staining of prostatic tissue have been overcome. The prostatic lobes and other accessory sex glands in male mice (Balb/c), rats (F_{344}), Chinese hamster (cricetulus), guinea pig, Mystromys albicaudatus, and in both male and female Mastomys natalensis have been morphologically identified and compared. Further knowledge of the basic (fine) structure of the prostate is being gained by examination at high resolution microscopy (Histo-Pathology-cooperating unit) and electron microscopy (Bionetics-cooperating unit) in prenatal, neonatal, pre-pubertal, pubertal, and adult stages. The mitotic activity of epithelial cell populations in the prostatic lobes of rats (F_{344}) and mice (Balb/c) of various ages is being studied and autoradiographs of prostatic tissue labelled with tritiated thymidine to demonstrate DNA

synthesis in epithelial cell populations of the prostatic lobes of F_{344} rats and Balb/c mice of the different age groups are being made.

A suitable site for growing prostate transplants has been found. Pieces of prostate transplanted to the cleared 4th mammary fat pad of 2-3 week old Balb/c mice have been surviving for more than 12 months. Histological examination of these transplants show that the epithelial height is maintained, there is increased secretary activity following treatment with testosterone, and the stroma is increased in both cells and fibers.

Based on the morphological and histological findings in our early work the following investigations have been started:

A. The effects of hormones (estrogen, prolactin, testosterone) etc. on morphology, mitoses, and carcinogenesis of the prostate.

B. The effect of excess mating on prostatic cancer.

C. Direct injection of carcinogen (1-ethyl-1 Nitrosourea) into prostate to induce adenocarcinoma. Work with other carcinogens is also planned.

These studies on excess mating, direct injection of carcinogens into the prostate, and investigation of the effects of hormone stimulation on prostatic carcinogenesis are in progress with cooperation of Microbiological Associates.

Significance to Biomedical Research and the Program of the Institute:

Despite the fact that the prostate is one of the most common sites of cancer in the male, there are at present no experimental models for this kind of cancer. The development of a suitable animal model for studies of prostatic adenocarcinoma and attempts to demonstrate similarities to and differences from the human gland are the immediate goals of this project. This is one of the several projects of the Biological Models Segment of the Experimental Pathology Branch of NCI. The long-term goal of this project is the etiology and pathogenesis of adenocarcinoma of the prostate.

Proposed Course of the Project:

All the experiments attempting to induce prostatic adenocarcinoma outlined in this report are being continued. Prostatic cancer has been called the "Cancer of Old Men" and the age "fifty" as the age after which prostatic cancer is most frequent. Although spontaneous prostate adenocarcinoma has rarely been observed in the rat, a study of older animals would seem appropriate in experiments aimed at comparing the animal/man situations. The following experiments are planned: (a) A study of weight increase of the prostate with age (two age groups have already been studied).

(b) A continuation of preliminary studies on the carcinogenicity of the prostate of old male breeders and non-breeders (rats).

(c) Endocrinological studies will include influences of hormones on prostate differentiation and function, changes in prostate sensitivity to hormones with aging, differential sensitivity of the lobes to various hormones, and measurement of serum hormone levels in normal rats at various ages and the effect of carcinogen administration on these levels.

(d) Based on the success in growing prostate transplants in the cleared fourth mammary fat pad and on preliminary experiments on organ culture of the prostate investigations of prostate transplants growth in organ culture under hormone stimulation and treatment with carcinogens will be undertaken and transplantation of prostate organs cultured under exposure to carcinogens and under various conditions of hormone stimulation to cleared fourth mammary fat pad are foreseen.

Serial No. NCI-4507
1. Experimental Pathology Branch
   OASDC, DCCP
2. Endocrine Carcinogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Endocrine Control of Mammary Growth, Differentiation and Carcinogenesis

Previous Serial Number: None

Principal Investigator: Dr. Douglas H. Janss

Other Investigator: Theresa Ben

Cooperating Units: None

Man Years:

Total	: 2.0
Professional	: 1.0
Other	: 1.0

Project Description

Objectives:

The project is designed to examine the influence of hormones on the process of mammary carcinogenesis by: examination of epidemiological data for indications of altered hormonal milieu which may be factors responsible and/or conducive either for the induction and growth or the prevention and regression of human breast cancer; elucidation of mechanisms involved in the series of hormone dependent cellular events which result in the "normal" progression of stem cell population to a differentiated cell population with specific milk synthesis function; elucidation of mechanisms whereby the exposure of the cell populations to carcinogens under the proper cellular environmental conditions alters the normal progression of cellular events, resulting in neoplastic transformation rather than in normal differentiation and function; correlation of information gained from animal and "in vitro" human tissue experiments such that model systems which adequately mimic the various aspects of human breast cancer can be developed and used to determine the cause(s) and to aid in the development of preventive measures.

Methods Employed:

Techniques for the isolation of mammary epithelium (ductal and alveolar origin) have been developed by the principal investigator. The epithelium freed from

the usual large mass of surrounding adipose and dense connective tissue is utilized for the study of a number of important areas. This cell isolation technique allows for the concentration of epithelial cells such that an adequate microscopic evaluation of the histological changes which accompany growth and differentiation can be made. Techniques for the growth and maintenance of the isolated epithelial cells in culture have been developed. Incorporation of radioactive precursors into nucleic acids is used to evaluate the hormonal stimulation and sensitivity of the alveolar and ductal epithelial cells of the mammary gland. These primary cell cultures are also used to examine malignant transformation induced by treatment of the culture with chemical carcinogens, interaction of chemical carcinogens with cellular constituents, the influence of hormones on these interactions and the effect of these hormone-carcinogen interactions on the normal cellular processes. Following in vivo treatment mammary epithelial cells can be isolated and examined for the interaction of hormones and carcinogens with cellular macromolecules as well as the host's metobolism-carcinogen-hormone interactions on epithelial cell functions. In addition, these cells are examined for their ability to metabolically activate carcinogens, and/or hormones which can then interact with cellular constituents. The potentially reactive hormone and carcinogenic molecules as well as hormone metabolites and non-carcinogenic detoxification products formed in these cells are isolated and identified.

Major Findings:

Although this project has only recently been initiated and has been hindered somewhat by the lack of utilizable laboratory space, significant progress has been made in the following areas:

The viability of rat mammary epithelium following the harsh collagenase separation procedure has been examined. These cells are still metabolically viable for when incubated with glucose-^{14}C, $^{14}CO_2$ can be detected. Furthermore, the addition of a physiologically active concentration of insulin to the system results in a 20 fold increase in the rate of $^{14}CO_2$ production. The incorporation of thymidine-^{3}H and uridine-^{3}H into DNA and RNA respectively has been detected. The addition of insulin, hydrocortisone and prolactin to the incubation media results in a 10 fold increase in the rate of DNA and RNA synthesis. Having demonstrated the viability of the epithelial cell preparation, a microsomal fraction of mammary epithelial cells which could be used for studies of carcinogen metabolism was prepared. 7,12-dimethylbenz(a)anthracene-^{3}H (DMBA-^{3}H) incubated in the presence of this microsomal fraction and NADPH was covalently bound to RNA. When NADPH was omitted from the incubation system, DMBA-^{3}H binding was not observed. Attempts have been made to isolate and identify possible metabolites of DMBA. Of the known liver metabolites of DMBA only the 7-OHM-DMBA derivatives has been isolated.

Techniques for the culture of mammary epithelial cells have been perfected. Epithelial cells are isolated from the fat and connective tissue of the mammary gland, and are freed of fibroblasts by centrifugation through a ficoll density gradient. Epithelial cells so purified can then be grown and maintained in culture. Active proliferation of the epithelial cells in culture depends

upon the addition of insulin, hydrocortisone and prolactin. However, the hormonal requirements (qualitative and quantitative) for growth and differentiation similar to that observed in vivo are still under investigation. Since it has been shown that the induction of mammary tumors can be blocked by stimulating the gland with high levels of certain hormones, it is necessary to ascertain what level of hormones in culture will give growth characteristics comparable to that found in vivo. It has been shown that mammary tumors cannot be induced in the absence of certain endocrine organs. Thus, this system will help us to dissect which hormones are involved and what reactions must result before transformation can occur. Involved in these same studies, is the attempt to transplant cells cultured in various hormone enriched media to adult animals. Preliminary evidence indicates that cells isolated from Sprague-Dawley rats and grown in cell culture can be transplanted to the intrascapular area of another rat of the same strain. Although the transplant growth has been small, histological examination reveals mammary morphology.

In order to examine the hormonal control of cellular differentiation of the mammary epithelium, attempts have been made to separate the two primary epithelial cell types. At present ductal and alveolar cells can be separated to same extent using ficoll density gradient centrifugation. Refinement of these techniques will take place in the coming year. Once separated these cells will be grown in culture and serve as a system to examine the hormonal requirements for differentiation and growth. This system can be utilized to answer the question of possible differentiation of ductal cells into alveolar cells with the capability of synthesis of milk proteins. Carcinogen, hormone, and hormone-carcinogen interaction in the two cell types can be easily examined. This may give some information as to the cell of origin of polycyclic hydrocarbon induced mammary tumors.

Significance to Biomedical Research and the Program of the Institute:

Recently published epidemiology data indicate that the incidence of breast cancer in North American women is greater than seven times that of certain Oriental countries. Although the incidence rate in young Oriental women is similar to that of American females, the risk in American women continues to increase linearly with age while the Oriental incidence plateaus. However, Orientals having migrated to North America (also Hawaii) tend to acquire the incidence of characteristics of their adopted environment. Other epidemiological data suggest that the age of parity can have a significant influence on the risk of developing breast cancer. Thus, pregnancy at an early age alters the susceptibility of the breast to "carcinogenic" stimuli. Taken together it appears that many factors, i.e., Physiological, Endocrinological, Genetic, Environmental, Sociological, Psychological and Geographical may be involved and responsible for breast cancer. Although much is known about the induction and hormonal requirements for growth of animal breast cancer, new and/or further development and refinement of model systems are necessary to correlate human epidemiological data and animal experiments. Human material, of necessity, must be used in an in vitro system to examine the influence of hormones and carcinogens on normal growth and differentiation and carcinogenesis. Thus, animal model systems which can be similarly studied to yield information on the mechanisms of hormonal

action and carcinogen transformation must be examined. The correlation of information gained from these animal systems with the in vitro human cell experiments should add to what is presently known, such that models which adequately mimic the various aspects of human breast cancer can be developed and used to determine the cause(s) and aid in the prevention of breast cancer.

Proposed Course of Project:

The proposed course of this project is in 5 parts. First, the factors involved in the changing sensitivity of rat mammary gland will be investigated. It has been shown the DMBA administration to Sprague-Dawley rats 50-65 days of age results in the formation of breast cancer in every animal treated. However, if the animals are younger (30-35 days old) or older (90-100 days old) the incidence is drastically reduced. What factors are responsible for this change? At present, a good morphological study of mammary growth and differentiation followed from the young to the old animal is not available. Thus, we proposed first to examine alveolar and ductal density at the three ages of different carcinogen susceptibility. This will be followed by a careful high resolution light and electron microscopic evaluation. Furthermore, the mitotic and labeling index of mammary epithelium from these three ages will be examined. Current studies measuring the sensitivity of the mammary epithelium obtained from rats of the three ages described to hormones such as estrogens, progestogens, and prolactin will be continued. The analysis of serum levels of prolactin, estrogen (estradiol and estrone and if possible estriol), and progesterone in the 30-35, 50-60- and 90-100 day old rat will be initiated. Information from these studies should indicate whether there are changes in the levels of hormones affecting the mammary epithelium which occur with age. Coupled with these studies will be an examination of the interaction of DMBA with the mammary epithelium at the different ages. We have previously shown that DMBA binds to the DNA, RNA and protein of these epithelial cells. Binding of DMBA to the DNA is persistant while that to RNA and Protein is not. Thus, we will examine the binding of DMBA to the macromolecules of epithelial cells of the three ages. Studies examining the cofactor metabolic activation and identification of metabolites using epithelial cells from ages susceptible and non-susceptible to cancer induction will be undertaken. These investigations should yield some information as to the factors involved in the changing susceptibility of the mammary epithelial cells to carcinogenic transformation with age.

The influence of the endocrine system on the induction of mammary cancer is well documented. Administration of high levels of hormones or drugs which stimulate the secretion of hormones which normally control mammary growth; inhibit the induction of mammary tumors. However, certain levels of hormones are required for transformation for ovariectomy prior to DMBA feeding drastically reduces tumor incidence while hypophysectomy completely eliminates the tumorigenic response. Thus, the binding of DMBA to cellular constituents following various endocrine system alterations will be examined. Furthermore, the influence of these hormonal changes on the metabolic activation and DMBA metabolite formation will be studied.

Cell culture procedures described will be used to examine the hormonal control of mammary epithelial cell growth and differentiation. With the isolation of ductal and alveolar cells the dissection of hormonal requirements in these populations will be feasible. In addition, the interaction of hormones with cytoplasmic receptor molecules, transfer of these complexes to the nucleus, and binding of the complex to DNA can be examined. The culture systems will also enable us to examine the effect of this hormone-complex DNA interaction on subsequent DNA function. Furthermore, the cellular processes of the mammary cell leading to cell division or differentiation and specialized milk synthesis can be more adequately studied.

The in vitro transformation of rat mammary cells will be examined. Since it is known that there is a requirement of a specific level of serum hormones for transformation in vivo, the level and type of hormones necessary for transformation will be followed. Furthermore, this system should lend itself to the careful analysis of hormone-carcinogen-interactions with cellular macromolecules and the role of these interactions on the process of transformation.

The logistics have been established for the procurement of human breast tissue. This tissue will be subjected to collagenase separation, ductal and alveolar cell isolation, and fibroblast removal techniques in preparation for cell culture. Once the cell culture of human epithelium has been perfected, studies as indicated for the rat above will be initiated. This will serve then as the point of correlation of animal and human breast growth, differentiation and carcinogenesis data.

Honors and Awards

Invited Participant and Speaker, Symposium on the Normal and Abnormal Growth of the Prostate, Southwest Foundation for Education and Research, San Antonio, Texas, March 1-3, 1973.

Serial No. NCI-4501
1. Experimental Pathology Branch, OASDC, DCCP
2. Perinatal Carcinogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Experimental Models of Wilms' Tumor

Previous Serial Number: NCI-4687

Principal Investigator: Sewa Ram Joshi, B.V.Sc., Ph.D.

Other Investigators: Jerry M. Rice, Ph.D.

Cooperating Units: Microbiological Associates, Inc., Bethesda, Md.

Man Years:
Total: 1.5
Professional: 1.1
Other: 0.4

Project Description

Objectives:

To develop an experimental animal model for Wilms' tumor of the kidney, which will duplicate as closely as possible the morphological and biological characteristics of the human neoplasm. The model, once established, will be used for studies of the physiological factors which modify induction of this neoplasm.

Methods Employed:

Organic compounds, both carcinogenic and non-carcinogenic, are synthesized or purified from commercial sources using standard procedures. Rats of various strains are exposed to these compounds at various times during pre- and postnatal life, and the carcinogenic response in various organs, especially the kidney, is evaluated histologically. Surgical techniques are used for transplantation of tissues and removal of endocrine organs to modify the hormonal balance. Organ culture techniques are being undertaken to determine the ability of apparently mesenchymal rat kidney tumors to respond to embryological inducers of renal differentiation.

Major Findings:

Initial studies in this area consisted of an effort to duplicate the results

reported by another laboratory on the induction of nephroblastomas in the Sprague-Dawley rat by 7,12-dimethylbenz[a]anthracene (DMBA) which was said to be effective only in ovariectomized animals. This indicated that sex hormones might have a regulatory role in the development of such tumors, and consequently transplacental studies were also initiated using DMBA and lead acetate, a known kidney carcinogen, in Sprague-Dawley rats which were then gonadectomized at birth. It has been found that the initial results reported by another laboratory cannot be duplicated, and attention is now shifting to the use of cycasin and nitroso compounds which have produced tumors identified as nephroblastomas in other laboratories. These tumors, however, have generally contained few or no identifiable renal epithelial elements, and their identification as nephroblastomas remains a subject for further investigation.

Major attention is now being focused on the production of viable offspring in various inbred strains of rats which have urogenital malformations as a consequence of maternal vitamin A deficiency during pregnancy. This is accomplished by raising female rats from birth on a vitamin A deficient diet in order to deplete body reserves of the vitamin and then maintaining the animals during pregnancy on just enough retinoic acid or retinoic acid supplemented with retinyl acetate to maintain pregnancy. Rats born to vitamin A deficient mothers will be exposed both in utero and neonatally to cycasin and to nitroso compounds in order to determine whether we can duplicate in the experimental rat system, the observed association in man of urogenital malformations, aniridia, and other congenital defects (known to be inducible in the rat by vitamin A deficiency) with an excess occurrence of Wilms' tumor.

Significance to Biomedical Research and the Program of the Institute:

Research for animal models of human childhood neoplasms should provide an insight into the types of causative agents and modes of exposures responsible for childhood cancer. It is to be expected that natural selection would tend to eliminate genotypes in the human population which predispose individuals to the development of fatal neoplasms before attaining reproductive age, yet the incidence of tumors in childhood is high. Epidemiological studies have pointed to the occurrence of childhood neoplasms in association with certain types of congenital malformation which are non-inherited, and suggest that environmental agents, alone or in combination, may play a role in the induction of such neoplasms. An animal model would allow the study in the laboratory of indications from epidemiological studies on man concerning factors important in the genesis of these tumors.

Proposed Course of Project:

The experiments outlined above should be continued to determine whether there is a synergism between maternal vitamin A deficiency and susceptibility to induction of renal tumors in the rat. The tumors observed in that species will be studied in organ culture to determine whether or not the morphologically mesenchymal tumors can be induced to form characteristic renal tubules and glomeruli, thus confirming the identification of such neoplasms as

nephroblastomas. Once the model is established, it should be used to determine the effects of manipulations of physiological parameters, including hormonal balance and immunological responsiveness, on the genesis and behavior of these tumors. Studies should also be made to determine what synergisms may exist between carcinogens and other non-oncogenic teratogens to determine the range of synergisms which may be demonstrable.

Honors and Awards

Instructor, Department of Civil Engineering, Howard University. Course titles: "Diseases Communicable to Man" and "Environmental Toxicology".

Serial No. NCI-4503
1. Experimental Pathology Branch, OASDC, DCCP
2. Perinatal Carcinogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Immunological Factors Responsible for the Differential Sensitivity Between Fetal and Adult Mice, and Between Different Inbred Strains, to the Induction of Pulmonary Tumors

Previous Serial Number: NCI-4687

Principal Investigator: W. Graeme Cotton, D.V.S.M., Ph.D.

Other Investigators: Jerry M. Rice, Ph.D.; Elaine Esber, Ph.D.; W. John Martin, Ph.D.

Cooperating Units: Laboratory of Immunology, NCI

Man Years:

Total:	3.5
Professional:	2.5
Other:	1.0

Project Description

Objectives:

To determine the manner and the extent to which immunological factors are responsible for the genetically determined differences in sensitivity to the induction and growth of pulmonary tumors among different inbred strains of mice, and between the fetus and adult within each strain.

Methods Employed:

Tumors are induced transplacentally by treatment of timed-pregnant mice with 1-ethyl-1-nitrosourea (ENU), which is synthesized in this laboratory. Tumors are transplanted to secondary hosts using trocar implants or counted cell suspensions, and to serial cell culture using standard media and procedures. Microcytotoxicity tests are carried out in the Laboratory of Immunology using lymph node and spleen cells from tumor bearing animals. In vivo studies of immunological cross-reaction are carried out by pre-immunizing mice with normal or tumor tissue, subsequently suppressing the primary (but not the secondary) immune response by 400 R whole-body X-irradiation, and challenging with the tumor to be tested.

Major Findings:

Some, but not all, pulmonary tumors induced transplacentally in strains of mice genetically resistant to these tumors grow poorly or not at all on transplantation to syngeneic recipients, but readily in F_1 hybrids between the strain of origin and another strain genetically more susceptible to the induction of the same type of tumor. Two such tumors from strain C3Hf, which is moderately resistant to pulmonary tumor induction, behave in this fashion and have been shown to be cross-reactive with each other and with normal lung tissue from genetically sensitive strain A, but not with a histologically unrelated tumor (sarcoma) or with lung tissue from genetically resistant strain DBA/2. Neither tumor is transplantable to another inbred strain in the absence of prior immune suppression, with the single exception that both grow extremely well in strain C3H, from which strain C3Hf was derived prior to 1950. Strains C3H and C3Hf reject reciprocal skin grafts and have been shown to differ from each other at non-H-2 histocompatibility loci. Normal lung tissue from strain C3H can immunize C3Hf animals against C3Hf lung tumor 85. This tumor grows uniformly well in strain C3H, the F_1 hybrid between the two strains, and in 80 percent of the F_2 generation, indicating that a single gene is responsible for the unexpected preferential growth of this tumor in an allogenic host. We conclude from studies such as these that genetically controlled sensitivity to pulmonary tumors in mice depends in part on whether the host animal can distinguish antigens characteristic of pulmonary tumor cells from its own normal lung tissue.

The question arises as to whether animals that are tolerant to and accept transplants of the transplanted C3Hf lung tumors, can be made responsive to these tumors by non-specific stimulators of the immune response, including chronic infection with Toxoplasma gondii, treatment of tumor cell inocula with neuraminidase, or by intralesional injection of Mycobacterium bovis (strain BCG). Experiments to test this point are in preparation. In the event that immune stimulation elicits a response to these tumors in susceptible mice, efforts will be made to determine whether autoimmune damage will occur in normal lung tissue which is cross-reactive with the tumor, though at a remote site.

The Hellstrom microcytotoxicity test has been used in the Laboratory of Immunology to show that both tumor-bearing and non-tumor-bearing, immune animals have lymphocytes active specifically against the tumor cells. However, at least some tumor-bearing susceptible tumor graft recipients have detectable levels of blocking factors in their serum, and efforts are under way to utilize the blocking factors to study the distribution of tumor-reactive antigens in different tissues of mice of susceptible strains as a function of age, both before and after birth.

Significance to Biomedical Research and the Program of the Institute:

This experimental system represents the first experimental demonstration of a role of immune factors in genetically determined sensitivity and resistance to a particular type of tumor. The existence of cross-reactive antigens on

tumors and normal lung tissue of susceptible individuals invites the question of whether a similar mechanism may explain tolerance (susceptibility) to other types of tumors in animals or in human beings. This in turn is of significance to any efforts to develop immunotherapeutic measures against neoplastic disease, since the problem of avoiding autoimmunization must be faced. This experimental model provides the means for exploring the significance of that problem, as well as of determining whether the non-specific stimulation of immune responsiveness will suffice to initiate tumor rejection in individuals who are intrinsically tolerant to tumor antigens.

Proposed Course of Project:

These studies should be extended to tumors obtained from other strains of mice in order to insure the generality of the conclusions drawn, and extended to other tumors of other organ systems. These should be chosen to reflect a pattern of susceptibility and resistance among mouse strains which differs from the lung tumor pattern. The studies of cross-reactivity should be extended to determine the tissue localization and time of appearance of cross-reactive antigens, and the efforts to immunize susceptible animals with these transplantable tumors using the adjuvants indicated above should be pursued.

Publications

Rice, J.M.: The biological behaviour of transplacentally induced tumours in mice. IARC Scientific Publication No. 3 (Lyon). (in press).

Serial No. NCI-4687
1. Experimental Pathology Branch, OASDC, DCCP
2. Perinatal Carcinogenesis Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Experimental Models of Human Childhood Neoplasms

Previous Serial Number: Same

Principal Investigator: Jerry M. Rice, Ph.D.

Other Investigators: William T. London, D.V.M.; Russell W. Madison, D.V.M.; Donald M. Jerina, Ph.D.; William O. Iverson

Cooperating Units: NINDS; Microbiological Associates, Inc.; and NIAMDD

Man Years:

Total:	2.0
Professional:	1.0
Other:	1.0

Project Description

Objectives:

To develop experimental animal models for human cancers of childhood, and to use these models to study the greater sensitivity of fetal and neonatal animal tissues to chemical carcinogens, and the role of factors other than the carcinogens themselves in enhancing or suppressing chemical carcinogenesis in experimental animals during intrauterine life and infancy. Carcinogenic organic compounds are synthesized where necessary using standard procedures; organic polymers are obtained commercially and characterized spectroscopically, and purified where necessary. Animals of various species and strains are exposed to these compounds at different times during pre- and postnatal life, and the carcinogenic response in various organs is evaluated histologically. Short and long-term cell and organ culture techniques are used where necessary to explore the role of interferon and other host defense mechanisms in resistance to carcinogenesis, and to study cocarcinogenic effects of microbial and viral infections in chemical carcinogenesis. Surgical procedures, including skin grafting, adult thymectomy, diffusion chamber implantation, etc., are used in evaluating antitumor defense mechanisms of the intact animal, and organic and biological chemical techniques are employed to determine the effects of modifications of chemical carcinogen structure and of metabolic factors on the response of host tissues to carcinogens.

Major Findings:

The paucity of reports on the induction of enzymes capable of metabolizing weak transplacental carcinogens such as nitrosamines and aromatic hydrocarbons in fetal tissues has prompted a study of chemicals that are known to affect the activity of various enzymes involved in aromatic hydrocarbon metabolism, and of substances capable of inducing the synthesis of these enzymes. It has been possible to prevent the acute hepatotoxicity of organic compounds which act through an epoxide intermediate (e.g. chlorobenzene), by treating adult animals with enzyme inhibitors which contain relatively unreactive organic epoxide rings. It may be feasible to extend these studies to carcinogenicity protocols in order to evaluate the role of various enzymes in the initial stages of carcinogenesis during pre- and postnatal life.

The polycation poly-D-lysine was previously shown to increase the interferon-stimulating abilities of the synthetic double-stranded RNA, poly I:C. Although this has proved to be effective in increasing the antiviral effects of poly I:C in vivo against both RNA (Semliki Forest) and DNA (Herpes) viruses in mice, poly-D-lysine has been shown to be nearly as effective as DEAE-dextran in inducing sarcomas at the site of repeated subcutaneous injections of the polycation into mice. Since polycations are extremely effective in the laboratory in increasing the apparent titers of many virus preparations, notably the murine leukemia-sarcoma complex, experiments on polycation carcinogenesis have been extended to investigate the possibility that these compounds work by setting up foci of infection by endogenous tumor viruses. Cell cultures derived directly from sarcomas induced by DEAE-dextran yield typical C-type RNA murine virus (XC test, EM, bouyant density) which after density gradient purification has produced myeloid and lymphocytic leukemias, but not soft-tissue sarcomas, on reinjection into mice. Efforts continue to isolate a sarcomagenic virus from these cell cultures. In vivo carcinogenesis experiments have demonstrated a synergism between the carcinogen 1-ethyl-1-nitrosourea (ENU) and the virus inducing agents 5-iodo- and 5-bromodeoxyuridine (IUDR; BUDR). No synergism has yet been detected, however, between these inducing agents and the cationic polymers, alone or in combination with ENU. The polycations, moreover, have been shown to be extremely effective in inducing subcutaneous sarcomas in mice following only two subcutaneous injections spaced two weeks apart, at DEAE-dextran concentrations identical with those proposed for inclusion in veterinary vaccines. However, the multi-injection regimen originally used to produce a high incidence of sarcomas in mice with these polymers has been completely ineffective after 15 months in the rat and in the hamster. This finding is remarkable in view of the known, pronounced susceptibility of the rat to sarcomagenesis by both solid plastic films and by the water soluble iron dextran preparations. The causes for this pronounced species difference are under investigation.

The viruses described above, isolated from NIH General Purpose (GP) Swiss mice, induce leukemias which do not regress in this random-bred line, in contrast to the lymphocytic and stem-cell leukemias inducible by ENU in animals of this line. The latter often undergo complete regression accompanied by the development of aplastic anemia. Studies with lower doses of ENU, which elicit a much lower total incidence of lymphomas in these mice, indicate that a smaller

proportion of those lymphomas which do develop subsequently undergo regression, which suggests the possibility that the regression-aplastic anemia phenomenon may be due in part to a long-term toxic effect of ENU on the hematopoietic and lymphoid stem cells. This does not explain, however, why efforts to repopulate the tissues of animals in regression have not succeeded, and the earlier hypothesis of an immune/autoimmune phenomenon remains tenable.

Significance to Biomedical Research and the Program of the Institute:

Easiest to assess is the identification of a new class of chemical carcinogen, the water-soluble organic polycations, which have proposed uses in medicine such as the inclusion in veterinary vaccines which are contraindicated by their carcinogenic activity. Furthermore, these are non-reactive chemical carcinogens, differing markedly in this respect from the usual low molecular weight organic compounds, and elucidation of their mechanism of action, particularly resolution of the question of the possible role of oncogenic viruses, may provide a useful tool in the study of combined effects in chemical carcinogenesis. The lymphoma regression/aplastic anemia syndrome, whose cause remains elusive, may provide new insights into mechanisms of host defense against neoplastic disease.

Proposed Course of Project:

The lymphoma regression experiments should concentrate on efforts to keep alive animals which have undergone the regression phenomenon in order to evaluate their immune response relative to both their tumors and normal tissue components. This can best be done by blood and bone marrow grafting procedures, preferably in germ-free or SPF animals. The search for sarcomagenic agents in cell cultures from polycation-induced mouse sarcomas should be pursued, with an effort to identify factors responsible for the pronounced species specificity of these substances among rodent genera.

Honors and Awards

Lecturer and co-organizer, Conference on Immunology of Carcinogenesis, Gatlinburg, Tennessee, May 8-11, 1972. Presented a paper entitled "Spontaneous regression of autochthonous malignant lymphomas induced in Swiss and NZW mice by 1-ethyl-1-nitrosourea."

Lecturer, annual meeting of the Teratology Society, Burlington, Wisconsin, May 14-17, 1972. Presented a paper entitled "An overview of transplacental carcinogenesis."

Member, NIH Fogarty International Center Committee on the Prevention of Fetal and Perinatal Disease, September 1972 - May 1973.

Participant and member of Panel on Reproduction, National Academy of Sciences/National Research Council Working Conference on the Principles of Protocols for Evaluating Chemicals in the Environment, San Antonio, Texas, February 11-16, 1973.

Lecturer, Workshop on Late Effects of Cancer Chemotherapeutic Agents, Boston, Massachusetts, October 19-20, 1972. Presented a paper entitled, "Carcinogenic effects of cancer chemotherapeutic agents in experimental animals".

Publications

Catalano, L.W., London, W.T., Rice, J.M., and Sever, J.L.: Prophylactic and therapeutic use of poly (I)·poly (C) [poly-D-lysine] against herpesvirus encephalitis in mice. Proc. Soc. Exp. Biol. Med. 140: 66-71, 1972.

Ulland, B.M., Weisburger, J.H., Weisburger, E.K., Rice, J.M., and Cypher, R.: Thyroid cancer in rats from ethylene thiourea intake. J. Natl. Cancer Inst. 49: 583-584, 1972.

Rice, J.M., Davidson, J.K., Madison, R.M., Kingsbury, E.W., and Turner, W.: Oncogenic water-soluble polycations. I. Induction of sarcomas in mice by diethylaminoethyl (DEAE)-dextran. J. Natl. Cancer Inst. 50: 387-401, 1973.

Oesch, F., Jerina, D.M., Daly, J.W., and Rice, J.M.: The role of epoxide hydrase in the metabolism, toxicity, and carcinogenicity of xenobiotic substances: induction, activation, and inhibition of hepatic epoxide hydrase. Chem. Biol. Interactions. (in press).

Rice, J.M.: Spontaneous regression of autochthonous malignant lymphomas induced in Swiss and NZW mice by 1-ethyl-1-nitrosourea. NCI Monograph No. 35. (in press).

Serial No. NCI-4500
1. Experimental Pathology Branch, OASDC, DCCP
2. Carcinogen Screening Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Mode of Action of Chemical Carcinogens -- The Mechanisms of the Appearance of α-Fetoproteins (AFP) in Serum of Rats Given Chemical Carcinogens

Previous Serial Number: Same

Principal Investigator: James M. Sontag, Ph.D. and Robert Kroes, D.V.M.

Other Investigators: John H. Weisburger, Ph.D.

Cooperating Units: None

Man Years:

Total	:	0.5
Professional	:	0.2
Other	:	0.3

Project Description

Objectives:

A fetal globulin, α-fetoprotein (AFP) has been demonstrated to be present in the serum of some patients with hepatocellular carcinoma and a few other lesions such as teratomas. Depending on geographic location, the percentage of AFP-positive patients varies from 30% in some areas like the United States or Great Britain, to 80% in Africa. The presence of AFP is being developed as a diagnostic tool for hepatocellular carcinomas. A number of questions on the mechanism of formation, of the derepression of this fetal protein are of interest in relation to the etiology of hepatocellular carcinoma in man. Do all hepatocarcinogens lead to tumors elaborating AFP? Does age play a role? Does the morphology and differentiation of the tumor affect production? Some of these basic questions were investigated in rats.

Methods Employed:

Utilizing amniotic fluid of inbred female Fischer strain rats at 16 to 20 days of gestation as antigen, antiserum was raised in rabbits. The antiserum was purified by specific techniques to secure an AFP-specific antiserum. Double diffusion (Ouchterlony) techniques were utilized to assess the

presence of AFP in serum of treated and control rats. In some cases confirmatory experiments were performed by electrophoresis and immuno-electrophoresis on cellulose acetate strips or on agarose coated slides.

In addition, radioimmune assays of selected serum samples have been performed by Dr. Sell, University of California, San Diego.

Major Findings:

Presence of AFP in serum of rats fed various hepatocarcinogens or hepatotoxins. A variety of hepatocarcinogens were administered to male Fischer 344 rats. At the dose levels given most of the carcinogens induced an early transitory rise in the titers of serum AFP, as determined by the Ouchterlony technique. The early elevation was found to be related to the dose level administered and was not dependent upon the carcinogen used.

Using the Ouchterlony technique, it was found that hepatocellular carcinomas induced by diethylnitrosamine produced high levels of α-fetoprotein, those induced by *N*-2-fluorenylacetamide and *N*-hydroxy-*N*-2-fluorenylacetamide produced moderate levels, while those induced by aflatoxin B_1 had low or undetectable levels. When the latter were reanalyzed by the radioimmuno-assay method for rat AFP, all of the serum samples were found to be elevated.

Significance to Biomedical Research and the Program of the Institute:

The current test series were inspired by the differences in the presence of a diagnostic antigen, α-fetoprotein (AFP) in the serum of human patients with liver cancer. The etiology of liver cancer in different parts of the world is unknown but presumably rests on a variety of inciting factors and chemical carcinogens. Model studies to pinpoint specific carcinogenic agents which would give rise to AFP and others which would not might give clues to etiologic factors operating in man in various parts of the world. At the same time an understanding of the factors controlling the release of a fetal antigen from normal or cancer cells might further our understanding of the mechanisms of carcinogenesis and thus eventually yield information which can be used to control and indeed prevent cancer development. Studies on the effect of immunocompetence in the carcinogenic process likewise bear on these same problems and their resolution would certainly augment means of controlling the progress of the disease.

Proposed Course of Project:

Studies are planned to determine the time relationship between the appearance of elevated levels of serum AFP and hepatocarcinogens. A wide variety of compounds will be investigated. Hopefully these studies will shed some light on the etiology of liver cancer as well as the use of AFP as a diagnostic tool for this disease.

Publications

Kroes, R., Sontag, J. M., Weisburger, J. H., Newberne, P. M., and Wogan, G. N.: Alpha-fetoprotein in rats bearing hepatomas induced by aflatoxin B_1. Nature 240: 240-241, 1972.

Kroes, R., Williams, G. M., and Weisburger, J. H.: Early appearance of serum α-fetoprotein during hepatocarcinogenesis as a function of age of rats and extent of treatment with 3'-methyl-4-dimethylaminoazobenzene. Cancer Res. 32: 1526-1532, 1972.

Kroes, R., Williams, G. M., and Weisburger, J. H.: Early appearance of serum α-fetoprotein as a function of dosage of various hepatocarcinogens. Cancer Res. 33: 613-617, 1973.

Kroes, R., Williams, G. M., and Weisburger, J. H.: On the precocious induction of α-fetoprotein by several hepatocarcinogens, including aflatoxin B_1, in rats. Proceedings of Primosten, Yugoslavia meeting (in press).

Mitchell, F., Yamamoto, R. S., and Weisburger, J. H.: Griseofulvin: Immunosuppressive action. Proc. Soc. Exptl. Biol. Med. 143: 165-167, 1973.

Serial No. NCI-4617
1. Experimental Pathology Branch, OASDC, DCCP
2. Carcinogen Screening Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Carcinogen Screening Operations

Previous Serial Number: Same

Principal Investigator: Elizabeth K. Weisburger, Ph.D.

Other Investigators: Lionel A. Poirier, Ph.D., James M. Sontag, Ph.D., John H. Weisburger, Ph.D., and Jerrold M. Ward, D.V.M., Ph.D.

Cooperating Units: None

Man Years:

Total	2.0
Professional	1.6
Other	0.4

Project Description

Objectives:

Management responsibilities are assumed on the design, performance and evaluation of studies in contract laboratories on the chronic effect and of the carcinogenicity of chemicals or of mixtures of chemicals. The aim of these investigations is to gather information on hazards involved in handling certain chemicals or drugs, and also on the mechanisms underlying joint effects in mixtures. Of particular interest are such mixtures which tend to give increased or decreased overall carcinogenic effect. Of concern also is the methodology of carcinogen screening, with emphasis on improvement in sensitivity, speed and economy. Short-term assays such as those based on mutagenesis are under development, taking into account the need to secure biochemical activation of most environmental agents.

Methods Employed:

Attempts are made to secure information on chemicals representing the greatest hazard to the largest number of people. Chemicals and drugs are rated as to priority on this basis as well as other criteria such as relationship to known carcinogens, epidemiologic observations, indications of specific toxicity and related factors. Standard protocols as well as

newly designed protocols attempting to increase the sensitivity and speed of such tests are utilized. For example, recent developments on *in vitro* transformations by chemical carcinogens, of select cell systems, of the newer relationships between mutagenesis and carcinogenesis, and similar advances in the fundamental underlying sciences suggest that a battery of short-term preliminary screens can be developed based on these basic areas. Exploratory meetings with relevant national and international organizations in industry, in government, and in university environments are setting the stage for a concerted developmental effort in this area.

Major Findings:

In the current year several of the long-term experiments in contract laboratories are nearing the data generation stage, even though in some cases statistical evaluation is yet to be done. A number of the chemicals, including drugs, industrial chemicals, pesticides, and environmental agents have been found to lead to cancer in test systems involving mice or rats.

1. Industrial chemicals. Indications from more definitive data are that the industrial compounds, 2,4,6-trimethylaniline, 2,4,5-trimethylaniline, 2,4-diaminotoluene, *o*-toluidine, 4-chloro-*o*-toluidine, and 4,4'-methylene-bis-2-chloroaniline are carcinogenic either in mice or rats or both.

2. Pesticides. During the past year we have indications that several simple halogenated agricultural fumigants and several pesticides may represent a carcinogenic hazard. However, most pesticides tested do not seem to have this effect. In contrast to previous reports Avadex and bis-2-hydroxyethyl-dithiocarbamic acid were not carcinogenic in more recent tests in rats.

3. Artificial sweeteners. Studies in two contract laboratories with cyclamate and saccharin indicate that under the conditions employed neither of these compounds is carcinogenic.

4. Drugs. Further data from a study of drugs used in cancer chemotherapy are now available. Indications are that an increase of over 50% from the spontaneous tumor incidence was noted with 6-mercaptopurine riboside, dichloromethotrexate, a mixture of Vincristine, methotrexate, 6-mercapto-purine and prednisone, a mixture of Melphalan and testosterone propionate, and a mixture of Imuran with prednisone. Most of the tumors were in the hematopoietic system especially in mice. In addition new drugs proposed for use as cancer chemotherapeutic agents are being studied. Some of these compounds have reached the stage of early clinical trials. It is hoped that our testing series would give information on any possible hazards from these drugs before very extensive clinical use is undertaken.

5. Food additives. Data from a short-term assay in Strain A mice of numerous food additives from the GRAS list became available. Only one compound, cinnamyl anthranilate, caused an increase in pulmonary adenomas. This substance is now under test in Fischer rats and C57Bl/C3H hybrid mice.

6. Natural products. Studies on approximately 30 fungi isolated from Asian foodstuffs are underway. Likewise, the possible carcinogenicity of fractions from plant materials used in Curacao, a region of high esophageal cancer incidence, is being checked.

Significance to Biomedical Research and the Program of the Institute:

Many factors in the environment have not yet been evaluated for chronic toxicity and possible carcinogenicity. It is important to secure such information after deliberate and judicious setting of priorities. Cancer in man has been observed in the past as a result of exposure to chemicals subsequently demonstrated to be carcinogenic. The aim of this program is to forestall such unintentional exposures of man which would result in neoplastic disease in the future.

Proposed Course of Project:

Continuing efforts are required 1) to assess relative priorities of materials to be tested, 2) to undertake studies on variations in the protocols to simplify or to develop novel test techniques which are more sensitive, more specific and more economic, and 3) to apply such tests to identify carcinogenic hazards in the environment.

Honors and Awards

Elizabeth K. Weisburger:

Member, Carcinogenesis Panel at National Academy of Sciences Working Conference on Principles of Protocols for Evaluating Chemicals in the Environment, San Antonio, Texas, February 11-16, 1973.

Member, Surgical and Dental Drugs Advisory Committee, Food and Drug Administration, Rockville, Maryland.

Acting Director, Bioassay Segment

Elected to Society of Toxicology

Invited Discussant, U. S.-Japan Cooperative Medical Science Program, Panel on Methods for Evaluating Environmental Mutagenesis and Carcinogenesis, Tokyo, Japan, August 25-27, 1972.

Invited Lecture: Inhibitory Effects in Chemical Carcinogenesis. National Cancer Center Research Institute, Tokyo, Japan, August 28, 1972.

Invited Lecture: Inhibitory Effects in Chemical Carcinogenesis. University of Hawaii, Honolulu, August 28, 1972.

Invited Lecture: Chemical Carcinogenesis. Symposium on Chronic Effects of Fluorides, National Institute of Dental Research, October 17, 1972.

Invited Lecture: Inhibitory Effects in Chemical Carcinogenesis. Department of Anatomy, University of Maryland, Baltimore, December 6, 1972.

Invited Lecture: Inhibitory Effects in Chemical Carcinogenesis. Michigan Cancer Foundation, Detroit, March 7, 1973.

John H. Weisburger:

Member, Editorial Committee, *Cancer Research*, American Association for Cancer Research

Member, Editorial Board, *Food and Cosmetics Toxicology*

Member, Editorial Board, *Chemico-Biological Interactions*

Member, Editorial Board, *Xenobiotica*

Invited participant: Conference on Host-Environment Interactions in the Etiology of Cancer in Man -- Implementation in Research. Primosten, Yugoslavia, August 27-September 2, 1972.

Invited lecture: Carcinogenesis and its Importance in Clinical Cancer. Seventh National Cancer Conference, American Cancer Society, Los Angeles, California, September 27-29, 1972.

Invited lecture: Chemical Carcinogens and their Mode of Action in Colonic Neoplasia. 58th Annual Clinical Congress, American College of Surgeons, San Francisco, California, October 2-6, 1972.

Publications

Ulland, B. M., Weisburger, J. H., Weisburger, E. K., Rice, J. M., and Cypher, R.: Thyroid cancer in rats from ethylene thiourea intake. J. Nat. Cancer Inst. 49: 583-584, 1972.

Weisburger, J. H.: Chemical Carcinogenesis. In "Cancer Medicine," J. F. Holland and E. Frei, eds., Lea and Febiger, Philadelphia (in press).

Weisburger, J. H. and Williams, G. M.: Cancer tests. The relation of cancer induction and genetic damage. Proc. Symposium on Evaluation of Genetic Risks of Environmental Pollutants, Stockholm, Sweden (in press).

Serial No. NCI-4618
1. Experimental Pathology Branch, OASDC, DCCP
2. Carcinogen Screening Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Mode of Action of Chemical Carcinogens -- Endogenous and Exogenous Factors in Chemical Carcinogenesis

Previous Serial Number: Same

Principal Investigator: Elizabeth K. Weisburger, Ph.D.

Other Investigators: John H. Weisburger, Ph.D., Richard S. Yamamoto, Sc.D., James M. Sontag, Ph.D., Jerrold M. Ward, D.V.M., Ph.D., and Lionel A. Poirier, Ph.D.

Cooperating Units: Dr. Russell M. Madison, Microbiological Associates
Dr. Borge M. Ulland, Litton-Bionetics, Inc.
Dr. J. D. Prejean, Southern Research Institute
Dr. Daniel P. Griswold, Southern Research Institute
Dr. Marcus M. Mason, Mason Research Institute
Dr. Hans Ruelius, Wyeth Laboratories, Inc.

Man Years:

Total	3.6
Professional	0.9
Other	2.7

Project Description

Objectives:

Investigations to discover the intimate factors involved in the initiation and development of neoplasia are explored by means of animal experiments in various species. The studies utilized a number of chemical carcinogens administered to animals under varying conditions of diet, endocrine situation or immunological status so as to gain insight into controlling mechanisms in the initiation and development of chemically induced tumors. Further efforts deal in the development of systems which lead to the prevention of the processes of chemical carcinogenesis in a study of the mechanisms related thereto.

Methods Employed:

Chemical carcinogens or unknown agents to be evaluated for carcinogenicity are administered by a number of routes to several strains of rats or mice on specific dietary regimens. Additionally, certain chemicals with specifically tailored structures or properties are given together with chemical carcinogens to determine whether tumor induction can be delayed or inhibited. Host factors such as the endocrine or immunologic status are being investigated in relation to the effectiveness of tumor formation. After judiciously selected treatment and observation periods, autopsies of the experimental animals are performed. Upon histologic processing of tissues, the results in experimental systems are carefully evaluated in relation to control groups.

Major Findings:

1. Mode of action of carcinogenic aromatic amines. Our group has reported earlier that acetanilide or *p*-hydroxyacetanilide could inhibit the induction of liver cancer with *N*-2-fluorenylacetamide or in some cases with *N*-hydroxy-*N*-2-fluorenylacetamide. In order to determine whether this effect held with other carcinogenic aromatic amines besides the fluorene derivatives, acetanilide or *p*-hydroxyacetanilide were fed to female Sprague-Dawley rats in conjunction with benzidine, 2-anthramine or DMBA. The objective was to inhibit the induction of mammary cancer in these rats which were very susceptible to such influences. In neither case did acetanilide or *p*-hydroxyacetanilide decrease either the mean number of tumors per tumor-bearing animal or the total number of animals with tumors. Whether acetanilide or *p*-hydroxyacetanilide could inhibit other tumors such as those of the ear duct induced by *N,N'*-dimethyl-4-aminostilbene was also investigated. In this case there did seem to be some inhibitory effect on ear duct tumor induction by the acetanilide derivatives. A series of other known potent carcinogens was also tested with acetanilide or *p*-hydroxyacetanilide. With 3'-methyl-4-dimethylaminoazobenzene, both acetanilide and *p*-hydroxyacetanilide had an inhibitory effect on liver cancer induction in male Sprague-Dawley rats on a 12% protein-low riboflavin diet. Likewise, the action of the very potent liver carcinogen 6-(*p*-dimethylaminophenylazo)quinoline was inhibited by either acetanilide or *p*-hydroxyacetanilide. However, with 4'-fluoro-4-aminobiphenyl and 3,2'-dimethyl-4-aminobiphenyl, acetanilide and *p*-hydroxyacetanilide appeared to have no inhibitory action. Other inhibitors previously employed in such cases, such as chloramphenicol, likewise were ineffective.

2. Effects of phenobarbital and other modifiers of diethylnitrosamine carcinogenesis in male Fischer 344 rats. Rats receiving 40 ppm of diethylnitrosamine in the drinking water were treated at various time periods during the process with phenobarbital, hydroxyurea, antilymphocytic serum, or dibenamine. Rats which received phenobarbital concurrently with diethylnitrosamine had a slightly lower degree of severity of liver lesions than the rats receiving diethylnitrosamine alone. Hydroxyurea had no effect. Phenobarbital given after diethylnitrosamine administration or a combination

of phenobarbital with hydroxyurea or antilymphocytic serum after diethylnitrosamine administration led to more severe liver lesions than in the rats which received diethylnitrosamine alone. The experiments with dibenamine are not yet concluded.

3. Effects of benzene in newborn mice. There have been many case reports in the literature ascribing leukemia or lymphomas in man to continued occupational exposure to benzene. Thus far no experimental model has been found to simulate this effect. We have therefore injected newborn C57Bl mice with benzene dissolved in corn oil repeatedly for a one year period. The mice are being followed until they are two years of age. Preliminary indications are that the benzene treatment leads to an earlier appearance of amyloidosis than in the controls. Thus far no tumors have apparently been induced by the benzene exposure.

4. Induction of thymic lymphoma with butylnitrosourea. Several Japanese investigators have reported that leukemia could be induced in mice by ingestion or injection of butylnitrosourea. In our laboratory butylnitrosourea was fed to C57Bl mice in the drinking water. Thymic lymphomas were found in 50% of the mice in 3 to 6 months.

5. Carcinogenesis study with nitrogen mustard and phorbol esters. Presently the therapeutic procedures used for treating patients with psoriasis include the use of nitrogen mustard. To determine whether there was any risk from this dermatological therapy, mice were treated with daily alternate doses of nitrogen mustard and the promoting agent, phorbol ester derived from croton oil. The experiment is still in progress.

6. Carcinogenicity of 1,1-bis(4-fluorophenyl)-2-propynyl-*N*-cycloctyl carbamate. This compound has originally been prepared for use as a cancer chemotherapeutic agent. During studies on this material Dr. Harris of Lilly found that it was a carcinogen. We have therefore fed some of this material to Charles River CD male rats at 3 dose levels to investigate the incidence of intestinal tumors as well as other pathological changes. Grossly the animals did develop tumors but the final results are awaiting the histopathological examination.

7. Use of Chinese hamster in carcinogenicity studies. Although the Syrian golden hamster is now employed to a great extent in carcinogenesis research, practically no studies have been done with Chinese hamsters. A supply of these animals was obtained from Dr. George Yerganian. To determine whether they were at all sensitive the animals were given 40 ppm diethylnitrosamine in the drinking water. Preliminary indications are that a high proportion of the animals developed esophageal tumors. In view of the distinct chromosomal number of this animal species and the fact that it does respond to known carcinogens, this may be an interesting model for use in carcinogenesis research.

8. Effect of various ureido compounds in the diet on tumor incidence. In order to determine whether some weakly active carcinogens such as Dulcin could be inhibited by arginine glutamate as we had found previously with acetamide, Dulcin and arginine glutamate were fed to rats. However, in this case the preliminary indications are that there was no striking difference in tumor incidence. In addition effects of other amides of environmental importance such as adipamide or various ureas fed alone or in conjunction with sodium nitrite are being studied. The experiments are still underway.

9. Effect of modification of chemical carcinogenesis by vitamin B-12 deficiency. Previous reports have indicated that vitamin B_{12} deficiency decreased the carcinogenic activity of *N,N*-dimethyl-4-aminoazobenzene in the livers of rats. To study the effect of this vitamin deficiency on the activity of other carcinogens such as methylazoxymethanol acetate, aflatoxin, *N*-2-fluorenylacetamide, and diethylnitrosamine, rats were fed a commercial B_{12} deficient diet with and without dietary supplementation by vitamin B_{12} and with the carcinogens mentioned. Preliminary results indicate that vitamin B_{12} deficiency decreases the average tumor yield from methylazoxymethanol acetate and increases the latent period of diethylnitrosamine hepatocarcinogenesis. The toxicity of aflatoxin, *N*-2-fluorenylacetamide and *N,N*-dimethyl-4-aminoazobenzene was increased in rats fed the B_{12} supplemented diet.

10. Structure-function correlations in mutagenicity and carcinogenicity. In the last several years there have been indications that the major chemical carcinogens act via a reactive electrophilic intermediate or a carbonium ion-like structure. The principles of organic chemistry may be applied as a guide for indicating the potential of a given drug. The mutagenic activity towards repair deficient *E. coli* of several carcinogens and related compounds was investigated in collaboration with Dr. Hans Ruelius. The bacterial mutagenicity system successfully detected several carcinogens including dimethylhydrazine, ethylmethane sulfonate and methylmethane sulfonate. Further a new mutagen benzyl chloride, which had been found carcinogenic on subcutaneous injection was discovered. Unfortunately the system did not detect the direct acting compound 2,3-epoxypropene aldehyde and several carcinogens requiring metabolic activation such as diethylnitrosamine and *N*-2-fluorenylacetamide. Further studies on the possible carcinogenicity of various alkyl halides are being done at the Frederick Cancer Research Center and as part of a contract activity with Dr. Gary Stoner at the University of California. Preliminary results show that several of the alkyl halides increased the production of lung adenomas in mice.

Significance to Biomedical Research and the Program of the Institute:

The prevention and cure of neoplasia hinges on an understanding of the intimate factors involved in the pathogenesis of cancer. The research performed within the framework of this report aims to produce data which will permit the comprehension of the underlying mechanisms involved in chemical carcinogenesis.

Proposed Course of Project:

A number of the tests for chronic toxicity and carcinogenicity presently underway or planned to go on stream shortly will require considerable effort for several years. Additional emphasis will be placed on methods for inhibiting the carcinogenic process and on understanding the various steps leading to such inhibitions. Thus studies will include variations in endogenous factors as well as select exogenous materials.

Honors and Awards

Richard S. Yamamoto: Received the Japanese Government Research Award for Foreign Specialists for a tour of duty at the National Cancer Center Research Institute in Tokyo, Japan.

Lionel A. Poirier:

Member, Bioassay Segment, Carcinogenesis, DCCP, NCI

Chairman, Subgroup on Short-term Bioassay Systems

Consultant, Pharmaceuticals Manufacturers Association, on short-term bioassay systems

Consultant, Health Protection Branch, Department of Health and Welfare, Government of Canada

Member, Interagency Panel on Environmental Mutagenesis

Organizer and participant, NCI Workshop on Chemical Mutagenesis, Bethesda, Maryland, July 18, 1972

Lecturer: Structure-function Correlations in Chemical Carcinogenesis. Health Protection Branch, Ottawa, Canada, June 13, 1972.

Participant: International Workshop on Mutagenicity Testing. Zurich, Switzerland, October 1-5, 1972.

Lecturer: One-Carbon Compounds and Cancer. Chester Beatty Research Institute, Pollards Wood, United Kingdom, October 10, 1972.

Publications

Deckers, C., Glass, R. M., Grantham, P. H., Yamamoto, R. S., and Weisburger, J. H.: A comparative study of the proteins of rat plasma, liver and hepatoma by agarose immunoelectrophoresis. Brit. J. Cancer 26: 190-200, 1972.

Grantham, P. H., Weisburger, J. H., and Weisburger, E. K.: Effect of the antioxidant butylated hydroxytoluene (BHT) on the metabolism of the carcinogens *N*-2-fluorenylacetamide and *N*-hydroxy-*N*-2-fluorenylacetamide. Food Cosmet. Toxicol. (in press).

Lepage, R., Poirier, L. A., Poirier, M. C., and Morris, H. P.: The enzymology of the formation and interconversion of labile 1-carbon groups in five hepatomas and in Walker Tumor 256. Cancer Res. 32: 1099-1103, 1972.

Morais, R., Poirier, L. A., and Dupuis, C.: Inhibition of mitochondrial 5'-endonuclease activity by carcinogenic amines and their *N*-oxidized derivatives. Chem.-Biol. Interactions 5: 391-399, 1972.

Poirier, M. C., Poirier, L. A., and Lepage, R.: The hepatic activities of 1-carbon enzymes during the chronic administration of diethylnitrosamine, 2-acetylaminofluorene, and *N,N*-dimethyl-4-aminoazobenzene to rats. Cancer Res. 32: 1104-1107, 1972.

Poirier, L. A. and Whitehead, V. M.: Folate deficiency and elevated formiminoglutamate excretion during chronic diethylnitrosamine administration to rats. Cancer Res. 33: 383-388, 1973.

Ward, J. M. and Hurvitz, A. I.: Ultrastructure of normal and neoplastic mast cells of the cat. Vet. Pathol. 9: 202-211, 1972.

Ward, J. M., Wright, J. F., Nelson, N. S., Berman, E., Liddle, C. G., and Hellman, A.: Bone and soft-tissue neoplasms in cats exposed to radiostrontium. J. Nat. Cancer Inst. 48: 1543-1546, 1972.

Weisburger, J. H.: Host-dependent factors in environmental carcinogenesis: Introductory remarks. In Environment and Cancer (24th Annual Symposium on Fundamental Cancer Research), Williams and Wilkins, Baltimore, 1972, pp. 349-354.

Weisburger, J. H. and Rall, D. P.: Do animal models predict carcinogenic hazards for man? In Environment and Cancer (24th Annual Symposium on Fundamental Cancer Research), Williams and Wilkins, Baltimore, 1972, pp. 437-452.

Weisburger, J. H., Weisburger, E. K., Madison, R. M., Wenk, M. L., and Klein, D.: Effect of acetanilide and *p*-hydroxyacetanilide on the carcinogenicity of *N*-2-fluorenylacetamide and of *N*-hydroxy-*N*-2-fluorenylacetamide in mice, hamsters and female rats. J. Nat. Cancer Inst. (in press).

Ulland, B. M., Weisburger, J. H., Yamamoto, R. S., and Weisburger, E. K.: Antioxidants and carcinogenesis: Butylated hydroxytoluene, but not diphenyl-*p*-phenylenediamine, inhibits cancer induction by *N*-2-fluorenylacetamide and by *N*-hydroxy-*N*-2-fluorenylacetamide in rats. Food Cosmet. Toxicol. (in press).

Yamamoto, R. S., Kroes, R., and Weisburger, J. H.: Carcinogenicity of diethylnitrosamine in *Mystromys albicaudatus* (African white-tailed rat). Proc. Soc. Exptl. Biol. Med. 140: 890-892, 1972.

Yamamoto, R. S., Williams, G. M., Richardson, H. L., Weisburger, E. K., and Weisburger, J. H.: Effect of *p*-hydroxyacetanilide on liver cancer induction by *N*-hydroxy-*N*-2-fluorenylacetamide. Cancer Res. 33: 454-457, 1973.

Serial No. NCI-4619
1. Experimental Pathology Branch, OASDC, DCCP
2. Carcinogen Screening Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Mode of Action of Chemical Carcinogens -- Studies on the Metabolism of Chemical Carcinogens

Previous Serial Number: Same

Principal Investigator: Elizabeth K. Weisburger, Ph.D. and Preston H. Grantham, M.S.

Other Investigators: John H. Weisburger, Ph.D., Lionel A. Poirier, Ph.D., Jerrold M. Ward, D.V.M., Ph.D., and Jane Idoine, M.S.

Cooperating Units: None

Man Years:

Total	: 4.1
Professional	: 1.9
Other	: 2.2

Project Description

Objectives:

The aim of this research is to gather data relevant to the etiology of neoplasia at the molecular level. To this end chemical carcinogens, especially those of the aromatic amine type, are being utilized. Their metabolism and interaction with host tissues and specific targets are studied. Simplified yet realistic model systems are devised in order to gain an understanding of the fundamental events operating in chemical carcinogenesis. Specific inhibitors, and accellerators of the carcinogenic process are applied as tools to develop information on and permit discrimination between biochemical events directly involved in the neoplastic change and those representing other reactions.

Methods Employed:

Biochemical and pharmacological techniques are applied to determine the metabolism of *N*-hydroxy-*N*-2-fluorenylacetamide and related compounds in various animal species. This includes isolation and purification of enzyme systems concerned with certain metabolic steps.

Procedures are developed and applied for the separation, purification and analysis of macromolecular constituents such as DNA, RNA and proteins from tissues of animals treated with chemical carcinogens and from control animals.

The metabolism of chemical carcinogens and related compounds, and interaction with host targets are examined *in vivo* and *in vitro* in the presence of various chemicals or antibiotics which affect the carcinogenic process.

Major Findings:

1. Water-soluble metabolites of aromatic amines, specifically acetanilide. Efforts have been underway for some years to identify the water soluble metabolites found as biotransformation products of many drugs. Acetanilide has been under investigation in our laboratory as a simple model for the more complex carcinogen *N*-2-fluorenylacetamide. Identification of two compounds as water soluble metabolites of acetanilide has been accomplished. These are S-(5-acetamido-2-hydroxyphenyl)mercapturic acid and S-(5-acetamido-phenyl)mercapturic acid. These metabolites were isolated from rat urine after dosing with tritiated labeled acetanilide. The structures were determined by mass spectroanalysis and nuclear magnetic resonance. The significance of these findings are that the position of attachment of the thio group has been confirmed as being meta to the acetylamino grouping. The formation of these metabolites strongly suggests that epoxides may be intermediates and that these epoxide intermediates subsequently react with glutathione. In connection with a study on the inhibitory effect of *p*-hydroxyacetanilide on the carcinogenic effect of *N*-2-fluorenylacetamide and *N*-hydroxy-2-fluorenylacetamide the water soluble metabolites of *p*-hydroxy-acetanilide were also investigated. By using tritiated *p*-hydroxyacetanilide as a marker it was found that S-(5-acetamido-2-hydroxyphenyl)mercapturic acid was also a metabolite of *p*-hydroxyacetanilide. This suggests that *p*-hydroxy-acetanilide may be a common intermediate from acetanilide.

2. Metabolism of *p*-hydroxyacetanilide under acute and chronic conditions. ^{3}H-*p*-Hydroxyacetanilide was prepared biosynthetically by injecting rats with ^{3}H-acetanilide. Urines were collected and the *p*-hydroxyacetanilide was isolated by DEAE-cellulose column chromatography. A metabolism study was undertaken with controls fed an ordinary diet and animals which had been prefed unlabeled *p*-hydroxyacetanilide for 4 weeks. There was very little difference in the percent of labeled material excreted in the urines over a 24-hour period from the control and prefed groups respectively. However, there was a 30% reduction in the amount of unconjugated material excreted in the urine in the prefed group compared with the control animals. There also was a slight increase in the glucuronide fraction in the prefed group. Despite the fact that *p*-hydroxyacetanilide is supposed to act as a sulfate trap the radioactivity excreted in the sulfate fraction was practically the same with each group. Water soluble metabolites were likewise about the same.

3. Biochemical reduction of nitroaryl compounds in relation to carcinogenicity. Confirming earlier studies from this laboratory the enzymatic reduction of 2-nitronaphthalene by rat liver extracts was a much slower process than the reduction of the corresponding hydroxylamine. Reduction of both compounds was linear with time for the first 15 minutes of incubation. These results preclude the detection of *N*-hydroxyl-2-naphthylamine from rat liver extracts incubated with 2-nitronaphthalene. Even in the presence of phosphoadenosine-phosphosulfate (PAPS) and rat liver nuclei no significant labeling of DNA occurred when 9-^{14}C-*N*-hydroxy-2-acetylaminofluorene was added to the incubation mixture. The results suggest that rat liver nuclei lack the sulfotransferase enzyme required for formation of the sulfate ester of *N*-hydroxy-2-fluorenylacetamide.

4. Metabolism of auramine and derivatives. Previous results indicating that auramine is a carcinogen have led to the supposition that auramine and its derivatives may be metabolized to a common reactive benzylic alcohol intermediate. This compound might then be esterified with sulfate or acetate to form the proximate carcinogen. However, no evidence for this pathway could be deduced in metabolism studies in rats that had been injected with auramine or derivatives. The chemical reactivity of auramine with nucleophilic amino acids *in vitro* indicates that auramine may be a direct acting carcinogen. This point will be pursued further.

5. Metabolism of 6-aminochrysene. Further studies with 12-^{3}H-6-aminochrysene have continued. A major observation was that the *N*-glucuronide of 6-aminochrysene was the chief biliary metabolite. The *N*-glucuronide is acid labile and decomposes rapidly under slightly acid conditions at 37°. In metabolism studies with rats injected with ^{3}H-6-aminochrysene, it was found that almost 60% of the urinary activity was in the form of tritiated water indicating that the label was lost during metabolism. For these reasons, 5,6-^{14}C-6-aminochrysene has been synthesized (Project 4620) and will be used to continue studies on metabolism of 6-aminochrysene.

6. Metabolism of fluorenylacetamide and *N*-hydroxy-fluorenylacetamide in rats prefed *p*-hydroxyacetanilide. The metabolism of the labeled FAA and N-OHFAA in rats which had been prefed with *p*-hydroxyacetanilide (*p*-OHAA), *p*-OHAA + FAA, *p*-OHAA + N-OHFAA, or FAA or N-OHFAA alone for 4 weeks was investigated. In the FAA study it was found that prefeeding *p*-OHAA increased the amount of FAA metabolites excreted into the urine during a 24 hour period. In addition the glucuronide fraction of the urine was increased in the *p*-OHAA and *p*-OHAA + FAA groups. As might be expected the amount of isotope bound in the liver as well as that bound to protein both in the liver and the blood plasma was lower. The N-OHFAA study showed that there is also an increased output of urinary glucuronides and an increase of fecal metabolites in the animals prefed *p*-OHAA. As was seen with the FAA study the distribution of ^{14}C in the liver was lower than in the controls. Protein bound isotope was also lower in both plasma and liver. The protective action of *p*-OHAA against the carcinogenic effects of FAA and N-OHFAA can probably be explained in terms of increased glucuronide formation with enhanced excretion in the urine and feces. The net effect is the reduction in level of carcinogen to which cells become exposed.

7. Glucuronyl transferase activity. As was mentioned in the preceding paragraph *p*-OHAA prefeeding increased levels of glucuronides in the urine. Therefore the levels of glucuronyl transferase in controls and in rats prefed *p*-OHAA for a period of 4 to 5 weeks were determined. The assay was done by the classical method using *p*-nitrophenol as substrate. The data showed that there was a 3-fold increase in the level of enzyme in the liver microsomes of the rats prefed *p*-OHAA compared with the controls not fed this substance. The increased enzyme level may explain the decrease in toxicity and carcinogenicity of FAA and N-OHFAA when also fed compounds such as *p*-OHAA or butylated hydroxytoluene.

Significance to Biomedical Research and the Program of the Institute:

By the utilization of certain chemical carcinogens, the molecular mechanism of steps leading to cancer is being explored. If the entire process is viewed as a series of steps going from 1) the primary interaction between an agent and a crucial cellular constituent or constituents, a reaction for which there are repair processes antagonizing the attack, followed by 2) multiplication of abnormal cells which in turn can 3) undergo further transformations, it is easy to visualize a number of points which with future effort could conceivably prevent or even reverse such interactions. Thus, the ultimate aim of these studies is to fully comprehend the sequence of complex reactions leading to cancer and hopefully to be in a position to prevent them under realistic conditions in man.

Proposed Course of Project:

The program of elucidating the biochemistry of carcinogenesis, our eventual goal, is a continuing, long-term effort. Certain promising leads which have been discussed in this account will be followed up in further experiments.

Honors and Awards

Lionel A. Poirier:

Lecturer: N-Hydroxylation and carcinogenesis. V International Congress of Pharmacology, San Francisco, California, July 23-28, 1972.

Lecturer: One-carbon compounds and cancer. University of California, Berkeley, July 22, 1972.

Lecturer: One-carbon compounds and cancer. UCLA, Los Angeles, California, August 2, 1972.

Publications

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Matsushima, T., Grantham, P. H., Weisburger, E. K., and Weisburger, J. H.: Phenobarbital-mediated increase in ring- and *N*-hydroxylation of the carcinogen *N*-2-fluorenylacetamide, and decrease in amounts bound to liver DNA. Biochem. Pharmacol. 21: 2043-2051, 1972.

Matsushima, T. and Weisburger, J. H.: Effect of carbon monoxide or of 3-aminotriazole on *C*- and *N*-hydroxylation of the carcinogen *N*-2-fluorenylacetamide by liver microsomes of hamsters pretreated with 3-methylcholanthrene. Xenobiotica 2: 423-430, 1972.

Poirier, L. A. and Weisburger, J. H.: *N*-Hydroxylation and carcinogenesis. Proceedings Vth International Congress of Pharmacology, S. Karger (in press).

Weisburger, J. H.: Cancer and environment - Mechanisms of carcinogenesis with emphasis on aromatic amines. Proceedings of Symposium on Metabolism and Disease, Ottawa, Canada (in press).

Weisburger, J. H. and Weisburger, E. K.: Biochemical formation and pharmacological, toxicological and pathological properties of hydroxylamines and hydroxamic acids. Pharmacological Reviews (in press).

Williams, G. M. and Yamamoto, R. S.: Absence of stainable iron from preneoplastic and neoplastic lesions in rat liver with 8-hydroxyquinoline-induced siderosis. J. Nat. Cancer Inst. 49: 685-692, 1972.

Serial No. NCI-4620
1. Experimental Pathology Branch, OASDC, DCCP
2. Carcinogen Screening Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Mode of Action of Chemical Carcinogens -- Chemical Investigations

Previous Serial Number: Same

Principal Investigator: Elizabeth K. Weisburger, Ph.D.

Other Investigators: Preston H. Grantham, M.S.

Cooperating Units: Laboratory of Chemistry, NHLI
Viral Carcinogenesis Branch, NCI
Drug Development Branch, NCI

Man Years:

Total	:	0.5
Professional	:	0.2
Other	:	0.3

Project Description

Objectives:

In order to gain an understanding of the etiology of cancer, chemical studies are performed on the properties of certain carcinogens. Model studies on the reactions of chemical carcinogens with potential targets are performed.

Methods Employed:

Properties of chemicals acquired or synthesized for the bioassay of chemical carcinogens are investigated to determine the purity of the materials. In model experiments the reaction of certain of these agents, particularly their primary and ultimate reactive forms with select potential targets are studied. The materials produced are made available for related studies on the biochemistry and biology of the carcinogenic process. Methods for purification and analysis of various chemicals are applied.

Major Findings:

During the past year this project has continued to prepare reference materials for metabolism studies on carcinogens or co-carcinogenic analogs. Additional compounds presumed to be metabolites of acetanilide were synthesized. Furthermore, various carcinogens unavailable commercially were synthesized for the use of other members of the Section (Project 4618). A synthesis of carbon-14 labeled 6-aminochrysene has been developed. The labeled material will be used for a metabolism study under Project 4619.

Significance to Biomedical Research and the Program of the Institute:

The studies performed are a necessary adjunct to the broad programs on the mechanism of action of chemical carcinogens pursued in this Section.

Proposed Course of Project:

This project will continue along the same lines as previously.

Honors and Awards

Elizabeth K. Weisburger:

Assistant Editor-in-Chief, *Journal of the National Cancer Institute*, 1971--

Editorial Board, *Excerpta Medica (Cancer)*

Board of Trustees, Lebanon Valley College, 1970-1973; Alumni Award 1973

Publications

Weisburger, E. K.: Industrial cancer risks. In Sax, N. Irving (Ed.): Dangerous Properties of Industrial Materials, 4th edition, Reinhold, New York (in press).

Serial No. NCI-4680
1. Experimental Pathology Branch, OASDC, DCCP
2. Carcinogen Screening Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Mode of Action of Chemical Carcinogens -- Development and Application of *In Vitro* Systems Involving Epithelial Cells

Previous Serial Number: Same

Principal Investigators: James M. Sontag, Ph.D. and Jane Idoine, M.S.

Other Investigators: Robert Kroes, D.V.M., Elizabeth K. Weisburger, Ph.D., John H. Weisburger, Ph.D., and Jerry Elliott, B.S.

Cooperating Units: Dr. Robert Huebner, Viral Carcinogenesis Branch, NCI
Dr. Aaron Freeman, Microbiological Associates
Dr. Rhim, Microbiological Associates

Man Years:

Total	: 1.8
Professional	: 0.6
Other	: 1.2

Project Description

Objectives:

Neoplasia in man and animals involves mostly epithelial cells. Thus far efforts to grow epithelial cells *in vitro* under conditions required for studies of neoplastic transformation have been mostly unsuccessful. Many efforts along these lines have been at the level of cultures derived from whole embryos or of fibroblasts. The transformation obtained in such systems with a few chemicals resulted in the production of mesenchymal tumors. Our principal aim was to secure cultures of epithelial cells from liver for studies of their biochemical properties and attempts at transformation, which would yield carcinoma, the principal type of neoplasia in man and animals. Once established such systems could serve to not only gain insight into the mechanism of action of chemical carcinogens under readily controlled conditions, but hopefully also assist in developing quick and reliable bioassay procedures for chemical carcinogens.

Methods Employed:

Classical tissue culture methods were applied but modified in such a manner to select epithelial cells on the basis of their lesser ability to attach to the culture dish. The cells were kept under conditions where continuing growth and proliferation was maintained. Procedures were applied to characterize these cells by biochemical and morphological tests such as synthesis of albumin, presence of aryl hydrocarbon hydroxylase, and structural similarity to liver cells. Select carcinogens were added and the results evaluated with reference to morphological structure and ability of the cells to induce tumors after injection into animals.

The activity of certain key enzymes, particularly those involved in the metabolism of chemical carcinogens located on the endoplasmic reticulum is determined on cell systems treated *in vitro* with chemical carcinogens with and without infection by viral agents and also after treating animals with chemicals.

Major Findings:

1. Transformation by various chemical carcinogens of epithelial hepatic cell cultures and development of hepatocellular carcinoma after injection of transformed cells into syngeneic hosts. As discussed in previous reports the liver cells derived from 8 to 10 day old Fischer strain 344 rats have been placed under long term culture. The cells have remained stable as indicated by their inability to give tumors following injection into syngeneic hosts. Malignant transformation of these cells has resulted following their treatment with a variety of chemical carcinogens such as DMBA, *N*-hydroxy-2-fluorenylacetamide, or nitrosomethylurea.

Characteristically, no morphological distinctions were apparent between the untreated non-transformed cell lines and ones recently transformed by chemical carcinogens. Thus, the only criteria by which a cell line could be judged to be transformed was by its ability to give rise to a tumor in a host animal. Since tumors often did not become apparent until many months after inoculation of the cells, a considerable time was required to determine if malignant transformation had occurred. This long lag time has been overcome by application of the soft agar technique developed in Sachs laboratory. Normal untreated cells do not grow in soft agar whereas transformed cells give rise to colonies.

The epithelial nature of these cells is indicated by their typical epithelial-like morphology, shown by both light and electron microscopy, and mosaic pattern of growth. In addition, malignantly transformed cells consistently produce hepatoma-like carcinomas. Characterization of these cells by histochemistry and immunoelectrophoresis provide evidence of their parenchymal nature.

2. Comparison of enzyme activity in virus-infected cell systems and control cultures. With the cooperation of Dr. Huebner and associates, particularly Drs. Freeman and Rhim at Microbiological Associates, Fischer rat embryo cell cultures were infected with the Rauscher leukemia virus (RLV). Arylhydrocarbon hydroxylase (AHH) activity in these virus-infected cell systems was investigated. Appreciable increases in specific AHH activity (enzyme activity per mg of cellular protein) were observed in a cell line (F-1706) that had been infected with RLV. Increased activity in infected cells was first noted at 21 days post-inoculation. In a more extensive investigation with this same line at high passage (some 100 or more population doublings) similar effects were noted. However, different effects of RLV infection were observed in another line F-111 of Fischer rat embryo cells at lower passage levels (44 to 74 population doublings). When these cells became chronically affected, AHH activity was decreased compared to that in the parallel control cultures. For a transient period, however, when virus growth was first apparent, an appreciable increase in AHH activity was observed in these infected cells of the F-111 line. These observations had been made with cell homogenates of confluent cultures that had been maintained for 7 to 14 days after subculturing. Parallel cultures of some of the passages have been maintained for 21 to 28 days before being assayed for AHH activity. Again the activity in infected cells compared to that in the controls was lower for F-111 cells and higher for F-1706 cells. Thus rat embryo cells infected with a C type RNA virus (RLV) demonstrated enhanced activity of the hydroxylase system that has been associated with formation of active intermediates during polycyclic aromatic hydrocarbon-induced carcinogenesis. The increased AHH activity in RLV infected cells may be one factor explaining their enhanced sensitivity to transformation by aromatic hydrocarbons.

Significance to Biomedical Research and the Program of the Institute:

An assay system which can be designed to give results more rapidly, specifically, and reliably than the customary long-term tests for carcinogenicity is highly desirable with the dual purpose of studying fundamental phenomena and the detection of harmful agents in our environment. Full exploitation of workable *in vitro* systems has not been achieved. It is our goal to develop such systems; an additional advantage of *in vitro* systems is that eventually they can be established utilizing cells from man. Hence carcinogenesis *in vitro* with human cells avoids the question of species difference, so often raised in evaluating the significance of carcinogen bioassay systems.

Proposed Course of Project:

These epithelial cells will be carefully examined for suitability as a carcinogen screening system. The relevant morphological properties in both normal and carcinogen treated cells will be investigated thoroughly. Extensive efforts will deal with the biochemical properties of both the normal and the transformed cells in culture. Basic biochemical mechanisms of the system will be investigated.

Honors and Awards

James M. Sontag, Damon-Runyon Fellow, Experimental Pathology Branch, January 1972 - December 1972.

Publications

Freeman, A. E., Weisburger, E. K., Weisburger, J. H., Wolford, R. G., Mawak, J. M., and Huebner, R. J.: Transformation of cell cultures as an indication of the carcinogenic potential of chemicals. J. Nat. Cancer Inst. (in press).

Williams, G. M., Elliott, J. M., and Weisburger, J. H.: Carcinoma after malignant conversion *in vitro* of epithelial-like cells from rat liver by chemical carcinogens. Cancer Res. 33: 606-612, 1973.

Serial No. NCI-4688
1. Experimental Pathology Branch, OASDC, DCCP
2. Carcinogen Screening Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Mode of Action of Chemical Carcinogens -- Investigations of Systems Leading to Cancer in the Colon and Small Intestine

Previous Serial Number: Same

Principal Investigator: Jerrold M. Ward, D.V.M., Ph.D.

Other Investigators: Richard S. Yamamoto, Sc.D., John H. Weisburger, Ph.D., and Carolyn Brown, M.S.

Cooperating Units: None

Man Years:

Total	2.1
Professional	0.6
Other	1.5

Project Description

Objectives:

In the United States the mortality rate due to colon and rectum cancer in males is second only to lung cancer, and in females second to breast cancer. In incidence, colon cancer is third after skin and lung in men, and in women, after breast and uterus. In Western Europe, the relationship is similar. However, in other countries such as Japan and Africa, colon cancer incidence is much lower, thus suggesting that environmental factors are associated with the etiology of this important disease. Because of the absence of adequate tools to induce colon and rectal cancer in animals, model studies could not be performed. However, recently several new agents were discovered by which colon cancer could be induced in animals. The goal of the studies to be described is to evaluate how select environmental factors affect the production of cancer of the colon in animals thereby gaining insight into conditions possibly affecting humans.

Methods Employed:

Rats, mice and other rodent species were treated with chemicals known to lead to colon cancer. These include 1,2-dimethylhydrazine and azoxymethane. The animals are placed on diets mimicking those utilized in various parts of the world with regard to quantity and quality of protein, fat, carbohydrates, micronutrients, and the amount of bulk material such as cellulose in the diet. After appropriate periods of time the animals are carefully autopsied and the lesions are examined microscopically.

Major Findings:

1. Development of a colon cancer model in rats and mice. A dose response to azoxymethane and dimethylhydrazine injection in rats and mice provided a highly reproducible experimental model. Rats injected with azoxymethane were used for the most detailed studies. The number of intestinal tumors per rat provided a method of statistical comparison between experimental groups. With large doses of azoxymethane, rats developed numerous colon tumors in just 6 months. However, many small intestinal tumors were also induced. Lowering the injected dose resulted in lesser numbers of small intestinal tumors and a longer latent period for the development of colon tumors.

In rats, colon tumors induced included both adenomas and carcinomas, the latter of which metastasized to various tissues. In mice, intestinal tumors (adenomas and carcinomas) were localized to the terminal colon, but did not metastasize as readily as those in rats.

2. Dietary studies with experimental colon cancer. Rats and mice injected with azoxymethane or dimethylhydrazine were fed one of the following diets high in one of the following: fat, proteins, protein and fat, added tryptophan or ground cellulose. None of these diets resulted in increased intestinal cancer incidence. However, rats fed diets high in ground cellulose (40%) had a significant reduction in small intestinal tumor incidence (average number of tumors per rat), but not in colon tumor incidence. Rats fed these diets consumed more food and had higher fecal weights than rats fed normal diets. The reduction in tumor incidence was thought to be a result of the excretion of the injected carcinogen or its metabolite in the bile and the dilution of the bile with the greater volume of food ingested by the rats fed undigestable ground cellulose.

3. Transplantation of chemically-induced carcinomas of the colon and small intestine. Four different chemically-induced carcinomas (3 small intestine, 1 colon) were transplanted to weanling rats. Three of the lines are in the second passage. Two of the lines are slow growing, 2 are fast growing, resulting in death 2 months after injection. The establishment of permanent transplantable intestinal tumors is needed for studies in immunotherapy and chemotherapy.

Significance to Biomedical Research and the Program of the Institute:

Establishment of a colon cancer model with similarities to the human disease was necessary to study the effects of diet on colon cancer induction. Dietary factors are thought to be responsible for the occurrence of human colon cancer. Our studies suggest that under our experimental conditions, diets containing nutrients thought to be important for the high incidence of human colon cancer in some parts of the world did not increase the incidence of chemically-induced cancer. The limitations of this model are obvious.

Proposed Course of Project:

To complete all the experimental studies noted above in order to a) characterize the pathology of the experimental model, b) determine the effects of various diets on colon cancer incidence in the experimental model, c) establish the transplantable carcinomas noted above.

Publications

Weisburger, J. H.: Model studies on the etiology of colon cancer. In Nakahara, W., Takayama, S., Sugimura, T., and Odashima, S. (Eds.): Topics in Chemical Carcinogenesis (Proceedings 2nd International Symposium, Princess Takamatsu Cancer Research Fund), University of Tokyo Press, Japan, 1972, pp. 159-174.

Weisburger, J. H.: Chemical carcinogens and their mode of action in colonic neoplasia. Dis. Colon Rectum (in press).

Weisburger, J. H.: Chemical carcinogenesis in the gastrointestinal tract. Cancer (in press).

III. COLLABORATIVE PROGRAM REPORTS

SUMMARY REPORT

OFFICE OF THE ASSOCIATE SCIENTIFIC DIRECTOR FOR CARCINOGENESIS

July 1, 1972 through June 30, 1973

Five specific contracts are reported under this Office: Three are large multifaceted contracts relating to the Carcinogenesis Program in general. Although portions of these activities relate to specific segments, they are included here in order to present a cohesive summary of the progress accomplished within the multidisciplinary efforts of each.

These programs are of additional interest because they bring staff from diverse organizational backgrounds into the Program: one is within a laboratory supported by another agency, (AEC-NCI Interagency Agreement, Oak Ridge National Laboratory); one is the Frederick Cancer Research Center which is staffed and managed by a contractor but remains under the direct auspices of the NCI and the third is university based (University of Nebraska, Eppley Institute for Research on Cancer).

The specific Frederick Cancer Research Center programs reported are those supported and monitored by the Carcinogenesis Program only. There are other task orders being performed at the Frederick Cancer Research Center but they are sponsored and reported by other NCI programs.

CONTRACT NARRATIVES

OFFICE OF THE ASSOCIATE SCIENTIFIC DIRECTOR
FOR CARCINOGENESIS

July 1, 1972 through June 30, 1973

AEC-NCI Interagency Agreement (Oak Ridge National Laboratory)(NCI-FS-64-13)

Title: NCI-AEC Carcinogenesis Program

Contractor's Project Director: Dr. Francis T. Kenney

Project Officer (NCI): Dr. Allen H. Heim

Objectives: The broad objectives are as follows: (A) to carry out large scale co-carcinogenesis studies in exposure chambers with controlled atmospheres containing selected chemical agents to be tested for direct carcinogenic effects on respiratory tissues or for co-carcinogenic effects with known carcinogens, radiation, and viruses; (B) to bring to bear on problems of chemical carcinogenesis the diverse scientific talents of the staff of the ORNL, Biology Division, and the scientific and engineering expertise of other ORNL components. A major part of the funding is associated with the first objective.

Specific objectives are as follows: (1) to develop and define animal models for study of the pathogenesis of human lung cancer with emphasis on interaction of exogenous and endogenous factors, using inhalation exposures as well as other methods for respiratory carcinogenesis studies; (2) to analyze the mechanisms for repair of damage to DNA by chemicals or irradiation in mammalian cells and the role of such damage and repair in carcinogenesis; (3) to correlate both the histopathological aspect of the systemic immune response and anatomic factors involving the progressive growth of chemically-induced tumors with functional immunologic factors directed against tumor growth.

As stated above, one of the major objectives is to incorporate the scientific talents of the ORNL staff into the Carcinogenesis Program. The degree to which this has been accomplished may best be realized by presenting the objectives, major findings and significance of each of the particular efforts.

1. Respiratory Carcinogenesis

Specific Project Director: Dr. Paul Nettesheim

Objectives: To analyze co-carcinogenic effects of physical and chemical agents in the induction of lung cancer. Identification and study of those environmental agents (e.g., irritant gases and dusts, infectious microbes) which may increase the susceptibility of respiratory tissues to normally ineffective doses of carcinogen or which may alter the "effective" tumorigenic dose by affecting distribution, deposition, and/or clearance of

inhaled carcinogenic particulates. To study host factors, which affect tumor induction and tumor progression. To identify the major steps in the morphogenesis of lung cancer on the histological and cytological level. To develop new experimental models for co-factor studies.

Major Findings: (1) A new inhalation co-carcinogenesis study is being started to determine whether nitrogen dioxide, formaldehyde and acrolein exhibit "co-carcinogenic" effects in three different lung tumor induction systems with polycyclic hydrocarbons (using hamsters and rats). No data are presently available. (2) High levels of viramin A partially protect the respiratory tract of rats from the metaplastogenic and tumorigenic effects of intratracheally injected 3-methylcholanthrene. (3) During influenza virus pneumonia, the base-level of aryl-hydrocarbon hydroxylase is significantly reduced in the lungs of mice; induction of this enzyme is also partially inhibited by the pneumonia.

(4) Further studies on the effect of influenza pneumonia on the deep lung clearance showed that a marked defect in clearance persists for as long as a year after initiation of the pneumonia. (5) N-Nitrosoheptamethyleneimine, which induces a high incidence of squamous cell carcinoma in the lungs of rats when given in the drinking water, causes only very few such tumors when administered by subcutaneous injection. (6) A lung colony forming assay with a respiratory tract squamous cell carcinoma was developed in mice. This system allows the assessment of the relative "tumor stem cell" pool size. (7) Exfoliative cytology obtained from hamsters intratracheally injected with Fe_2O_3 + BaP allows early and accurate diagnosis of bronchogenic carcinoma. Studies designed to detect carcinoma in situ and preneoplastic lesions by cytological techniques are presently underway.

Significance to Biomedical Research and the Program of the Institute: Epidemiologic data indicate a multifactorial etiology in human lung cancer. This work is a study, using animal models, of the role of inhaled chemicals and of physical and biological agents influencing the respiratory tract in the development of lung cancers. It is expected that this approach will help to define the decisive environmental and host factors in the pathogenesis of lung cancer.

Current Annual Level: $700,000

2. Immunology of Carcinogenesis

Specific Project Director: Dr. Michael G. Hanna, Jr.

Objectives: To correlate both the histopathologic aspect of the systemic immune response and anatomic factors involving the progressive growth of chemically-induced primary and secondary tumors with functional immunologic factors directed against successful tumor growth.

Major Findings: The process of BCG-mediated regression of established tumor in guinea pigs has both local and systemic features, both of which require host specific immunity. A requirement for an early sensitization to BCG

antigens was demonstrated in antilymphocyte serum pretreated guinea pigs having depleted peripheral and central lymphocyte compartments. Locally in the tumor and regional lymph node activated histocytes are the major effector cells of tumor destruction, tumor cell death involving a histiocyte-tumor cell membrane association. The control of distant metastasis was attributed to development of tumor specific humoral and cell-mediated immunity. Cell-mediated immunity was demonstrated to be a classic lymphocyte-mediated homograft rejection.

Significance to Biomedical Research and the Program of the Institute: This work provides an essential analysis at the histological and pathologic level of the role of the immune system in combating development and metastasis of chemically-induced tumors. It may lead to a more definitive mode of cancer therapy based upon fortification of the capacity to reject tumors by immunological mechanisms.

Current Annual Level: $185,000

3. Repair Mechanisms in Carcinogenesis

Specific Project Director: Dr. R. B. Setlow

Objectives: (A) To understand the role of DNA-repair mechanisms in determining the responses of various human cells to carcinogenic agents; (B) to develop and use a model system that is capable of relating quantitatively UV and chemical changes in DNA to tumor induction.

Major Findings: (A) Two types of DNA repair in human cells exposed to a wide variety of chemical carcinogens have been observed: (1) the UV-type, in which $\sim$ 100 nucleotides of DNA are replaced per lesion, is observed in normal but not xeroderma pigmentosum fibroblasts, and (2) the γ-ray type, in which only a small number ($\sim$ 4) of nucleotides are replaced, is observed in both normal and XP cells. Intercalating reactive compounds are type 1, whereas simple alkylating agents are type 2.

(B) Preliminary results using fish clones show that (1) UV-irradiated cells injected into isogenic recipients give rise to tumors. The tumor induction is UV-dose-dependent, and (2) visible light illumination of the irradiated cells before injection reduced markedly the number of tumors. These data imply that pyrimidine dimers in cellular DNA give rise to tumors.

Significance to Biomedical Research and the Program of the Institute: (A) Chemically-induced cancers may develop as a result of failure of cellular mechanisms for repair of damage to DNA. This work provides knowledge of the cellular mechanisms operative in repair and may lead to measures for prevention of cancers induced by chemical or physical agents.

(B) The implication that UV- induced pyrimidine dimers in DNA result in tumors is the first presumptive demonstration of a quantifiable lesion in DNA that gives rise to tumors.

Current Annual Level: $150,000

Proposed Course: These programs will continue along the paths described above in the objectives of each. In the respiratory studies a greater emphasis will be placed on investigating correlations between animal models and human being cancer.

Date Contract Initiated: January 14, 1963

Current Annual Level: $1,035,000

COLUMBIA UNIVERSITY (NIH-NCI-72-3234)

Title: Development of a Tissue Culture Transformation System for Aromatic Amine Carcinogens

Contractor's Project Director: Dr. I. Bernard Weinstein

Project Officer (NCI): Dr. Stuart Yuspa

Objectives: To develop an epithelial cell line, namely rodent liver, for the study of aromatic amine carcinogenesis at a cellular level.

Major Findings: (1) Several epithelial cell cultures were established from normal adult rat liver and from rat hepatomas induced in vivo with aromatic amine carcinogens. (2) Criteria for assessing transformation of epithelial cells in culture were established. (3) Normal diploid rat epithelial cells were transformed by a single in vitro exposure to N-acetoxy-acetylamino-fluorene and were tumorigenic. (4) Type C RNA viruses were discovered in the hepatoma cultures but not in the normal cultures.

Significance to Biomedical Research and the Program of the Institute: The majority of human tumors are epithelial in origin and techniques for studying epithelial carcinogenesis at a molecular level are just now being developed. This project will utilize liver cells in vitro to study aromatic amine carcinogenesis and aromatic amine interaction with macromolecules. This approach is now receiving great emphasis in the overall Carcinogenesis Program.

Proposed Course: (1) The optimal conditions for obtaining transformation of rat epithelial cells with a minimum latent period will be explored. (2) The type C Virus discovered in the rat hepatoma cultures will be characterized and its possible role in hepatic carcinogenesis will be studied. (3) Studies will be performed to determine if the epithelial cultures retain biochemical functions characteristic of liver parenchymal cells. (4) The methods developed will be applied to the cell culture of human liver and human hepatomas.

Date Contract Initiated: April 1, 1972

Current Annual Level: $131,384

FREDERICK CANCER RESEARCH CENTER (NCI-72-3294)

Each Division of the National Cancer Institute has the potential resources of the Frederick Cancer Research Center available to support or extend its program requirements.

Being a government-owned, contractor-operated facility many of the limitations of space and personnel inherent in the NCI Bethesda operation do not exist at the Frederick Cancer Research Center.

Limited only by availability of funds and the lack of clinical facilities, the contract provides a variety of services including management of scientific and technical projects, support services, and resource management.

A broad program of laboratory research is conducted under individual task orders and is related to the National Cancer Institute's research program on carcinogenesis by chemical and physical factors in our environment.

The specific task orders listed below are those supported and monitored by the Carcinogenesis Program.

Task Order #7

Title: Selected Bacteria Species

Contractor's Project Director: Dr. Milton Slein

Project Officer (NCI): Dr. Sidney Silverman

Objectives: The objective is to investigate the metabolism of anaerobic bacteria indrogenous to the human colon to determine their capabilities to produce carcinogens or precursors in vitro and to identify such substances chemically.

Major Findings: A laboratory facility has been established and staffing has been completed. Tests with selected bacterial species have been initiated. Tryptophane, bile acids and neutral steroids are being studied as natural metabolites for the possible bacterial etiology of colon cancer.

Significance to Biomedical Research and the Program of the Institute: The information concerning the ability of the intestinal bacteria to produce carcinogenic substances will help in explaining the etiology of colonic cancer. Knowledge of the substrates used and the pathways of metabolism may aid in control. The usefulness of the host-mediated assays for the rapid screening of carcinogens will be determined; other in vitro assay techniques will be assessed.

Proposed Course: Development of chemically defined media for growing anaerobes in underway with the aim of simplifying the metabolic studies. Labeled compounds will be used to determine metabolic pathways.

Date Contract Initiated: June 26, 1972

Current Annual Level: $330,700

Task Order #8

Title: Large-Scale Bioassay

Contractor's Project Director: Dr. Borge Ulland

Project Officer (NCI): Dr. Norbert Page

Objectives: The objectives of the Large-Scale Bioassay task is to conduct screening of selected environmental chemicals to determine their oncogenic potential. This task will also provide histopathology services for the FCRC. The scope of work consists of staffing, facility renovation and equipping, and procurement of animals and designated chemicals. Studies on approximately 60 selected chemicals during the first two-year period will include acute toxicity, subacute toxicity, chronic studies and terminal kill-pathology.

Major Findings: As of March 1, sixteen compounds have completed acute testing. Twelve compounds are currently undergoing or have completed subacute studies in rats and/or mice. In rats, Agerite has been completed and Ledate, ethyl tuads, Perthane, and ethyl telurac are in progress. In mice Agerite and ethyl tuads have been completed and Ledate, Perthane, piperonyl butoxide, piperonyl sulfoxide, azobenzene, Cyanamide, ethyl telurac, and Omal are in progress. Four more chemicals (azobenzene, Cynamide, ethyl telurac and Omal) have been started in rats. Additional chemicals have been assigned and are being procured and processed by Task 9. Fischer 344 rats and B6C3F1 hybrid mice are being obtained from the FCRC animal farm (Task 12) for these studies. Building 539 is being extensively modified for a barrier-type operation and scheduled for operation in late April 1973.

Significance to Biomedical Research and the Program of the Institute: It has been estimated that the etiology of cancer is primarily due to external and environmental chemicals. This contract will be one of the largest single efforts in the Bioassay Program to screen materials for carcinogenecity.

Proposed Course: By the end of Fiscal Year 1973, it is anticipated that: (1) studies will be completed in both rats and mice on 45 chemicals for acute toxicity and 35 for subacute effects (2) chronic testing of 14 chemicals will have been initiated in both rats and mice. By the end of the second contract year (June 1974), a total of 60 chemicals should be on chronic testing.

Date Contract Initiated: June 26, 1972

Current Annual Level: $617,300

Task Order #9

Title: Preparation and Characterization of Carcinogens

Contractor's Project Director: Dr. Walter L. Zielinski, Jr.

Project Officers(NCI): Dr. John Cooper
Dr. Marcia Litwack

Objectives: To provide state-of-the-art analytical expertise towards the total characterization of chemicals undergoing study in the large scale bioassay program as potential carcinogens; to provide quality control of the chemical aspects of the bioassay program in the examination of feed for mycotoxin and pesticide residues, the monitoring of blending homogeneity, and the determination of chemical stability in dosed feed, including the degradation kinetics and elucidation of the decomposition products formed; to prepare (by procurement or synthesis, followed by purification) ultra-pure carcinogens for biochemical and immunologic studies of the cancer process.

In addition, studies in collaborative investigation with NCI staff, have been initiated to determine the metabolic fate and the mechanism of action of the carcinogen.

Major Findings: A major analytical facility has been developed which includes full chromatographic and spectrophotometric competences, in addition to fluorescence, atomic absorption and flame emission, a gas chromatograph-mass-spectrometer-computer system, as well as purification and synthesis laboratories. A radioisotope laboratory is under preparation for metabolism studies.

Significance to Biomedical Research and the Program of the Institute: The evaluation of bioassay data from studies which investigate the carcinogenicity of chemicals requires not only knowledge of the major ingredient and its associated concentration in feed, but a spectrum of information related to the presence of mycotoxin and pesticide residues in the base feed, the stability and decomposition products of the chemical under study, and the identity of impurities initially present in the chemical. This latter information is of major importance since chemical contaminants which are present, can likewise be unknowingly administered throughout a chronic feeding study along with the major ingredient under study. Such contaminants may be carcinogenic in their own right, and may exert independent or synergisitc effects. The development of the total chemical contaminant picture is essential to avoiding misinterpretation of bioassay and pathology results.

It is equally necessary to provide ultra-pure carcinogens for biochemical and immunologic studies of the cancer process.

The carcinogenicity of nitrosamines is well established. Dimethylnitrosamine is an important member of this class, and elucidation of its metabolic fate is needed to provide basic information on the mechanism of action for this chemical class. The isotope effect protocol planned for this topic may provide a basic procedure for future metabolic studies as well as providing

insights into the metabolism of the particular substance under investigation.

Proposed Course: This project will provide a continuing resource in support of the large scale bioassay program at the Frederick Cancer Research Center (FCRC). The analytical expertise has been established in the past year and the laboratory is in full operation for this aspect of the contract. Ultra-purification and synthetic-characterizational capabilities will be applied to the support of the biochemical and colon bacteria studies, respectively, at FCRC. The preliminary metabolism studies of dimethylnitrosamine should be completed in the Fall of 1973.

Date Contract Initiated: June 26, 1972

Current Annual Level: $250,000

Task Order #10

Title: In Vitro Bioassay

Contractor's Project Director: Dr. Roman Pienta

Project Officer (NCI): Dr. Allen Heim

Objectives: The objective is to develop standardized in vitro bioassay methodology for detecting potential carcinogens.

Major Findings: A laboratory facility has been established and staffing has been completed. Two cell systems, one hamster and one human, have been selected for development and further study of in vitro bioassay methodology. Standard chemical carcinogens have been processed for routine use and early passage hamster embryo cells as well as other normal cell lines have been propogated and frozen in liquid nitrogen for future use.

Significance for Biomedical Research and the Program of the Institute: Increasing numbers of chemicals are being introduced into use each year thereby increasing the number of potentially carcinogenic chemicals which should be tested. In vivo testing is costly and time consuming. Consequently, the development of a rapid, relatively inexpensive, reliable in vitro test for carcinogens would provide a system for prescreening chemicals which could be tested in vivo for confirmation. Use of the prescreen introduces a much needed method for improving the selection of chemicals entered into the costly in vivo bioassay system thereby improving the efficiency and overall effectiveness of the entire carcinogen detection system.

Proposed Course: Efforts will continue to establish a reproducible, reliable test system and to optimize conditions in order to apply the method to mass screening. After test conditions are established, mass screening will be initiated.

Date Contract Initiated: June 26, 1972

Current Annual Level: $322,500

Task Order #12

Title: Production of Inbred and Hybrid Laboratory Animal Strains

Contractor's Project Director: Dr. Melvin Rabstein

Project Officer (NCI): Dr. Thomas Cameron

Objectives: To provide for production of inbred, hybrid and outbred laboratory rodents of various species and strains, in healthy condition and of known genetic background.

Major Findings: Staffing has progressed at a rate comensurate with the starting up of activities, and the addition of tasks at a reasonable and logical progression. A total of 24 positions have been filled, of which 18 were filled by former Ft. Detrick Animal Farm employees thoroughly familiar with the facilities and equipment and able to function effectively from the beginning of their rehiring. Fifteen of the 24 available animal buildings have been refurbished and made operational by the staff. Seven buildings are undergoing extensive contract renovations (1 for gnotobiote operations; 6 for barrier containment) and will be available at the close of the fiscal year. Two other buildings are held available for primate capabilities. Guinea pig and rabbit caging available from the previous Ft. Detrick operation, and the expedited purchase of polycarbonate cages with filter bonnet tops, permitted the establishment of 5 inbred mouse strains (AKR, Balb/c; C_3H, C57Bl/6, DBA_2), the $B6C3F_1$ hybrid mouse, Fischer/344 inbred rat strain, strains 2 and 13 inbred guinea pigs and the continuance of the Ft. Detrick Hartley Guinea pig outbred stock and the New Zealand outbred Rabbit stock. These colonies have been able to fill all requests to date from FCRC, as well as to issue many animals to meet the intramural needs of the NIH reservation which has not been satisfied by the NIH (DRS) inhouse colonies. Animals have also been supplied to a limited degree to the collaborative effort of Carcinogenesis and the other areas of NCI.

Significance to Biomedical Research and the Program of the Institute: Biomedical research of the scope and the quality envisioned for FCRC must be solidly based upon an unlimited resource of various animal species of the highest quality of health and genetic integrity. This has been essentially accomplished within this fiscal year. The FCRC Bioassay task, much of the NCI intramural research and some of the collaborative effort have been given continuity by their use of the animals produced by the FCRC animal farm with the cooperation and direction of the NIH in-house animal genetics unit. The previous animal supply for Carcinogenesis, via DCT contracts, has not been able to satisfactorily provide animals disease-free and of the quantity required for the Bioassay Program. Maintaining genetic consistency between animal colonies separated in distance and in time of establishment has been difficult. Establishing the FCRC colonies based upon the NIH breeding stock should allow increased confidence in genetic and microbial stability so that the results of these various bio-research efforts can be compared

to one another.

Proposed Course: This project will supply all of the animals required for the Large Scale Bioassay task at FCRC. It will supply a large share of the animals consumed by the NCI intramural effort. If sufficient space can be allocated, it will provide most, if not all, of the animals required by the collaborative program. As the seven buildings are released from the renovation contract and subsequently filled with breeding stock to their capacity, the protection of this invaluable animal production resource will be insured.

Date Contract Initiated: June 26, 1972

Current Annual Level: $291,450 (30% of Task 12)

MEMORIAL HOSPITAL (NIH-NCI-72-3286)

Title: A Study of Oncogenesis and Other Late Effects of Cancer Therapy

Contractor's Project Director: Dr. Giulio D'Angio

Project Officer (NCI): Dr. Curtis Harris

Objectives: The late effects of cancer therapy are little known. This study will examine the possible carcinogenic effects and other late effects of radiation and/or cancer chemotherapeutic agents in man. Many of the cancer chemotherapeutic agents have been shown to be carcinogenic in rodents. Radiation is a well-known carcinogenic agent in man and experimental animals. The contract is conducting an epidemiological investigation of children cured of Wilms's tumor and young women cured of trophoblastic tumors.

Major Findings: The first year was devoted to a pilot study to test the feasibility of various approaches to the problem. A workshop on Late Effects of Cancer Therapy in Children and Young Adults was held in Boston, Massachusetts, on October 19-20, 1972. The Proceedings of this workshop are being prepared for informal publication. The workshop succeeded in bringing together knowledgeable people in disparate discipline and resulted in a useful interchange of information relative to the psychic, somatic, and genetic effects of chemo- and radio-therapy.

Preliminary data being accumulated in the pilot investigation indicate that benign and possibly premalignant lesions are often not displayed on summary data sheets, and their identification requires a detailed review of the hospital chart. In fact these lesions are sometimes not detected on routine follow-up physical examination, because the examining physician devotes his attention exclusively to detecting signs of recurrence of the primary tumor.

Significance to Biomedical Research and the Program of the Institute: Since radiation and many cancer chemotherapeutic agents have been shown to be carcinogenic in man and/or experimental animals, the risk of second neoplasms occurring in patients cured of cancer may be high. In addition to

evaluating the human risks involved, this study may provide information relating findings in man to carcinogenesis data in experimental animals. Contract studies are in progress at the Southern Research Institute (NIH-NCI-68-998) to investigate the oncogenicity of cancer chemotherapeutic agents in rodents.

Proposed Course: Conduct epidemiological study as outlined above.

Date Contract Initiated: June 26, 1972

Current Annual Level: $80,383

NEBRASKA, UNIVERSITY OF (EPPLEY INSTITUTE FOR RESEARCH IN CANCER) (PH43-68-959)

Title: A Resource for Carcinogenesis Bioassays and Related Research

Contractor's Project Director: Dr. Philippe Shubik

Project Officer (NCI): Dr. Gio B. Gori

Objectives: The purpose of this contract is twofold in nature: the bioassay of agents suspected of being carcinogenic, and fundamental research on mechanisms of carcinogenesis.

(1) Bioassay - Compounds are selected for carcinogenic screening on the basis of several criteria, including (a) suspicion as a result of epidemiological studies uncovering suspected high risk to cancer in defined environmental exposures; (b) chemical properties and structures or physical properties suggesting a high index of suspicion for potential carcinogenic potency, and (c) potential or actual universal distribution or use of agents suggesting the need for carcinogenic bioassay. The techniques of bioassay include administration by various routes. Selection of various modes of administration is determined by properties of the materials, comparative response in various species, determination of dose response parameters, and the desire for maximum extrapolation to man. Protocols for these experiments take into account the current state of knowledge in the field as well as the properties of the agents being tested. Experimental design also utilizes the skills of the chemistry group and provides data analysis systems. A large number of compounds are under bioassay, using several dose levels and multiple species.

(2) Research on the Mechanism of Carcinogenesis - Objectives include fundamental research at the chemical and host levels for the purpose of elucidating biological mechanisms involved in the carcinogenic process, and to identify the factors controlling or modifying the natural history of induced neoplasms. Methods employed include the determinations of anatomic and metabolic fates of environmental carcinogens to determine sites of elective localization and paths of detoxification and excretion. Identification of the active form of the carcinogen, characterization of hormonal or genetic host factors critical to the carcinogenic responses, and comparison of response in different species are integral parts of this program. Molecular

aspects of carcinogenesis are actively investigated.

Major Findings: (1) Bioassays - Highlights of selected bioassay studies are as follows: (a) 7-H-dibenzcarbazole, a heterocyclic component of cigarette tobacco tar, was previously shown to be far more carcinogenic to hamster lung when administered in combination with ferric oxide than benzo(a)pyrene. The compound has now been shown to be carcinogenic to the lung when administered alone. Dibenzo!a,il pyrene is also carcinogenic in this same manner. (b) Seven substituted hydrazines have now been demonstrated to be carcinogenic. The carcinogenicity of methylhydrazine was shown for the first time in mice and also in hamsters. 1,1-diemthylhydrazine was also proven unequivocally to be a tumor producer (previously it was doubtful). Both are components of rocket fuel. Finally, the main metabolite of isoniazid, 1-acetyl-2-isonicotinoylhydrazine, was found to be a lung tumor producer in mice.

(c) The studies on 3 artificial sweeteners (sodium saccharin, sodium cyclamate, and calcium cyclamate) are continuing.

(d) The intraperitoneal studies on aflatoxin fractions (B_1, G_1, B_2, G_2) were completed. Of importance was the induction of gastrointestinal and adrenal neoplasms. Interpretation of the findings is difficult due to the carcinogenic activity of the solvent, dimethylformamide.

(2) Research on the Mechanism of Carcinogenesis - Selective examples of research projects include the following: (a) The hepatocarcinogenicity of DDT in the mouse and its absence of effect in the hamster may be a consequence of the accumulation of the neutral metabolite DDE in the mouse liver. Although the mouse after 6 weeks on a 250 ppm DDT diet accumulates 5 to 10 times as much DDT or DDD (a second neutral metabolite) as does the hamster on the same diet, the ratio of DDE deposition in the 2 species is 50 to 100. There is evidence that DDE is a stronger hepatocarcinogen to the mouse than is DDT.

(b) Studies on the kinetics of nitrosation reactions to produce nitrosoamides are continuing.

Aminopyrine, which is widely used in Europe as an analgesic, is a tertiary amine containing a diemthylamino group. While at this Institute W. Lijinsky and M. Greenblatt showed that the drug is readily nitrosated *in vitro* and *in vivo* to yield dimethylnitrosamine. Kinetic examination has now shown that nitrosation of aminopyrine gave measurable amounts of dimethylnitrosamine within 5 seconds; The rate of nitrosation was as rapid as that for the most rapidly nitrosated secondary amine, viz. N-methylaniline. Thus, administration of aminopyrine together with nitrite could be hazardous. The antibiotic oxytetracycline, which is also a dimethylamino tertiary amine, was nitrosated very much slower than aminopyrine to give dimethylnitrosamine. Thus, the rate of nitrosation of tertiary amines, like that of secondary amines, varies enormously depending on the chemical structure.

Since at least 15 urea derivatives occur in nature, their nitrosation to form carcinogenic nitrosoureas could present a human hazard. We are therefore testing several nitrosoureas related to naturally occurring ureas. The most

interesting finding to date is that nitrosodihydrouracil induced liver carcinomas in nearly 100% of the treated rats (of both sexes) and also induced some kidney adenomas. Dihydrouracil is a product of the metabolism of uracil and occurs in the urine. Thus the formation of its nitroso derivative in vivo or in food is a possibility, though a kinetic study showed that the nitrosation proceeds relatively slowly. This is the first nitrosourea found to induce tumors of the rat liver.

(c) Studies have continued on the induction of lung adenomas in strain A mice as a test system for the in vivo formation of N-nitroso compounds. Quantitative experiments have been previously described in which the dose of nitrite and the amine piperazine were varied, when both compounds were given at the same time. Part of these experiments have now been repeated using nitrite given with the amine morpholine. As for piperazine, the adenoma yield was more closely proportional to the square of nitrite concentration than simply to nitrite concentration, in agreement with the kinetic equations for chemical nitrosation.

(d) In vitro experiments showed that vitamin C (ascorbate) blocked the formation of several nitrosamines and nitrosamides, because it reacted with amines or ureas. It was, therefore, suggested that ascorbic acid might be administered to subjects receiving nitrosatable drugs, to block the in vivo formation of nitroso compounds. Three levels of ascorbate have been found to significantly inhabit the induction of lung adenomas in strain A mice treated with nitrite together with the amine piperazine. The inhibition was very effective for tthe higher dose of ascorbate. For the lowest does of ascorbate, the inhibition was much less though still statistically significant.

As a control, ascorbate was administered together with the performed mononitrosopiperazine (MNP). Instead of inhibiting the induction of lung adenomas, the ascorbate produced a significant increase in the lung adenoma yield. This preliminary experiment shows that caution will have to be used before ascorbic acid can be used in man.

(e) Ultraviolet light as a tumor-inducing agent, as well as its modifying effect on carcinogens as well as noncarcinogenic agents, has been studied for the last few years. The results demonstrate that a small dose of shortwave ultraviolet light (3 exposures of 3 hours each) induced a number of tumors, though the latent period was long. Ultraviolet light with a spectrum similar to sunlight failed to induce tumors even after a year's treatment with the maximum tolerated dose administered twice a week. Experiments using combined ultraviolet light and carcinogens showed that the light is a weak initiator when followed by a promoting agent and a weak promoter of carcinogen-induced neoplastic transformation. Ultraviolet light exposure prior to carcinogen applications, even a single irradiation, significantly increased the number of tumors. If applied after carcinogen applications, the neoplastic response was reduced. Combined ultraviolet treatment and applications of noncarcinogenic chemicals showed the response to be entirely dependent on the dose of the light and the wavelength used.

(f) Strong evidence has been obtained for the binding of benzo!alpyrene

to protein via the "K-region" atoms (nos. 4 and 5) of the hydrocarbon. The 4,5-dihydrodiol of benzo!alpyrene was found along with other free metabolites in a post-lysosomal in vitro system. Protein bound metabolites were also found. Chemical degradation of both the free 4,5-dihydrodiol and the bound metabolites by alkaline periodate leads to the formation of chrysene. Chrysene can only be produced from the bound metabolites if both K-region atoms are in an oxidized state and at least one of them is involved in the binding.

Significance to Biomedical Research and the Program of the Institute: The Eppley Institute has provided over the years a considerable output of major research results in chemical carcinogenesis and represents a unique national resource in which chemistry, pathology, and biology aspects of carcinogenesis are studied in an integrated program.

Proposed Course: This contract will continue to be a prime resource in the study, improvement, and conduct of bioassay techniques in carcinogenesis. The project will continue as outlined in the objectives above.

Date Contract Initiated: March 19, 1968
(Formerly: Chicago Medical School, December 1, 1964)

Current Annual Level: $2,049,269

SUMMARY REPORT

BIOASSAY OPERATIONS SEGMENT

July 1, 1972 through June 30, 1973

The Bioassay Operations Segment was established in March 1973 as a result of a reorganization of the Collaborative Contract Program. One of the objectives of the reorganization was to centralize coordination of resources and data analysis with management of the Bioassay Program. As a result, the Bioassay Operations Segment has incorporated the majority of the previous Bioassay Segment's standardized bioassay contracts, plus several support contracts (animal, chemical, and information) from the Information and Resources Segment. At this same time, a Carcinogen Metabolism and Toxicology Segment was created and assigned those bioassay segment contracts dealing with metabolic studies as well as research contracts on nitrosamines and mycotoxins.

The primary goal of the Bioassay Operations Segment is the identification and evaluation of carcinogenic chemical and physical agents in the environment to which the human population may be exposed. This requires the designing and conducting of long-term standardized bioassay. This entails (1) identifying and selecting chemical and physical agents for bioassay; (2) establishing logistical capabilities for testing; (3) acquiring, characterizing and purifying these agents; (4) identifying, developing and selecting biological models for carcinogenesis bioassay including improved animal models and short-term bioassay procedures; (5) identifying carcinogenic activity of selected agents by the appropriate bioassay tests; (6) monitoring testing progress and performance; (7) developing a data bank to include results of bioassay testing and information on use and characteristics of the agents being tested; (8) analyzing and evaluating the test results; and (9) deciding on further action required of the tested agents.

Dr. N. Page has been appointed Director of the Bioassay Operations Segment and Dr. J. Sontag as Acting Manager. An advisory group, whose members possess expertise in toxicology, pathology, biometry, etc., has been formed to provide advice on program needs and priorities as well as technical review of contract proposals.

The Bioassay Operations Segment is now responsible for 23 contracts as well as tasks 8 and 12 (large-scale bioassay studies and the animal farm) of the NCI Frederick Cancer Research Center. The bioassay contracts cover the testing of pesticides, industrial intermediates, drugs, natural products, etc. To effectively and economically manage such a large program requires close coordination between the resource

support and management activities. Now that both of these activities are controlled by the Bioassay Operations Segment, it is expected that past coordination problems will be eliminated. In addition, to insure maximum input from other areas, a close working relationship will be maintained between the Bioassay Operations, Carcinogen Metabolism and Toxicology and Information and Resources Segments.

As resources and funds are not available to test all chemicals introduced into the environment, a judicious selection must be made. A selection committee, headed by Dr. E. K. Weisburger, will select chemicals based on human exposure, production data, and structure-activity relationships. Once selected, compounds are tested in contract laboratories using standardized protocols wherever possible. Prior to being provided to the contractors, the chemicals must be procured, often specially synthesized, purified, and analyzed. Such capabilities have been developed under contract. (Contractors: Midwest Research Institute and NCI Frederick Cancer Research Center). Standard reference samples are now also available. (Contractor: Starks Associates). Dr. M. Litwack, Executive Secretary of the Selection Committee, has been assigned the responsibility of coordinating the chemical resources.

Simultaneous with the provision of testing facilities and chemical resources, animals must be provided in sufficient quantity and quality for the studies to commence on schedule. Dr. T. Cameron is responsible for this aspect. The main animals used in the bioassay program are the Fischer/344, Osborne-Mendel rats and the B6C3F$_1$ Hybrid mouse. These animals, in the past, have been procured primarily through the Animal Supply System developed by the Division of Cancer Therapy. Funds have been transferred from Carcinogenesis to DCT for this purpose. At this time, we are developing SPF colonies at the Frederick Cancer Research Center. Animals from those colonies will be used to supply the large-scale Bioassay Program at the Frederick Cancer Research Center and hopefully to other bioassay contractors. Stock animals for the Frederick Cancer Research Center breeding colonies were supplied by the NIH Veterinary Resources Branch (DRS).

Prior to the initiation of the long-term experiments, it is usually necessary to determine the acute toxicity and maximum tolerated dose levels to properly select the chronic treatment levels. Careful attention is given to experimental design and methods used to assure adequacy of sample size, controls, dose levels, animal care, pathology, and hazard control. Once the chronic studies are initiated, data on experimental design, clinical and survival observations, and pathology results are provided to the Carcinogenesis Bioassay Data System (CBDS). Data analysis of the results can be conducted at any time during the study, but usually at their conclusion. Finally, the results are evaluated by a panel to consider

the significance of the results and to recommend further action. Reports of the testing results are published in the open literature and in progress reports submitted by the individual contractors.

The Carcinogenesis Bioassay Data System (CBDS) designed by the Program and Data Analysis Unit in collaboration with the NIH Division of Computer Research and Technology is used to collect, monitor, and store experimental data and results from bioassay operations. It is designed for the complete or selective recall of bioassay data. The system is now in full operation with new bioassay experiments being entered as they are initiated. Data from about 20 bioassay contractors are now on file with several other contractors preparing data for entry into the system. During the coming year the staff will expand the system to permit the analysis of data using those methods best suited to the interpretation of carcinogenesis data. In addition, the system contains chemical and bioassay contract information sub-systems which list data on material presently under test or projected for study, date initiated, etc. The routine data entry, SNOP coding, etc. are provided under contract. (Contractor: Wolf Research and Development Corporation).

A close working relationship has been developed with Dr. H. Kraybill, Scientific Coordinator for Environmental Carcinogenesis. Dr. Kraybill, responsible for liaison with other government agencies, is kept informed of chemicals that are being tested and the results as they are known. He also provides suggestions of chemicals to be tested as a result of interest expressed by other agencies.

To effectively manage and monitor the Bioassay Program requires expertise in and coordination of various disciplines including animal care, chemistry, toxicology, biometry and data analysis, pathology, information handling, and fiscal operations. While the Program and Data Analysis Unit and cooperating groups in Carcinogenesis possess several of these disciplines, the need for more staff is urgent in view of the projected further expansion of bioassay and carcinogenesis activities. We had planned to add several additional personnel to the staff to meet current and anticipated needs. It does not appear at this time that these personnel requirements can be met due to the reduction in staffing level for the Carcinogenesis Program. To partially offset this setback, it is hoped that certain functions can be accomplished by the contract mechanism. We will attempt to meet many of the expanded requirements by use of pathology, information and bioassay support contracts. It should be apparent that more personnel are needed to do an effective job in managing and coordinating this major and important research program. While the use of support contracts may help considerably, additional staff will still be desperately needed to adequately perform the tasks required and monitor the overall program.

Many of the achievements reported below were accomplished by the Bioassay Segment prior to the reorganization of the segments. Dr. John H. Weisburger directed the segment until his retirement in November, 1972. Dr. Elizabeth K. Weisburger became the Acting Director from that time until the reorganization in March. She now serves as Director of the Carcinogen Metabolism and Toxicology Segment.

During the past year continuing efforts have been made to outline protocols for the bioassay of environmental chemicals so that results in one laboratory can be compared with those in others. The Bioassay Segment included 21 contracts in which a total of 445 compounds were under test. These included 142 chemical or industrial intermediates, 49 environmental chemicals, 75 food additives, 61 pesticides, 8 plant extracts and 100 drugs. A total of some 48,000 rats and 56,000 mice plus 2000 other animals - Guinea Pigs, Hamsters, and Rabbits - were employed for these tests. These contracts were designed to test various industrial and environmental chemicals for carcinogenicity or co-carcinogenic effects, to study the possible carcinogenicity of fungal and plant products, and the effect of diet on chemical carcinogenesis. In addition, projects are now underway at Papanicolaou Cancer Research Institute and Medical College of Georgia to study the response of different strains of rats and mice to chemical carcinogens of varying structures and organotropic effects. It is hoped that these projects will lead to knowledge of more sensitive strains which still have a low spontaneous tumor rate.

New contracts were awarded to Mason Research Institute, Litton-Bionetics, Dow Chemical Company, Medical College of Georgia and Papanicolaou Cancer Research Institute. A large-scale bioassay program, Task 8, has been initiated at the Frederick Cancer Research Center in which sixty chemicals will be tested during the first two years. Under Task 9, Preparation and Characterization of Carcinogens, a laboratory with state-of-the-art analytical capabilities has been set up at FCRC. Among the functions of this laboratory is the provision of well-characterized chemicals for the large-scale bioassay program. This includes purification of chemicals, characterization of inpurities in the compounds and identification of decomposition products. The capability for this synthesis of highly pure chemicals for research exists. In addition to the analyses required for the Bioassay Program, studies on the metabolism of Dimethylnitrosamine have been initiated in collaboration with the Carcinogenesis staff.

Some major findings in the contract program during the past year include the following:

Bio-research Consultants has reached the terminal phase of a study dealing mainly with a number of monocyclic aromatic amines, important industrial intermediates in dyestuff components, which had never been tested thoroughly. Several of them are carcinogenic in mice or rats including the epoxy 4,4'Methylene-bis-(2-chloroaniline), which is a resin hardener, Toluene-2,4-Diamine, a hair dye component and an important intermediate for Polyurethan foam production.

At Litton-Bionetics one project has now been completed but two others are continuing. Several industrial chemicals were found to be carcinogenic, but other environmentally useful materials, including Saccharin, were not carcinogenic under the test conditions.

At Hazelton Laboratories studies are underway on the chronic toxicity and possible carcinogenic action of several pesticides and of various halogenated compounds. Preliminary data indicate that some of the pesticides and some of the rather simple halogenated compounds may be hazardous.

Illinois Institute of Technology Research Institute is studying the possible carcinogenic or co-carcinogenic effects of the chlorinated Dibenzodioxins. Some of these materials are extremely toxic. Thus far, although none of them seem to be complete carcinogens, they do seem to have a co-carcinogenic effect. Of interest is the finding that Dioxane, a useful industrial solvent which has previously been reported to induce liver and nasal tumors in rats given this material in the drinking water, was an effective promoter when applied with a known carcinogen in skin painting studies.

Plant materials from medicinal plants suspected of being responsible for the increased incidence of esophageal cancer in Curacao are being investigated by a group at Howard University Colleges of Medicine and Pharmacy. The total aqueous extracts and the tannin containing fractions from three plant species appear to be carcinogenic, especially that from Acacia villosa.

At Mason Research Institute industrial intermediates, mostly dyestuff chemicals, are being tested for long-term toxicity. In a previous short-term study we have found that in Sprague-Dawley rats, 2-amino-anthraquinone induced kidney cysts which persisted for nine months after the last dose of the chemical. This effect is being investigated further at Mason Research Institute. It appears that although this compound is given orally, it precipitates in the kidney tubules and causes changes in the blood chemistry of the animals.

The testing of 37 clinically used chemotherapeutic agents and related chemicals at the Southern Research Institute indicates that 25 of these compounds have carcinogenic properties. These results are being further evaluated to determine their significance.

CONTRACT NARRATIVES

BIOASSAY OPERATIONS SEGMENT

July 1, 1972 through June 30, 1973

BIO-RESEARCH CONSULTANTS, INC. (NIH-NCI-68-1311)

Title: Determination of the Carcinogenicity of Several Chemicals Present in Man's Environment

Contractor's Project Director: Dr. Freddy Homburger

Project Officers (NCI): Dr. Jerrold Ward
Dr. Elizabeth K. Weisburger

Objectives: The long-term effect of chronic administration of a number of chemicals to mice and rats is being evaluated.

Major Findings: (1) Aromatic amines - o-Toluidine HCl (8) and o-phenylene diamine 2 HCl (25) were strongly carcinogenic in both rats and mice. m-Toluidine HCl (9) was negative. m-Phenylene diamine 2 HCl (24) was equivocal in rats, negative in mice. Tetrafluoro-m-phenylene diamine 2 HCl (23) was negative in rats but produced hepatomas in mice. p-Toluidine HCl (10) was equivocal in rats and produced hepatomas in mice. 2,4-Diaminotoluene (5) was carcinogenic in both rats and mice. 4-Chloro-o-toluidine (11) produced fewer hepatomas in rats but increased vascular tumors in mice. 2,4,5-Trimethylaniline (4) produced a few hepatomas in rats, numerous hepatomas in mice. 2,5-Xylidine HCl (7) produced a few hepatomas in rats but fewer hepatomas in mice than did 2,4,5-trimethylaniline. 2,4-Xylidine HCl (6) was negative in mice, equivocal in rats. 2,4,6-Trimethylaniline HCl (3) was strongly carcinogenic in both rats and mice and produced cirrhotic livers in rats. 2,4,6-Trichloroaniline (22) was negative in rats but increased hepatomas and vascular tumors in mice.

(2) Benzidine derivative - 3,3',4,4'-Tetraaminodiphenyl 4 HCl (12) produced hepatomas in rats and female mice. Tumors were not significantly increased in male mice.

(3) Diphenyl methane and diphenyl ether derivatives - 4,4'-Methylene-bis(2-chloroaniline) HCl (13) and 4-chloro-4'-aminodiphenyl ether (18) were both carcinogenic in both rats and mice.

(4) Aminostilbene derivative - 2,5-Dimethoxy-4'-aminostilbene (21) was strongly carcinogenic in rats, weakly carcinogenic in mice.

(5) Nitrobenzene derivatives - 1-Chloro-2-nitrobenzene (16) was equivocal in rats but produced numerous hepatomas in mice. 1-Chloro-4-nitrobenzene (15) was negative in rats, equivocal in male mice, and probably carcinogenic in female mice. 1-Chloro-2,4-dinitrobenzene (17) was negative in rats, equivocal in mice.

(6) Benzoguanamine (14) was not carcinogenic in either rats or mice.

(7) Dicyclopentadiene dioxide (20) was equivocal in rats and male mice, negative in female mice.

(8) Sweeteners - Assays on sodium cyclamate (Na cyclohexyl sulfamate - 1,2) and saccharin (benzosulfimide - 26, 27) were run in duplicate. A few transitional cell carcinomas of the bladder were found in rats receiving both compounds. These were low grade noninvasive lesions which were not dose-related. (Histological diagnoses have been confirmed by Dr. G.H. Friedell.) Ova were found in the sections of some bladders. A few rats receiving other compounds had granulomatous lesions of the lung characteristic of infection with Trichosomoides crassicauda. Papillary neoplasms of the bladder were found in several control rats. In view of this evidence suggesting the presence of bladder parasites, no valid conclusions as to the effects of these sweeteners on rat bladder epithelium can be drawn. Neither sweetener was carcinogenic in any other organ system in the rats. Sodium cyclamate was negative in both male and female mice. One group of male mice (27) fed saccharin exhibited an apparent increase in vascular tumors which was not confirmed by the duplicate assay (26).

Significance to Biomedical Research and the Program of the Institute: One primary effort in the prevention of cancer consists in determining potential carcinogenic hazards in the environment. The aim of this program is to gather such data for a number of widely used materials. Additionally, the test procedures and the results obtained will be examined critically with the aim of improving the methodology by which such assays are conducted. Information on the teratogenic properties of these same chemicals will likewise assist the areas of preventive medicine, and simultaneously yield data of significance with respect to the problem whether the carcinogenic and teratogenic properties are related.

Proposed Course: Autopsies on all animals, processing of tissues and histological evaluation have been completed. Manuscripts on the various classes of compounds studies are now in preparation.

Date Contract Initiated: June 28, 1968

Current Annual Level: $97,788

CINCINNATI, UNIVERSITY OF (NIH-NCI-73-3202)

Title: Study of the Carcinogenic and Co-Carcinogenic Properties of Industrial Chemicals

Contractor's Project Director: Dr. E. Bingham Mattheis

Project Officers (NCI): Dr. Henry Hennings
Dr. Norbert P. Page

Objectives: The purpose of this project is to test for skin carcinogenicity and co-carcinogenicity of selected industrial chemicals to which there is significant human exposure.

Major Findings: Due to the recent epidemiological investigation that has demonstrated an increased risk of developing lung cancer among coke oven workers, an attempt is being made to determine the factors contributing to the carcinogenic potency of the effluent from coke ovens. Experiments at this laboratory demonstrate that benzo[a]pyrene is present in a high concentration in many (but not all) samples of the coal tar that is a by-product from a coke oven. However, other compounds, probably heterocyclic nitrogen compounds, also contribute to the carcinogenic potency of coal tar. Co-carcinogenic compounds are also suspected as contributing to the total potency. Samples of coke oven effluent have been collected. Certain fractions have been prepared and are being tested for carcinogenic potency by topical application to skin. Hamsters are being exposed by inhalation to certain combinations of these tars.

It has been necessary to determine the dose-response curves for the production of mouse skin tumors by ultraviolet light of wavelengths 254 and 290-320 nm. At high levels of carcinogenic wavelengths in sunlight (290-320 nm), the carcinogenic potency at this wavelength can be affected by treatment with chemicals used as optical brighteners in one of the following ways: 1) inhibition, 2) Enhancement, or 3) no affect. The differences are not significant at this time and must await further results of experiments that are still in progress. At 350 nm, a non-carcinogenic wavelength, it was observed that n-dodecane induced tumors in C3H mice. Experiments are underway to determine whether a series of long-chain paraffins enhance the carcinogenic potency of ultraviolet light at 254 nm and 290-320 nm.

Significance to Biomedical Research and the Program of the Institute: The chemicals under investigation in this contract are found in the environment (home or occupational) of human beings. It is essential to know whether these materials are carcinogenic or markedly enhance (or inhibit) the potency of a "natural carcinogen", ultraviolet light. A most important function of this contract is to determine how combined exposure to various agents may affect their carcinogenicity. It has been demonstrated, for example, that chemicals which are not carcinogenic when tested by themselves may markedly enhance the carcinogenicity of other materials. This observation has very important implications for programs designed to restrict human exposure to carcinogens.

Proposed Course: The experiments described above will be completed and other experiments on the carcinogenic and co-carcinogenic potency of industrial chemicals will be started.

Date Contract Initiated: September 11, 1963

Current Annual Level: $79,199

DOW CHEMICAL COMPANY (NIH-NCI-72-3254)

Title: Carcinogenesis Bioassay of Environmental Chemicals

Contractor's Project Director: Dr. J. L. Emerson

Project Officer (NCI): Dr. Thomas P. Cameron
Dr. Jerrold Ward

Objectives: To study the chronic toxicity and possible carcinogenicity in rats and mice of a number of chemicals used in commerce and industry.

Major Findings: The acute oral median lethal dose and subacute 6 weeks dietary studies have been completed in rats and mice for 2-amino-5-nitrothiazole, 1,3-dichloro-5,5-dimethylhydantoin, 3-nitropropionic acid, proflavine dihydrochloride, and resorcine blue. In addition studies on scopolamine hydrobromide have been completed in mice. These acute and subacute studies will also be conducted on N,N'-dicyclohexylthiourea as soon as the chemical is available. Resorcine blue has been dropped from further testing and has been replaced with N,N'-dicyclohexylthiourea. Due to the cost of scopolamine hydrobromide it was not considered economically feasible to do chronic studies at this time.

From the results of the subacute dietary studies, the maximum tolerated dose (MTD) has been established for each chemical. The MTD and 1/2 MTD are being used in the chronic rat studies of 110 weeks duration and mouse studies of 90 weeks duration. Due to a lack of stability of 1,3-dichloro-5,5-dimethylhydantoin and 3-nitropropionic acid in the diet, these chemicals are being administered 5 days per week via gavage. N-2-fluorenylacetamide is being used as a positive control in the chronic rat and mouse studies.

Chronic rat studies have been initiated using 2-amino-5-nitrothiazole, 1,3-dichloro-5,5-dimethylhydantoin, 3-nitropropionic acid and N-2-fluorenylacetamide. Chronic mouse studies with these same chemicals were initiated in March.

Significance to Biomedical Research and the Program of the Institute: A primary effort in the prevention of cancer consists of determining potential carcinogenic hazards in the environment so that human use and exposure may be minimized. The aim of this program is to gather such data for a number of widely used materials.

Proposed Course: The acute and subacute studies on N,N'-dicyclohexylthiourea were completed in May. Proflavine dihydrochloride and N,N'-dicyclohexylthiourea were started in the chronic studies in June 1973. Therefore, all acute and subacute tests have been completed and all chemicals are currently in the stage of chronic testing. All animals will be examined at the time of death or at the termination of the study for evidence of neoplasia and chronic toxicity. This is the first year of the contract which is projected to continue through Fiscal Year 1975.

Date Contract Initiated: June 12, 1972

Current Annual Level: $88,776

FREDERICK CANCER RESEARCH CENTER (NIH-NCI-72-3294)

Task Order #8

Contract narrative is reported under the Office of the Associate Scientific Director for Carcinogenesis

GEORGIA, MEDICAL COLLEGE OF (NIH-NCI-72-3256)

Title: Lifetime Carcinogenic Bioassays on Small Rodents

Contractor's Project Director: Dr. Yasuyuki Akamatsu

Project Officers(NCI): Dr. Thomas P. Cameron
Dr. James M. Sontag

Objectives: To determine the suitability of several strains of mice for possible use in bioassay of chemicals for carcinogenicity. This study is being done by treating the different strains with a variety of chemical carcinogens, which affect different organs, and comparing their susceptibilities.

Major Findings: At this time subacute toxicity tests of 23 chemicals are in progress on the following strains of mice: C57BL/6, BALB/c, C3H/He, DBA/2, CBA and the B6C3F1 hybrid. All the chemicals under test have been shown to be carcinogenic under previous conditions of study.

Significance to Biomedical Research and the Program of the Institute: As part of the program of the Bioassay Operations Segment, many compounds are now being tested in various pure or hybrid strains of mice. Most of the effort is now being concentrated on the (C57BL/6 x C3H/Anf)F_1 hybrid strain. However, if a more sensitive strain could be found, particularly to certain classes of compounds, and one with a low spontaneous cancer incidence, this would facilitate the carcinogen screening program of the Bioassay Operations Segment.

Proposed Course: Subacute toxicity tests will be completed on the 23 known chemical carcinogens being used in this study. After which chronic toxicity studies will be initiated using each of the chemicals at dose levels determined to be maximally tolerated (MTD) and 1/2MTD. The responses of each of the different strains will be carefully evaluated.

Date Contract Initiated: June 29, 1972

Current Annual Level: $99,924

GULF-SOUTH RESEARCH INSTITUTE (NIH-NCI-70-2210)

Title: Carcinogenesis Bioassay of Pesticides and Other Environmental Chemicals

Contractor's Project Director: Dr. Harry Burchfield

Project Officers(NCI): Dr. Herman Kraybill
Dr. Thomas Cameron

Objectives: The objectives of this project are to test a number of pesticides for carcinogenicity in mice and rats by administration in the diet.

Major Findings: Rangefinding studies on the toxicity of 20 pesticides and three positive controls have been completed using Osborne-Mendel random bred rats and C57Bl x C3H inbred mice. All of the compounds are being tested for carcinogenicity at the highest tolerated dose and at one half of this value using a total of 5000 rats and 5000 mice. Pesticides will be withheld from the diet at the end of 80 weeks, and the mice will be sacrificed at 90 weeks and the rats at 110 weeks for gross and histopathology. Sufficient photodieldrin for the entire program has been synthesized. All new batches of pesticides received for toxicology studies have been analyzed chemically to establish identity and the presence of impurities. Methods have been developed and applied to the analysis of endogenous pesticides and PCB's in feed as well as the amounts of pesticides added to feed.

Significance to Biomedical Research and the Program of the Institute: Many of the pesticides under study have widespread usage resulting in extensive human exposure to them. The chemicals selected for testing have either been tested inadequately for carcinogenicity in the past, or the results of such tests have been equivocal. These extensive experiments are designed to yield definitive data and help resolve these issues.

Proposed Course: All chemicals have been analyzed and are presently being evaluated for carcinogenicity to rats and mice. Sacrifice of the animals for histopathologic examination will commence during the summer of 1973.

Date Contract Initiated: June 15, 1970

Current Annual Level: $499,958

HAZLETON LABORATORIES (NIH-NCI-72-3278)

Title: Carcinogenesis Bioassay of Environmental Chemicals

Contractor's Project Director: Dr. William A. Olson

Project Officers(NCI): Dr. Norbert P. Page
Dr. Jerrold Ward

Objectives: To determine the carcinogenicity of common volatile halogenated chemicals administered chronically to rats and mice at the maximum tolerated dosage.

Major Findings: The chemical purity and preliminary dosage determinations for the 18 test and control chemicals have been completed. Chronic dosing tests with all compounds in both Osborne-Mendel rats, and C57BLXC3H mice at maximum tolerated dose and 1/2 maximum tolerated dose have been initiated. The compounds are given via stomach tube, five times per week. Two compounds, dibromochloropropane and ethylene dibromide have been determined to be highly active carcinogens in rats and gross observations indicate them to also be carcinogens in mice. Histopathological examination of rats found to be moribund, having excessively large visible tumors, or found dead in the female animals given 30 mg/kg dibromochloropropane for periods up to 38 weeks resulted in the finding of mammary adenocarcinoma in eight out of 14 animals.

Histopathological examination of tumors from 39 male and female animals given 40 mg/kg continuously or 80 mg/kg ethylene dibromide for the first 14 weeks, no compound for 16 weeks, then resumed treatment at 40 mg/kg resulted in induction of squamous cell carcinomas which apparently started in the esophageal portion of the cardiac stomach, then invaded the stomach wall and metastasized peritoneal surfaces.

Tumors were observed in sacrificed or dead animals as early as the tenth week of exposure; however, most of the animals on which the diagnosis is based were on the study for forty weeks.

Significance to Biomedical Research and the Program of the Institute: The chemicals being evaluated under this contract are widely used industrial solvents, economic poisons, and in some cases a component of household products. The two compounds which have been found to be carcinogenic under the conditions of this experiment are used in fumigating buildings, foods, and other items which are later used by man. Tolerances for residues of these chemicals in various foods have been previously established at levels up to 400 ppm as bromine in specific foods. These new findings will provide a basis for establishing precautionary handling and use patterns for the chemicals, and for consideration by the appropriate regulatory agencies for limiting public exposure.

Information regarding other chemicals being evaluated will also be valuable in determining safe handling and usage, whether the compounds are shown to be carcinogenic or not.

Proposed Course: If possible, the test chemicals will be given to both species for a total period of 78 weeks. The groups of mice will be held for an additional 12 weeks and the rats an additional 32 weeks. Histopathologic diagnosis of tumors and other toxicological effects will be made, and the results reported.

Date Contract Initiated: May 1, 1971

Current Annual Level: $429,393

HAZLETON LABORATORIES (NIH-NCI-73-3225)

Title: Carcinogenesis Bioassay of Pesticides and Other Environmental Chemicals

Contractor's Project Director: Dr. William A. Olson

Project Officers(NCI): Dr. Herman F. Kraybill
Dr. Elizabeth K. Weisburger

Objectives: To determine the carcinogenicity of selected chemicals administered in the diets of rats and mice at the maximum tolerated dosage.

Major Findings: The chemical purity and preliminary dosage determinations for the 17 pesticide and control chemicals have been completed. Chronic feeding tests are underway with all compounds in both Osborne-Mendel rats and C57BL X C3H Mice, at maximum tolerated dose (dietary ppm basis) and 1/2 of the maximum tolerated dose. One positive control compound, AAF, was demonstrated to be a potent carcinogen in the rat tests. All other tests are continuing. The occurrence of externally palpable tumors, and grossly observable tumors of animals prematurely sacrificed or found dead is greater in groups of animals fed certain test chemicals, however, the histopathological diagnosis is yet incomplete, and conclusions cannot be made at this time.

Significance to Biomedical Research and the Program of the Institute: The chemicals being evaluated under this contract are primarily economic poisons, mostly of the chlorinated hydrocarbon type, which have been shown to be persistent in the environment, and frequently found as contaminants in food. In addition to the dietary exposure of the entire population to ppm quantities of these chemicals, some industrial workers, and many agricultural workers are regularly exposed to much greater quantities of these chemicals. These studies are an attempt to determine if the compounds are likely to be involved in the causation of human cancer; and if found to be carcinogenic, the users of the compounds and appropriate regulatory agencies will have a basis for limiting exposures.

Proposed Course: The test chemicals will be given to both species of animals for a total period of 78 weeks. The groups of mice will be held an additional 12 weeks and the rats 32 weeks, unless it is necessary to terminate the tests early because of morbidity or mortality. Histopathological diagnosis of tumors and other toxicological effects will be made, and the results reported.

Date Contract Initiated: December 1, 1970

Current Annual Level: $413,392

HOWARD UNIVERSITY (NIH-NCI-71-2167)

Title: Chemical and Biological Investigation of Potential Carcinogens from Plants

Contractor's Project Director: Dr. Govind J. Kapadia

Project Officer (NCI): Dr. Elizabeth Weisburger

Objectives: (1) To prepare purified fractions from certain herbs that are used habitually in Curacao and South Carolina and isolate and characterize crystalline compounds from these fractions, (2) to test each fraction and compound for carcinogenicity in experimental animals and identify substances responsible for the carcinogenic effects, (3) to extend such chemical and bioassay studies to plant species related to the above as well as to other plants that are suspected of possessing carcinogenic activity,and (4) to elucidate the chemical structure of any carcinogenic substance that may be isolated.

Major Findings: There is a high frequency of esophageal cancer in the natives of Curacao and the Gullahs' of South Carolina that is possibly related to the ingestion of unknown carcinogens of plant origin. Certain herbs used as folk remedies are especially suspect. In earlier studies, Dr. O'Gara, NCI, observed an elevated tumor incidence in rats on administration of crude plant material as well as concentrated extracts of Krameria, Acacia, and Annona, some of which were prepared by Dr. Kapadia. The work has been extended to 46 plant samples (belonging to 39 genera and 43 species, procured from Curacao and South Carolina) that were received. Thirty-four plant extracts (representing total water-extractable material, tannin-containing and tannin-free fractions from 12 plant species) have been prepared. Of the ten extracts and fractions that have been biologically evaluated thus far, the total aqueous extracts and tannin-containing fractions derived from Krameria ixina, Krameria triandra and Acacia villosa appeared to be carcinogenic, the latter being the most potent. Histologically, the induced tumors showed characteristics of malignant fibrous histiocytoma, that manifested low grade malignancy, but usually multi-nodular growth pattern. Further chemical studies and biological testing for carcinogenic activity are underway with other suspect plants.

Significance to Biomedical Research and the Program of the Institute: This project is directly related to the major objective of the Carcinogenesis Program, i.e., the identification of unknown carcinogens in natural plant materials. This project should serve as the ultimate check to ascertain the carcinogenicity of materials implicated by epidemiological studies on the island of Curacao. Information gained from this study could conceivably lead to definitive studies that should be conducted in other high incidence areas to identify the carcinogens involved.

Proposed Course: This contract is complementary to the University of Miami, Contract Number NIH-NCI-71-2274 which provides for the collection of the plants in Curacao and South Carolina. It is estimated that this contract will extend for two additional years.

Date Contract Initiated: June 25, 1971

Current Annual Level: $91,332

IIT RESEARCH INSTITUTE (NIH-NCI-71-2338)

Title: Carcinogenesis Bioassay of Chlorinated Dibenzodioxins and Related Chemicals

Contractor's Project Director: Dr. Maurice E. King

Project Officers(NCI): Dr. Herman Kraybill
Dr. Richard Bates

Objectives: To determine the carcinogenicity and co-carcinogenicity of chlorinated dibenzodioxins and related compounds by application to the skin of mice and by oral administration to mice and rats.

Major Findings: Kilogram quantities of the relatively nontoxic dioxins (unsubstituted, dichloro- and octachloro-) were prepared, analyzed and made available for animal bioassay. Approximately 10 grams of the highly toxic 2,3,7,8-tetrachlorodibenzo-p-dioxin has been prepared and will be used in toxicity and carcinogenicity trials as soon as chemical analysis is complete. Initial studies of preparative routes for the 2,3,7-trichloro dioxin synthesis have been completed and preparation of the quantity required for bioassay has begun. Synthesis of the hexachloro derivative will shortly be investigated to see whether preparation of the required amount is practical. Initial studies of possible synthetic routes for chlorinated dibenzofurans have begun.

Feeding studies with the unsubstituted dibenzodioxin and the 2,7-dichloro and octachloro derivatives are underway by admixture of the test compound(s) to the diet. The octachloro test results illustrate the necessity for being concerned with low levels of contamination with the more toxic members of this series. Most rats and mice fed octachlorodioxin at levels of 1.0% and 0.5% died early in the course of the study. Analysis of the octachlorodioxin preparation revealed the presence of hexachlorodioxin at a concentration of less than 0.1% which may be responsible for the deaths observed. On the other hand, previous estimates of low toxicity of octachlorodioxin were based on acute studies. In the present study, toxic effects were only seen after about two months of exposure. Thus, it is also possible that there may be a delayed toxicity from octachlorodioxin itself. Feeding studies with the octachloro compound have been reinitiated at lower doses using a batch with no detectable hexachlorodioxin.

These same compounds are also being assayed in skin carcinogenesis trials which have been in progress for more than one year. Compounds are being tested as complete carcinogens and for promoting ability following DMBA initiation. Of considerable interest is the finding that dioxane is an effective promoter in skin painting studies, producing effects only slightly less in degree than those of the positive control compound, croton oil.

Significance to Biomedical Research and the Program of the Institute: Some chlorinated dibenzodioxins are extraordinarily toxic contaminants in certain pesticides and other environmental chemicals. In addition to their extreme toxicity, they are highly stable under environmental conditions and tend to accumulate in the food chain. They have been tested for acute toxicity and teratogenicity but have not been examined for carcinogenicity. This program is designed to accomplish the latter and is part of the NCI program of testing chemicals to which man is exposed for carcinogenicity.

Proposed Course: As sufficiently pure samples of the other chlorinated dibenzodioxins selected for study become available, they will be entered into chronic tests. Preliminary investigations on the feasibility of testing the related chlorinated dibenzofurans will be made. Proposed long term experiments should require three more years for completion.

Date Contract Initiated: June 25, 1971

Current Annual Level: $319,178

LITTON BIONETICS, INC. (NIH-NCI-69-2085)

Title: Carcinogenicity of Chemicals Present in Man's Environment

Contractor's Project Director: Dr. Borge M. Ulland

Project Officer (NCI): Dr. E. Weisburger

Objectives: To study the chronic toxicity and possible carcinogenicity in rats of a number of chemicals used in commerce and industry. Also, to investigate the possible teratogenic effects of some of these environmental agents.

Major Findings: As reported last year in a preliminary way, two chemicals proposed for industrial development, propylene imine and propane sultone, are highly active carcinogens leading to tumors in a number of tissues of rats, but most interestingly they produced gliomas in male and female rats and mammary cancer in female rats. Ethylene thiourea, a pesticide contaminant and rubber chemical gives a high yield of thyroid cancer. Preliminary information, in some cases based on incomplete data, suggest that the following chemicals were not carcinogenic: bis-(2-chloroethyl)-ether, bis-(2-hydroxyethyl)-dithiocarbamic acid, avadex, all of which were found active in an earlier study at Litton Bionetics, Inc., in mice. Also inactive were sodium azide, ethylene carbonate, sodium sulfhydrate, thiosemicarbazide, dithiooxamide, semicarbazide hydrochloride, glycerol monochlorohydrin, mirex, saccharin, and vinylene carbonate.

The positive control N-2-fluorenylacetamide led to the expected number of types of tumors in male and female Charles River CD rats employed in this study. The antioxidant butylated hydroxytoluene (BHT), but not the antioxidant diphenyl-p-phenylenediamine, reduced tumor formation in the liver with the positive control compound N-2-fluorenylacetamide and its N-hydroxy

metabolite. Other combinations involving butylated hydroxytoluene and diphenyl-p-phenylenediamine with diethylnitrosamine and diphenyl-p-phenylenediamine with propane sultone failed to prohibit tumor growth at various sites.

Even though propylene imine and propane sultone were highly active carcinogens when administered chronically to rats, they failed to induce cancer transplacentally. When pregnant female rats of the same strain were exposed in the latter part of their pregnancy, the offspring kept lifetime had no excess cancer over controls. These two active carcinogens likewise exhibit little if any evidence of teratogenicity in rats. Ethylene thiourea, however, when fed in the diet during days 9 through 19 of pregnancy induced teratogenic effects.

Significance to Biomedical Research and the Program of the Institute: One primary effort in the prevention of cancer consists in determining potential carcinogenic hazards in the environment. The aim of this program is to gather such data for a number of widely used materials. Additionally, the test procedures and the results obtained will be examined critically with the aim of improving the methodology by which such assays are conducted. Information on the teratogenic properties of these same chemicals will likewise assist the areas of preventive medicine, and simultaneously yield data of significance with respect to the problem whether the carcinogenic and teratogenic properties are related.

Proposed Course: Histologic evaluation and data compilation has been completed on all groups. Statistical analyses will be performed at the NCI.

Date Contract Initiated: June 28, 1968

Current Annual Level: $60,288

LITTON BIONETICS, INC. (NIH-NCI-71-2146)

Title: Carcinogenesis Bioassay of Environmental Chemicals

Contractor's Project Director: Dr. F.M. Garner

Project Officers(NCI): Dr. Norbert P. Page
Dr. Elizabeth K. Weisburger

Objectives: To assess in rats and mice the carcinogenicity and chronic toxicity of selected chemicals present in man's environment. Secondary objectives may deal with the pathogenesis of cancer induction.

Major Findings: Investigations relating to the determination of the maximal tolerated dosages of assigned chemicals in rats and mice are complete in all but three compounds. These compounds are presently being obtained in bulk quantities.

Chronic studies have been initiated for fifteen assigned chemicals and the two positive controls in rats and for sixteen chemicals and both positive controls in mice.

Significance to Biomedical Research and the Program of the Institute: By identifying potentially hazardous substances present in the environment, using the rat as a model, it may be possible to minimize man's exposure by prohibiting or restricting the use of these agents or modifying the method in which they are processed and handled.

Proposed Course: Chemicals are fed to rats and mice over their life span at a maximally tolerated dose and one-half that level. All animals will be examined at death or at the end of the study for evidence of neoplasia and chronic toxicity.

Date Contract Initiated: May 13, 1971

Current Annual Level: $240,479

LITTON BIONETICS, INC. (NIH-NCI-72-3252)

Title: Lifetime Carcinogenic Bioassays on Small Rodents

Contractor's Project Director: Dr. F. M. Garner

Project Officers(NCI): Dr. Norbert P. Page
Dr. Elizabeth K. Weisburger

Objectives: To study the chronic toxicity and possible carcinogenicity in rats and mice of a number of chemicals used in commerce and industry.

Major Findings: Investigations relating to the determination of the maximal tolerated dosages are underway for 13 of the assigned chemicals in both rats and mice.

Significance to Biomedical Research and the Program of the Institute: A primary effort in the prevention of cancer consists of determining potential carcinogenic hazards in the environment so that human use and exposure may be minimized. The aim of this program is to gather such data for a number of widely used materials.

Proposed Course: After preliminary determinations of the maximum tolerated doses of the suspect compounds over a 6-week period, the long term tests will be phased in so that the facilities and manpower can be utilized most efficiently.

Date Contract Initiated: June 29, 1972

Current Annual Level: $265,104

MASON RESEARCH INSTITUTE (NIH-NCI-71-2144)

Title: Carcinogenesis Bioassay of Environmental Chemicals

Contractor's Project Director: Dr. Marcus Mason

Project Officers(NCI): Dr. Thomas P. Cameron
Dr. Elizabeth K. Weisburger

Objectives: To study the chronic toxicity and possible carcinogenicity of environmental chemicals.

Major Findings: This program deals with the chronic toxicity and potential carcinogenesis of environmental chemicals in Fischer 344 rats, C57BL/6 and B6C3F$_1$ mice. Animals on Series 1 compounds have 10% of their number necropsied after 12 months of treatment. Necropsies have commenced and will continue throughout the year. It is too early to assess the probable carcinogenicity of Series 1 compounds but preliminary findings indicate that acetamide and hexanamide may be potential hepatocarcinogens. One Series II compound, 2-aminoanthraquinone has been found to initiate renal uremia in female rats with subsequent demise in 20 weeks. A pilot study of the effects of diethylnitrosamine (DEN) on highly inbred Chinese hamsters shows that 40 ppm DEN initiates papillomas of the esophagus and stomach, accompanied by liver cirrhosis.

Significance to Biomedical Research and the Program of the Institute: One of the functions of the Carcinogenesis Program is to evaluate the potential carcinogenic risk that is represented by chronic exposure to environmental chemicals. This contract will be significant in identifying and minimizing potentially hazardous substances to which man is exposed.

Proposed Course: In this study rats and mice will be fed chemicals at a maximally tolerated dose and one-half that over a two year period. The overall project is expected to continue until approximately 1974.

Date Contract Initiated: May 1, 1971

Current Annual Level: $329,949

MASON RESEARCH INSTITUTE (NIH-NCI-72-3255)

Title: Lifetime Carcinogenic Bioassays on Small Rodents

Contractor's Project Director: Dr. Marcus Mason

Project Officers(NCI): Dr. Thomas P. Cameron
Dr. Elizabeth K. Weisburger

Objectives: To study the chronic toxicity and possible carcinogenicity in rats and mice of a number of chemicals used in commerce and industry.

Major Findings: All chemicals received have been tested for purity and stability. Investigations relating to the determination of the maximum tolerated doses of assigned chemicals in Fischer 344 rats and $B6CF_1$ mice are under way or have been completed. Of the 14 compounds received 8 are in progress or completed in subacute studies. Six chronic studies have been initiated in rats and mice with corresponding diet, vehicle, and positive control groups. The remaining 8 compounds are on order or being synthesized.

Significance to Biomedical Research and the Program of the Institute: A primary effort in the prevention of cancer consists of determining potential carcinogenic hazards in the environment so that human use and exposure may be minimized. The aim of this program is to gather such data for a number of widely used materials.

Proposed Course: After preliminary determinations of the maximum tolerated doses of the eight remaining compounds over an 8 week period, long term chronic studies will be phased in and continue in effect for 18 months. Then 10% of all groups will be necropsied and preliminary observations will be made. After that animals will be held for six more months and necropsies and histopathological analyses will be undertaken.

Date Contract Initiated: June 29, 1972

Current Annual Level: $188,328

MIDWEST RESEARCH INSTITUTE (NIH-NCI-72-3270)

Title: Analytical Chemistry Resource

Contractor's Project Director: Dr. Evelyn Murrill

Project Officers(NCI): Dr. Marcia Litwack
Dr. Larry Keefer

Objectives: The objectives of this program are to purchase, store, and distribute bulk chemicals for use in bioassay studies and to analyze chemicals, chemical/feed mixtures and feeds for the Carcinogenesis Program.

Major Findings: During the first eight months of the contract, 72 requests have been received from the National Cancer Institute for procurement, distribution, storage, and analysis, and 23 requests for analysis only. Suppliers have been located for 70 chemicals and the chemicals have been purchased; two requests have been cancelled because of synthetic difficulties. Analysis has been completed on 33 chemical samples and 20 chemical/feed mixtures. Currently, 21 other samples are being analyzed.

Significance to Biomedical Research and the Program of the Institute: The literature of Carcinogenesis bioassay studies contains many examples of experiments which cannot be meaningfully interpreted because the test substances had not been adequately characterized chemically. When such an

experiment results in low tumor incidence, for example, the question will always remain as to whether the tumors observed might not have arisen from small concentrations of highly potent impurities, rather than from the allegedly weakly potent nominal ingredient. Other questions of a chemical nature which are central to the interpretation of bioassay data include such problems as decomposition of the chemical in feed or intubation mixtures and levels of mycotoxins or pesticides in food and bedding. For these reasons it is essential that the Carcinogenesis Program have available to it a facility capable of characterizing those materials entered into the testing program as well as determining the effectiveness of administration of these substances to the test animals.

Proposed Course: To continue the purchase, storage and distribution of compounds used in the bioassay program, and the analysis of bulk chemicals, chemical/feed mixtures and feed.

Date Contract Initiated: June 28, 1972

Current Annual Level: $384,050

NEBRASKA, UNIVERSITY OF (EPPLEY INSTITUTE FOR RESEARCH ON CANCER) (NIH-NCI-68-959)

Title: A Resource for Carcinogenesis Bioassays and Related Research

Contractor's Project Director: Dr. Philippe Shubik

Project Officer (NCI): Dr. Gio B. Gori

Contract narrative is reported under the Office of the Associate Scientific Director for Carcinogenesis

NEW YORK UNIVERSITY (NIH-NCI-71-2020)

Title: Alkylating Agents as Carcinogens and Anti-Carcinogens

Contractor's Project Director: Dr. Benjamin L. Van Duuren

Project Officer (NCI): Dr. Lionel Poirier

Objectives: A wide variety of alkylating agents are being introduced into the environment with the recent advances in chemical technology. Some of these compounds are encountered in manufacturing processes where they may be occupational hazards. Others become widely distributed in the environment. The purpose of this work is to determine the possible carcinogenic activity of some of these compounds in laboratory animals using several routes of exposure. The aims of the work are to determine the carcinogenicity of such alkylating agents, chemical structure-carcinogenicity relationships, and to

yield information that can lead to the reduction or elimination of human exposure to these agents.

Major Findings: A total of 25 alkylating agents have been tested or are currently on test for carcinogenic activity by one or more routes of exposures. The systems used were skin application in mice, as initiating agents on mouse skin, intraperitoneal injection in mice, and subcutaneous injection in mice and/or rats. Six of these compounds have shown significant carcinogenic activity. They are chloromethyl methyl ether, bis(chloromethyl)ether, epichlorhydrin, glycol sulfate, dimethylcarbamyl chloride and 2,3-dichloro-p-dioxane. Four of these compounds have been used in the chemical industry. The present work was the first to demonstrate the potential occupational hazard of these compounds and has resulted in the removal of some of these compounds from the work environment. In addition, the studies carried out with these compounds showed that a-chloro ethers are carcinogenic whereas their β-chloro isomers are usually not; also, bifunctional a-chlorinated ethers are more active than their monochloro analogs. The relationship between carcinogenicity and chemical reactivity with several nucleophilic agents was carried out as part of this work.

Significance to Biomedical Research and the Program of the Institute: The future accomplishments are expected to be in the realm of identifying the carcinogenic hazard to man of industrial chemicals, some of which find their way into the human environment, e.g., as pesticides.

Proposed Course: To continue to examine epoxides, halo-ethers and other alkylating agents particularly those of industrial and environmental importance in relation to cancer causation in man.

Date Contract Initiated: October 1, 1970

Current Annual Level: $92,084

PAPANICOLAOU CANCER RESEARCH INSTITUTE (NIH-NCI-72-3253)

Title: Lifetime Carcinogenic Bioassays on Small Rodents

Contractor's Project Director: Dr. Wilhelmina Dunning

Project Officer (NCI): Dr. James Sontag
Dr. Thomas Cameron

Objectives: To determine the suitability of several strains of rat for possible use in bioassay of chemicals for carcinogenicity. Each strain is being treated wtih a variety of chemical carcinogens, which affect different organs, and a comparison is being made of their susceptibility to each agent.

Major Findings: Comparative chronic toxicity tests are underway with the chemical N-hydroxy-N-2-fluorenyacetamide in six inbred strains of rats. These include Fischer line 344, A x C line 9935 Irish, August line 990, Marshall line 520, S x F line 40814, and Zimmerman line 61. Subacute toxicity

tests have been done with Safrole and beta-propiolactone and the chronic studies are being developed.

Significance to Biomedical Research and the Program of the Institute: As part of the Bioassay Operations Segment's program, many compounds are now being tested in various pure strains of rats. However, of the many strains available the most suitable ones for use in bioassay have never been experimentally determined. As an outcome of this study, more sensitive strains of rats than those presently used, particularly to certain classes of chemicals, may be found. This would facilitate the bioassay testing program.

Proposed Course: Various known carcinogens, of diverse chemical types and affecting different organs, will continue to be administered to the rat strains. Their responses will be carefully evaluated.

Date Contract Initiated: June 16, 1972

Current Annual Level: $133,704

SAN FRANCISCO, UNIVERSITY OF (NIH-NCI-73-3229)

Title: Studies of Carcinogenicity of Metallo-Organic Compounds

Contractor's Project Director: Dr. Arthur Furst

Project Officers(NCI): Dr. John Cooper
Dr. Richard Bates

Objectives: This contract was designed to test metals and their compounds especially metallo-organic derivatives for their potential carcinogenicity. Different routes of administration are being investigated in various species of rodents. Studies on excretion of these compounds are also being conducted; this information may shed light on the mechanisms by which these metals cause cancer.

Major Findings: The control Fischer-344 rat spontaneous tumor incidence has been evaluated in greater detail over the 900 day observation period. Nine groups from three different suppliers show a lower spontaneous leukemia and hepatoma rate than observed by other investigators.

Although titanocene was previously shown to be lymphogenic, no other titanium compound has yet to induce tumors. After 570 days, no noticable tumors were found in animals administered various derivatives of mercury. Neither gold, silver, zinc, or lead gave any fibrosarcomas.

Manganese, organic derivatives of which are under consideration as additives to aviation gasoline to eliminate smoke, produced a possible 42% incidence of tumors when administered as the acetylacetonate derivative intramuscularly. One third of these tumors are confirmed fibrosarcomas.

Significance to Biomedical Research and the Program of the Institute: The use of non-ferrous metals is continuously expanding in our industrial society. Metal refining industries are increasing in size and scope, as is the plating industry. Cadmium metal is being used in much greater quantities. Manganese may be used in aviation gasoline, thus altering the atmosphere around airports significantly. Select groups of workers are being exposed to these metals; also, the pollution of air and water by heavy metals, as described above, is greater than ever before. It is necessary to determine which of these metals pose a human cancer hazard.

Proposed Course: The contractor is a major resource to the Carcinogenesis Program as an expert on the industrial usage of metals and their carcinogenicity. Long-term studies, along the lines outlined, will continue on those metals to which humans receive significant exposure. Emphasis will be placed on metal-containing chemicals such as various pesticides and those metallic compounds used as preservatives or as additives. Further work and emphasis will be placed on determining the rate of excretion of metals from animals after oral administration.

Date Contract Initiated: June 3, 1964

Current Annual Level: $93,946

SOUTHERN RESEARCH INSTITUTE (NIH-NCI-73-3214)

Title: Carcinogenicity Studies of Chemotherapeutic Agents and Related Compounds

Contractor's Project Director: Dr. D.P. Griswold

Project Officers (NCI): Dr. Norbert Page
Dr. Elizabeth K. Weisburger

Objectives: To evaluate the potential carcinogenicity of chemotherapeutic agents and related chemicals in rats and mice following long-term exposure.

Major Findings: Histological evaluation of rats and mice exposed to 39 clinically used chemotherapeutic agents indicates a statistically significant increase ($p < 0.01$) in the number of tumors that occurred in those animals exposed to the following 25 compounds: 6-mercaptopurine, azathioprine, actinomycin D, mitomycin C, Daunomycin, streptozotocin, dibromomannitol, dibromodulcitol, DIC, BIC, chlorambucil, Melphalan, cyclophosphamide, uracil mustard, cytoxal alcohol, o-merphalan, CCNU, BCNU, 1-acetyl-2-picolinylhydrazine, procarbazine, n-nitrosodiethylamine, mixture B (prednisone, vincristine, and cyclophosphamide), mixture E (arabinosyl cytosine, and CCNU), mixture I, (melphalan and testosterone propionate) and mixture J (azathioprine and prednisone). An increased tumor incidence ($p < 0.05$) was also noted in the rats or mice injected with dichloromethotrexate, mixture C (vincristine, methotrexate, 6-mercaptopurine, and prednisone), and mixture G (actinomycin D, chlorambucil, and methotrexate). No statistically significant increase ($p > 0.05$) in the tumor incidence was

observed in the animals treated with Methotrexate, arabinosyl cystosine, 6-mercaptopurine riboside, prednisone, hydroxyurea, o,p-,DDD, vincristine, vinblastine, carbanilide, 4,4'-diacetyl-bis-(amidinohydrazone), dimethane sulfonate, mixture A (6-mercaptopurine riboside and 6-mercaptopurine) and mixture F (CCNU and prednisone).

Significance to Biomedical Research and the Program of the Institute: Results will provide clinicians with a basis for the selection of agents to be used in long-term chemotherapy in man.

Proposed Course: To continue assaying the carcinogenic potential of those agents to which man is currently exposed or may be exposed for extended periods to time. Those compounds showing an increased tumorigenesis will be further evaluated for carcinogenic potential at various lengths of exposure.

Date Contract Initiated: June 24, 1968

Current Annual Level: $325,218

WOLF RESEARCH AND DEVELOPMENT CORPORATION (NIH-NCI-72-3302)

Title: Support Contract to Provide Data Control Operations Services and Microfilming Services to the Carcinogenesis Bioassay Data System (CBDS)

Contractor's Project Director: Mr. Dalton Tidwell

Project Officers (NCI): Dr. John Cooper, II
Mr. Terence Kuch
Dr. Norbert P. Page

Objectives: This project links the CBDS bioassay contractors and the CBDS computer subsystem implemented through the Data Management Branch, Division of Computer Research and Technology. The contractor is to develop and document a system to accept, log, and process data collection forms submitted by the scientific research organizations participating in the CBDS. He is to provide a trained and qualified staff to operate this system with functions of: project management, forms control, editing and coding, computer software, maintenance and development of an automated and microfilmed dated base.

Major Findings: An operational system to control and process CBDS bioassay contractor data from initial data entry to updating the CBDS data base and creating microfiche records for all data processed has been developed and documented. It is projected that by the completion of the current contract period the Support System will have processed over 30,000 data collection forms for entry in the CBDS data base, and recorded them on microfiche. These records are available for analysis by bioassay contractor participants and NCI investigators.

Significance to Biomedical Research and the Program of the Institute: Data collected by bioassay contractor pathologists will be made available for examination by any qualified researcher.

Proposed Course: The system designed and imolemented by the contractor is functioning and proposed to continue in operation during the data collection phase of the Carcinogenesis Bioassay study. The contractor support provided to the Data Management Branch, Division of Computer Research and Technology, will be funded under this contract to maintain the integrity of this ongoing effort.

Date Contract Initiated: June 26, 1972

Current Annual Level: $100,126

SUMMARY REPORT

BIOLOGICAL MODELS SEGMENT

July 1, 1972 through June 30, 1973

The Biological Models Segment has the responsibility for developing experimental models for studying the etiology and pathogenesis of the major forms of human cancer and utilizing these models for developing methods to prevent human cancer. Separate segments exist for lung cancer and cancer of the colon and rectum, but the remaining types of human cancer fall within the area of responsibility of the Biological Models Segment.

Where appropriate experimental models exist, these are utilized in the Segment program. However, in the case of a number of types of cancer found commonly in humans, including pancreas and prostate, no adequate experimental models are available. Under these circumstances, Segment activities are designed to develop such models. Additional efforts involve attempts to study the process of carcinogenesis directly in human tissues through the development of adequate cell and organ culture techniques. An additional, potentially fruitful approach which is under consideration within the Segment is more extensive application of laboratory procedures to analyze biochemical, physiological, and morphologic variables among populations known to have different susceptibilities to the development to cancer.

The Segment now has 27 contracts in existence including 8 on the pancreas, 6 on the prostate, 2 on breast, 3 on perinatal carcinogenesis, 2 on development and analysis of approaches to assay environmental chemicals, and 6 of miscellaneous nature. When the Segment was established in the Fall of 1971, a group of consultants from academic and research institutions were selected. These individuals, representing a variety of disciplines, are interested in carcinogenesis in a variety of organ systems. These individuals have played a major role in the development of the program since that time. Only 8 of the contracts now in existence were in effect before the Segment was established. The choice of priorities in awarding contracts since that time have depended heavily on advice by Segment Advisors.

Areas of activity in which the Segment program can expand in the near future are dictated largely by the availability of qualified intramural staff who can develop and monitor the program. Thus, during the coming fiscal year, there should be some expansion of research on perinatal and breast carcinogenesis. During this past year, a small working group met to discuss the need for research on breast carcinogenesis. The group included both intramural and consultant members of the Biological Models Segment, a representative from the Breast Cancer Task Force, and several ad hoc consultants. Academic disciplines represented included endocrinology, epidemiology, pathology, biochemistry, and surgery. The meeting was highly productive in developing experimental approaches utilizing both laboratory animals and human material and in developing experiments to bridge the gap between the two. It was the consensus of the group that there were a large number of urgently needed projects which are not being supported by other groups and which fall within the responsibility of the Biological Models Segment. As resources become available, a high priority will be given to establishing this program. Initial studies will involve

comparison of morphology, and hormone responsiveness of normal and abnormal breast tissue, and of serum hormone levels between Oriental and American women in order to investigate factors which may cause the much greater incidence of breast cancer in the latter population. These will be complemented by related studies in animal systems which exhibit similar phenomena.

Most of the contracts within the Segment are less than one year old and are designed as long-term projects for which early results were not anticipated. However, significant observations have been made in a number of contracts including the following:

At Boston University hyperplasia in pancreatic duct epithelium within 6-9 weeks after surgical implantation of a diffusing chamber containing dimethylhydrazine has been induced. The DMH continuously bathes the cells at a constant rate dependent upon the fluid and its solutes in the chamber and the physical characteristics of the chamber (designed to permit long term exposure of the duct to the carcinogen). Although time only will tell if such lesions are preneoplastic, this exciting observation is worthy of note at this time.

A high incidence of tumors in rat kidney induced by N-2-(4'-fluorobiphenyl) acetamide has been demonstrated at the University of Maryland. The pertinent point to be made here is that there is a significant resemblance of these lesions to human clear cell carcinomas of the kidney. Much investigation is still necessary in evaluating the histogenesis and transplantation behavior of these tumors and observed preneoplastic lesions. Particular emphasis will be placed on searching human kidney tissue for early lesions resembling those observed in the rat.

A significant increase in the incorporation of tritiated methylnitrosourea into RNA and DNA of guinea pig pancreas undergoing regeneration has been shown at the University of Kansas. If increase binding of carcinogen is consistently associated with the regeneration process, this would have extreme import to our understanding of pancreatic adenocarcinoma induction, particularly if such incorporation can be correlated with a demonstrated increase in malignant disease incidence in these experimental animals.

Mason Research Institute has been unable to show any concentration of cadmium in prostate tissue or fluid. It does accumulate within renal cortex, pancreas, heart or lung of rats and dogs. If suggestive epidemiologic data that this metal is associated with increased carcinoma of the prostate in man is experimentally verifiable, it does not seem likely that simple preferential concentration is a necessary prerequisite. Other factors such as sensitivity to cadmium and key molecular events will need to be examined in greater detail.

Finally, the Alton Oschner Medical Foundation has now firmly established the synergism between X-rays and exogenous estrogens in the induction of mammary gland cancer. Significantly, the Special Virus Cancer Program of NCI has been unable to detect any viruses in these tumors. Furthermore, preliminary evidence suggests that progesterone may inhibit DES-induced mammary cancer and radiation synergism, and ovariectomy may modify this progesterone inhibition

CONTRACT NARRATIVES

BIOLOGICAL MODELS SEGMENT

July 1, 1972 through June 30, 1973

ALTON OCHSNER MEDICAL FOUNDATION (NIH-NCI-71-2131)

Title: Carcinogenesis by Radiation plus Estrogen

Contractor's Project Director: Dr. Albert Segaloff

Project Officers (NCI): Dr. Norbert Page
Dr. Jane Taylor

Objectives: (1) To investigate interaction or synergism of radiation and hormones in the induction of mammary tumors. (2) To develop a small animal model to simulate the human menstrual cycle.

Major Findings: The contractor has been able to show that a single dose of X-rays delivered to one mammary chain of A x C inbred rats which had also received a continuous exogenous source of diethylstilbestrol (DES) resulted in a significant increase in mammary tumors over that resulting from the estrogen alone. None of the non-estrogen treated control animals have as yet developed tumors, even those which were radiated. When early mammary tumors alone are considered, there is a dosage response between radiation in the range used (50,150 or 450 r) and tumor induction in the DES-treated rats. However, when the total number of tumors induced is considered, the greatest synergism was found with the median dose employed, 150 r. Although preliminary evidence in female A x C rats exists that continuous administration of progesterone will inhibit both estrogen-induced mammary cancer and radiation (800 r) synergism, evidence in castrated female A x C rats is beginning to indicate that progesterone may not inhibit estrogen-induced mammary cancers in the absence of the ovaries.

It has been found that the induced mammary cancers contain many mast cells and it is believed that data analysis will show that their numbers and distribution will correlate with tumor growth rates. Using electron microscopy, the Special Virus Cancer Program has been unable to demonstrate virus particles in the induced tumors.

Studies are underway to ascertain if the DES or the high level of plasma prolactin from DES are necessary at the time of radiation for the synergism to manifest itself.

Significance to Biomedical Research and the Program of the Institute: Both radiation and ovarian hormones, given individually in large doses, have been implicated in causing an increase in breast cancer of both laboratory animals and women. It has been speculated that X-rays, routinely used for diagnosis and radiotherapy, plus estrogens and progresterones, now being widely used in birth control pills or for postmenopausal therapy, may interact synergistically and be contributing to the alarming rise in the incidence of breast cancer.

In recent years the Carcinogenesis Program of NCI has devoted some efforts and funds to studies of the hormones, progestogens and estrogens which are often combined in contraceptive pills. This study is an extension of efforts in this field.

Proposed Course: The radiation/estrogen studies now underway are expected to be completed by mid-1974. It is expected that additional studies will be initiated to vary dosages of estrogen administered by various routes which would mimic more closely the estrogen content in the human female contraceptives.

It is also planned to continue research to set up an artifical estrus cycle of the same length as the natural cycle of the rat. The point of major importance here would be to determine the dosage of estrogen (estradiol-17B, ethynyl-estradiol, etc.) necessary to duplicate the natural estrus cycle in order to determine precisely the quantity of estrogens required (with the additional amount found in the contraceptives) to influence the induction of mammary tumors in combination with a known dosage of x-ray.

With the completion of the initial experiments and the establishment of an artifical estrus cycle developed as discussed above, it is proposed to cycle the estrogen therapy and retain the radiation dose and quality that also has been established as effective. It is important here to determine if estrogen induction of breast cancer can be demonstrated in the absence of the host's ovaries. All other studies have been done in the intact host.

The final experiment, as now projected, would be to extend the radiation-estrogen work to the Fischer/344 strain of inbred rats. With data from the other studies as outlined above it should be possible to determine more rapidly the carcinogenesis by radiation and estrogen in this inbred strain of rats whose incidence of spontaneous mammary tumors is "high risk" in comparison to the "low risk" of the A x C rats. This is of interest because of the data of the human disease showing high and low risk populations.

Date Contract Initiated: May 25, 1971

Current Annual Level: $67,742

BOSTON UNIVERSITY (NIH-NCI-72-3297)

Title: Controlled Methods for the Delivery of Chemical Carcinogens to the Pancreas

Contractor's Project Director: Dr. Richard Elkort

Project Officer (NCI): Dr. Richard Bates

Objectives: To examine techniques of surgically implanting a carcinogen containing permeation chamber into the pancreatic duct as a means of inducing adenocarcinoma.

Major Findings: Design specifications for the implantable permeation delivery

system have been completed, and techniques developed for in vitro carcinogen assay. Techniques have been developed for implanting the chamber without having to ligate the pancreatic duct. Microanalytical glucose and insulin assay techniques have been perfected and baseline values obtained for these substances, which will be correlated with any tumor induction. It has been demonstrated that the implantation of the device is tolerated by the animal and produces no significant effect on the normal functioning or structure of the pancreas. Changes induced by the operative introduction of the catheter have reverted to normal in all control animals at the end of a four to six week period. In a small number of animals implanted with catheters containing the alkylating agent dimethylhydrazine, hyperplasia and proliferation of pancreatic ductal cells and goblet cells are seen. By nine weeks, heavy lymphocytic infiltration is noted around the pancreatic duct as well as in the liver and regional lymph nodes, in addition to continued hyperplasia and evidence of cellular activity. The significance of these findings and their relationship to carcinoma induction are currently being investigated.

Significance to Biomedical Research and the Program of the Institute: The establishment of an animal model for pancreatic adenocarcinoma of man is a prime goal of this contract, representing one approach to reach this goal. If induction occurs by physically implanting the carcinogen at the target cells of interest, a detailed study of the neoplastic process may then subsequently occur. In addition, development of a biomedical marker based on changes in glucose or insulin metabolism may have direct application to earlier diagnosis of pancreatic carcinoma in humans.

Proposed Course: Contractor will continue to determine the optimal concentrations of various carcinogens with which the ductal cells are to be challenged. A subcontractor (Abcor, Inc.) has designed the implanting device with properties which permit a controlled diffusion of the encapsulated carcinogen so that the target cells are continually bathed with the inducing agent. Abcor will also develop analytical techniques so that time/dose relationships of carcinogen can be calculated if tumors are successfully induced. Surgical implantation will be performed in rabbits for long-term studies. In addition, canine pancreas will be perfused with a carcinogen shunted through an implantable dialysis device that will minimize systemic distribution of the carcinogen, if indicated.

Date Contract Initiated: June 28, 1972

Current Annual Level: $113,069

CASE WESTERN RESERVE UNIVERSITY (NIH-NCI-72-3284)

Title: Enhanced Induction of Guinea Pig Pancreatic Adenocarcinoma

Contractor's Project Director: Dr. Samuel S. Epstein

Project Officer (NCI): Dr. Richard R. Bates

Objectives: This project is designed to confirm earlier reports of induction

of adenocarcinoma of the pancreas in guinea pigs by nitrosamides and to find ways to enhance the incidence and decrease the latency of these tumors.

Major Findings: The major finding of this contract to date is that there is relatively similar uptake of the pancreatic carcinogens, nitrosomethylurea (NMU) and nitrosomethylurethane (NMUt) in the maternal pancreas, liver, kidney and brain of the guinea pig. Similarly, there is relatively similar uptake of labeled NMU and NMUt in fetal tissues, with the brain having greater uptake than other organs.

Maximally tolerated doses of NMU and NMUt in the pregnant guinea pig have been determined; 2.5 mg/kg and 1.25 mg/kg per day, respectively.

Significance to Biomedical Research and the Program of the Institute: The elucidation of the mechanisms of induction of adenocarcinoma of the pancreas in the guinea pig is likely to aid in the determination of the biological factors critically involved in the pathogenesis of human pancreatic cancer. Factors such as transplacental exposure; increased cell division and effects of environmental pollutants, such as asbestos which has been found in the pancreas of humans dying of asbestosis or mesotheliomas in relatively high concentrations, will also be investigated, with particular reference to the possibility of *in vivo* interactions with nitrosamide carcinogens.

Proposed Course: The proposed course will involve determination whether an enhanced incidence of pancreatic adenocarcinoma results from transplacental exposure of guinea pigs to NMU and NMUt. Similarly, the effects of pancreatitis (chemically induced) and enhanced cell division (induced by partial pancreatectomy) will be studied on maternal animals and on their F1 progeny. Concurrent biochemical studies on the metabolism of NMU and NMUt in maternal and fetal tissues will also be studied. Similar studies will be carried out with human and guinea pig pancreatic slices *in vitro*. The effects of asbestos on the uptake and metabolism on NMU and NMUt and on pancreatic cell division will also be studied.

Biochemical studies on the localization, uptake and metabolism of labeled NMU and NMUt will be studied in the normal and regenerating pancreas of guinea pigs.

Date Contract Initiated: June 29, 1972

Current Annual Level: $98,238

CHICAGO, UNIVERSITY OF (NIH-NCI-72-3290)

Title: Route of Carcinogen Administration in Pancreatic Adenocarcinoma Induction

Contractor's Project Director: Dr. Stan D. Vesselinovitch

Project Officer (NCI): Dr. Richard R. Bates

Objectives: The objective of this project is to explore several animal species and various routes of administration in an effort to enhance the carcinogenic dose to the pancreas or the susceptibility of the target tissue.

Major Findings: The primary emphasis in the early stages of this project has been the study of the acute and chronic changes in the pancreas of the mouse, rat and dog after administration of carcinogens by parenteral, oral, inturbation, and direct administration into the ductal system. Morphological changes in the pancreas of the mouse were observed four days after single dose administration of methylnitrosourethane (MNUt) by inturbation. However, such changes disappeared by the eighth day. MNUt given orally induced obvious toxicity on the liver parenchyma.

Significance to Biomedical Research and the Program of the Institute: This approach to the problem of establishing a biological model of pancreatic carcinogenesis is most important to the Program. Until such time that a reproducible, experimental protocol is available which characteristically induces cancer of the pancreas, the research necessary to enable an understanding of the cause and ultimate prevention of this disease will be markedly restricted. Model development is one of the major goals, and this project along with others is designed specifically to reach this goal.

Proposed Course: It is too early to expect definitive results at this stage of the project. Experience gained will be used to adjust doses and further manipulate the administration regimens. Surgical techniques will be mastered for laparotomy and intraductal injection of the carcinogens to avoid high death rates experienced. Furthermore, induction of chronic pancreatitis using ethanol may be a useful approach to reach the project's objectives.

Date Contract Initiated: June 28, 1972

Current Annual Level: $160,696

CHICAGO, UNIVERSITY OF (NO1-CP-33287)

Title: Definition of Sensitivity of Carcinogenesis Bioassay Systems

Contractor's Project Director: Dr. Stan Vesselinovitch

Project Officer (NCI): Dr. Jerry Rice

Objectives: To establish standard bioassay systems in mice for screening substances suspected of potential carcinogenic hazard to man, making best use of the age-dependent variation in sensitivity to chemical carcinogens exhibited by F-1 hybrid mice (first generation crosses of C3HeB x A/He and C57B1/6 x C3HeB) and to provide definition of diagnostic criteria and of detailed biologic behavior of the neoplasms produced, especially those of the liver and lung.

Major Findings: Initial studies on the sensitivity to various carcinogens

of both hybrid strains of mice are nearly concluded, and in process of compilation for publication. The appearance in these mice of large numbers of renal epithelial tumors, which are uncommon in this species, has allowed a study of the morphogenesis of these tumors. The development of neurogenic tumors as a consequence of exposure to ENU is most pronounced in animals exposed transplacentally early in gestation (day 12-14), in contrast to experience in the rat, and there is a higher proportion of CNS gliomas and a significant proportion of cerebellar medulloblastomas, also in sharp contrast to the predominance of peripheral neurinomas and the absence of medulloblastoma in the rat. Studies of continuous pre- and postnatal exposure of hybrid mice to known human carcinogens, which require enzyme-mediated conversion to reactive metabolites, is nearing completion and should indicate the feasibility of including perinatal exposure in the usual carcinogen screening programs in order to increase the sensitivity of such tests. Transplantation studies with hepatomas induced by different agents have revealed that a massive, hyperplastic reaction to amitrol is generally not neoplastic and serves as a model for the type of reactive lesion which must be distinguished from neoplasia.

Significance to Biomedical Research and the Program of the Institute: A major deficiency in carcinogen screening programs has been their lack of uniformity. It has been in the past quite impossible to estimate the relative carcinogenic potency of different compounds, even those tested in the same species, because of uncontrolled variables associated with testing protocols. These include the intrinsic differences in neoplastic response of different tissues in various strains of mice, the pronounced quantitative differences in response of fetuses, infants, and adults, and the marked effects of route of administration and dosage regimen. The dissimilarity between the histological features of murine versus human tumors of corresponding cell type has also led to disagreements, which require resolution, concerning the significance of certain neoplasms of the mouse to assessment of cancer risk in man.

The purpose of this contract is to evaluate the significance of these variables within the context of an optimal screening protocol, and to develop improved standardized procedures which will be used for future carcinogen screening programs. Definition of biologic behavior and diagnostic criteria for the histologic identification of the neoplasms that can be induced in these animals will provide needed reference standards for the evaluation of endpoints in carcinogenesis tests.

Proposed Course: New starts will occur only in the area of mouse hepatoma biology, with the result that this contract will continue for some years at a reduced level of funding somewhat below the current budget. Collaboration of experienced morphologists from other institutions who are interested in mouse liver tumors will be established in an effort to define standard diagnostic criteria for these tumors, taking into account possible variables such as the age of the host in which a tumor appears and the nature of the inducing agent. Other studies will be concluded in an orderly fashion and prepared for publication as rapidly as possible.

Date Contract Initiated: June 25, 1969

Current Annual Level: $276,068

CORBEL LABORATORIES, INC. (NIH-NCI-72-3299)

Title: Provide Animal Holding Facilities and Services

Contractor's Project Director: Mr. William Rickman

Project Officer (NCI): Dr. Jerry Rice

Objectives: The goal of this project is to demonstrate transplacental chemical carcinogenesis in the Old World monkey, Erythrocebus patas, as a representative primate species. It is uniquely suited for this purpose because of its relative freedom from bacterial and latent viral infections; the readiness with which it breeds in captivity; the availability of a nucleus colony of this species; and the active participation of an experienced primatologist, Dr. William London, of the Infectious Diseases Branch, National Institute of Neurological Diseases and Stroke to whom the nucleus colony mentioned above belongs.

Major Findings: Although importation of additional breeding monkeys has been slower than originally anticipated, the nucleus colony has been sufficient for preliminary studies of dosage. The pregnant patas tolerate single or repeated injections of 0.1 mmoles/kg 1-ethyl-1-nitrosourea, either intravenously in pH 3 isotonic citrate buffer or intraperitoneally in trioctanoin, if administered after the 50th day of gestation (normal gestation: 160 days). Larger doses cause abortion, which may not occur until several weeks after treatment. A total of eight monkeys exposed in utero to ENU are now under observation for tumor development.

Significance to Biomedical Research and the Program of the Institute: Cancer is the third most frequent cause of death among infants and children in the United States and most industrialized countries, taking a lesser toll than either accidents or infectious diseases, but claiming nearly as many lives as the latter. Many of the specific varieties of cancer encountered in children are found uniquely or predominately in this age group and not infrequently develop so early in life that a prenatal origin is either certain or highly probable. With the recognition of the association between exposure to diethylstilbestrol in utero and the development of vaginal adenocarcinoma during the second decade of life, the possible significance to human health of exposure to transplacental chemical carcinogens has become increasingly a matter of concern. Experimental studies on transplacental chemical carcinogenesis have been limited to rodent species, which differ significantly from man in many ways likely to be of importance for understanding the different consequences of exposure to chemical carcinogens during fetal versus adult life. Among these are the very much more rapid rates of fetal and neonatal development and maturation in rodents, a factor which may be responsible for the failure so far to observe any tumors strikingly different from those inducible in adults in rodents exposed in utero to potent chemical carcinogens.

Proposed Course: This project is an exploratory program to demonstrate transplacental chemical carcinogenesis in a subhuman primate species. Particular

emphasis will be placed on defining the periods of maximal sensitivity of different organ systems and demonstrating parallels between both the morphological and clinical behavior of corresponding human tumors, and the results obtained in rodents with the same carcinogen. Comparative pharmacologic studies of the rate and extent of transplacental passage of ENU in rodent species and in patas monkeys will also be made.

Date Contract Initiated: June 28, 1972

Current Annual Level: $104,859

DARTMOUTH COLLEGE (NIH-NCI-72-3296)

Title: Enhanced Delivery of Synthetic Nitroso Compounds to the Pancreas in Rats

Contractor's Project Director: Dr. Daniel S. Longnecker

Project Officer (NCI): Dr. Richard R. Bates

Objectives: The principal goal of this contract is to determine the inducability of pancreatic adenocarcinoma in rats using specific chemicals having two ideal characteristics: 1) the base molecule is known to concentrate in the normal pancreas of man or animals, e.g. certain amino acids, neutral red, puromycin, and 2) these molecular species readily lend themselves to synthetic modification with the formation of nitroso derivatives, a functional derivative of many compounds known to be carcinogenic.

Major Findings: The screening system for detection of mutagens and carcinogens based upon inhibition of growth of a mutant Escherichia coli strain which lacks DNA polymerase has been established. Two preparations of a nitroso derivative of neutral red have been tested in this system and shown to have no activity. O-(N-nitrososarcosyl)-L-serine appears to have a mutagenic-carcinogenic potential when tested in this system, although relatively large amounts are required to inhibit growth of the mutant. Attempts to demonstrate in vitro transformation in fetal hamster cells following exposure to known polycyclic carcinogens and the nitroso derivative of neutral red have been equivocal. Preliminary evidence furthermore suggests that the nitroso derivative of neutral red may not be selectively absorbed by the pancreas to the same extent as the base molecule. It is possible that chemical modification of neutral red also modifies its behavior in a biological system.

Significance to Biomedical Research and the Program of the Institute: This approach to the problem of establishing a biological model of pancreatic carcinogenesis is most important to the Program. Until such time that a reproducible, experimental protocol is available which characteristically induces cancer of the pancreas, the research necessary to enable an understanding of the cause and ultimate prevention of this disease will be markedly restricted. Model development is one of the major goals, and this project along with others is designed specifically to reach this goal.

Proposed Course: Nitroso derivatives of amino acids and other base molecules known to concentrate in the pancreas will continue to be evaluated in the E. coli mutant and in vitro hamster cell system for potential carcinogenecity in an effort to discern the usefulness of these systems for carcinogens known to require metabolic activation. In addition, compounds such as azaserine, melphelan, and ethyl-N-nitrososarcosine will be studied. Preliminary toxicology and long-term animal studies will be initiated, particularly with compounds having the desired characteristics.

Date Contract Initiated: June 28, 1972

Current Annual Level: $60,066

HAZLETON LABORATORIES (NIH-NCI-72-3287)

Title: Induction of Adenocarcinoma in Dog Prostate

Contractor's Project Director: Dr. Dan W. Dalgard

Project Officer (NCI): Dr. Richard R. Bates

Objective: The objective of this contract is the search for a reproducible technical procedure for the induction of hormone-dependent adenocarcinoma of the prostate of the dog.

Major Findings: The initial approach was to inoculate the carcinogens directly into the prostate gland and monitor the response by repeated biopsies and rectal palpation. This procedure unfortunately produced extensive adhesions making subsequent biopsies impractical. Efforts aimed at developing systems for sustained release of carcinogens appear promising.

Significance to Biomedical Research and the Program of the Institute: A study of prostatic adenocarcinoma is a goal of the Institute. It is necessary then to have an animal model in which such studies can be performed. At the present time no experimental model is available.

The dog is the most likely animal species to provide a model, since it has a higher incidence of spontaneous adenocarcinoma of the prostate than other non-human species.

Proposed Course: Dogs of various age groups will be used. Each age group has its own unique physiology and morphology of the prostate gland. Three carcinogens will be examined, a polycyclic hydrocarbon, and alkylating agent and cadmium. Castration and androgen administration will be used in conjunction with carcinogen administration to develop tumors which can be studied in detail.

Date Contract Initiated: June 24, 1972

Current Annual Level: $161,600

KANSAS, UNIVERSITY OF (NIH-NCI-72-3271)

Title: Histogenesis of Guinea Pig Pancreatic Adenocarcinoma

Contractor's Project Director: Dr. Janardan K. Reddy

Project Officer (NCI): Dr. Richard R. Bates

Objectives: The studies of Druckery and colleagues have shown that methylnitrosourea and methylnitrosourethane can induce carcinoma of the stomach and pancreas when administered in the drinking water. However, an induction period of 800 days is required, and the incidence of the pancreas tumor is less than 20%. The objective of this project is to study the histogenesis of this reported disease in an attempt to determine the primary site of cancer induction and to manipulate this model so as to reduce the induction period and increase the incidence of pancreatic adenocarcinoma.

Major Findings: The use of tritiated methylnitrosourea has permitted the detection of its incorporation into the RNA and DNA of guinea pig pancreas. This incorporation is significantly increased in a gland which is undergoing regeneration and other toxic damage by ethionine. There appears to be increased binding of this carcinogen when regeneration, and its concomitant increased metabolic activity, is occurring.

Significance to Biomedical Research and the Program of the Institute: This approach to the problem of establishing a biological model of pancreatic carcinogenesis is most important to the Program. Until such time that a reproducible, experimental protocol is available which characteristically induces cancer of the pancreas, the research necessary to enable an understanding of the cause and ultimate prevention of this disease will be markedly restricted. Model development is one of the major goals, and this project along with others is designed specifically to reach this goal.

Proposed Course: Inasmuch as much of the preliminary studies have been completed, long-term carcinogenic experiments will be initiated to meet the objectives of this project. Sequential morphological studies to trace the progression of cancer will be initiated. Within these studies, the use of pancreaticotoxic agents such as cobaltous chloride, ethanol, lasiocarpine or 4-hydroxyaminoquinoline 1-oxide will be used.

Date Contract Initiated: June 10, 1972

Current Annual Level: $43,306

LITTON-BIONETICS, INC. (NIH-NCI-72-2063)

Title: Preparation and Examination of Experimental Biological Material

Contractor's Project Director: Dr. Elizabeth Kingsbury

Project Officer (NCI): Dr. Curtis Harris

Objectives: The principal objective is the preparation and examination of tissues for light and electron microscopy, studying the following: (1) the pathogenesis of chemically induced tumors of various organs such as prostate, pancreas, mammary gland and lung, and (2) the autoradiographic localization of labeled carcinogens or other relevant biological compounds, such as vitamin A.

A secondary objective is to provide support services, such as preparation of biological specimens for light and electron microscopy and autoradiographic slides for study and evaluation by NCI investigators.

Major Findings: In order to study the pathogenesis of chemically induced tumors of the rat prostate, the normal morphology of the various lobes, accessory glands and surrounding tissue must be carefully defined, including developmental changes from infancy to adulthood. Light microscope studies of the whole prostatic complex indicate that the dorsal, ventral and lateral lobes may be defined on a morphological basis. However, studies of very young rats show that development proceeds from an undifferentiated state to the more easily defined state of the adult. These findings constitute a firm morphological basis for future studies on the pathogenesis of chemically induced tumors in this organ. Fixation studies have shown that the prostate is an osmotically sensitive tissue, requiring fairly exacting conditions of fixation for electron microscopic studies.

The following short-term studies have been completed: (1) electron microscopy (EM) of cell lines derived from DEAE-dextran induced tumors, and treated with IUDR. Virus particles were observed and characterized as C-type; (2) EM studies of normal and transformed epithelial-like cell cultures from ten day old rat liver and its induced tumors. Three cell lines and 17 induced tumors were characterized. Glycogen was detected histochemically in all cell lines and numerous tumors; (3) in support of studies on susceptibility states of various organs, electron micrographs have been prepared, documenting the effects of N-Methyl-N-Nitrosourea (NMU) on the pancreas duct cells of the guinea pig, the major effect being nucleolar lesions; and (4) five sets of slides for autoradiography and light microscopic studies have been prepared and were evaluated by NCI scientists. Experimental findings are reported elsewhere.

Significance to Biomedical Research and the Program of the Institute: As man's environment continues to become more crowded and complex, with increasing technological changes, the day-to-day exposure to chemical carcinogens increase. In spite of efforts to eliminate such hazards to human health, enough of such chemicals remain in the environment that procedures are needed to protect humans and decrease his susceptibility to such carcinogens.

Proposed Course: The morphological description of normal rat prostate should be completed at the ultrastructural level by electron microscopy. Once, this base line is established, experimentation can proceed to determine the susceptibility of the various cell types to chemical carcinogens and the possible protective effects of various biological compounds. A second area of investigation has been initiated in order to determine why rat mammary tissue becomes highly susceptible to chemical carcinogenesis at a particular

age. A third project to be initiated concerns a search for viral particles in monkeys exposed transplacentally to ethylnitrosourea. Such viral particles, if found, should lead to attempts to isolate them and determine biological activity, if any. Supportive services for the pancreatic and lung studies will be continued as designated by the Project Officer.

Date Contract Initiated: December 1, 1971

Current Annual Level: $125,377

MARYLAND, UNIVERSITY OF (NIH-NCI-72-3206)

Title: Studies of the Histogenesis of Renal Carcinoma

Contractor's Project Director: Dr. Melvin Reuber

Project Officer (NCI): Dr. Richard R. Bates

Objectives: This project is designed to explore an animal model for the induction of kidney tumors and to describe the morphological alterations occurring from the time the carcinogen is first administered until the malignant tumors develop. Tumors will be induced in rats by feeding of N-2-(4'-fluorobiphenyl) acetamide. The tumors will be compared with human kidney tumors and the remaining kidney tissues from patients with tumors of this organ will be searched for preneoplastic lesions to compare with the preneoplastic lesions in the rats. Various types of lesions in the rat occurring after varying latent periods will be transplanted in order to gain further information on their biologic behavior. Host factors, such as age and co-carcinogens, in the development of renal carcinomas will be evaluated. The overall objective of the entire project is to gain further knowledge of the morphology and pathogenesis of the human kidney tumors and to develop an animal model which will be useful for studying the pathogenesis of the human tumor.

Major Findings: The changes in the kidney preceding the development of carcinomas take place slowly over a long period of time. Minimal focal hyperplasia of the distal convoluted tubules is seen after 24 weeks of carcinogen feeding. At the end of 36 weeks there is also hyperplasia of proximal convoluted tubules, whereas the distal tubules tend to become atrophic and scarred. There are also a few discrete lesions measuring between 2 and 3 mm. in size. At this time it still cannot be determined which specific lesions involving the proximal or distal tubules are preneoplastic. Electron microscopic studies on renal tissue from animals killed after 12 weeks on the carcinogen showed large collections of bizzare myelin-like figures in the cytoplasm of either or both proximal and distal tubular lining cells. Simplification of cytoplasm was observed in some cells.

Significance to Biomedical Research and the Program of the Institute: A major goal of the Biological Models Segment is the development of animal models of cancer induction which are as similar as possible to their human counterparts in their morphologic, biologic, biochemical, and physiologic characteristics. These models will then be used to study the factors which

determine sensitivity of the tissue to carcinogens and the factors, which affect the progression of carcinogen-induced lesions. It may be possible to prevent the development of renal carcinomas in humans. This project is designed to determine more about the morphology of human kidney tumors and to investigate an animal model which may be appropriate for the goal stated above.

Proposed Course: It is anticipated that this contract will require three years for completion. The nature of this project is such that similar evaluations at periodic intervals will occur for 48 or more weeks. Animal sacrifice, light and electron microscopic evaluation, laparotomies, autologous transplantation and histochemical studies will be carried out.

Date Contract Initiated: February 1, 1972

Current Annual Level: $82,400

MASON RESEARCH INSTITUTE (NIH-NCI-72-3238)

Title: Uptake and Excretion of Carcinogens in and Their Effects on the Prostate

Contractor's Project Director: Dr. Emil R. Smith

Project Officer (NCI): Dr. Richard R. Bates

Objectives: The principal goal of this contract is to measure the levels of selected carcinogens in the blood, the prostate gland, and in some cases, in the prostatic fluid of rats and dogs after systemic administration of these substances. Those agents having the highest affinity for the prostate gland will be employed in chronic studies in rats and dogs in an attempt to induce adenocarcinoma of the prostate.

Major Findings: Experiments with cadmium have been initiated. In rats given single subcutaneous doses of 30 mg/kg of cadmium chloride, peak plasma levels occurred 15 minutes after treatment and declined slowly thereafter. Plasma:erythrocyte ratios were consistently less than one. In animals necropsied at intervals up to 24 hours after treatment, cadmium accumulated in some tissues, particularly the kidney, liver and pancreas, but only very small amounts were found in the prostate glands. The dorsalateral gland contained more cadmium than the ventral gland.

In dogs given single subcutaneous doses of 10 or 30 mg/kg of cadmium chloride there was at 2 and 24 hrs an accumulation of cadmium in the liver, the renal cortex, the heart and lungs but only low levels were found in the prostate gland. No cadmium was detected in the prostatic fluid of dogs given single or repeated subcutaneous doses of 3 or 30 mg/kg of this salt.

Thus, it has been found that cadmium does not readily accumulate in the prostate gland of rats or dogs, and the small amounts which do enter the prostate gland of the dog do not pass into prostatic fluid.

Significance to Biomedical Research and the Program of the Institute: It is estimated that there will be 35,000 new cases and 17,000 deaths from adenocarcinoma of the prostate this year. In spite of its role as a major cause of human deaths for cancer, there are no experimental models for this kind of cancer and very little work is being done to develop such models. This project is one of several developed by the Biological Models Segment in order to stimulate research on the etiology and pathogenesis of adenocarcinoma of the prostate. This specific project is designed to aid in the selection of the appropriate chemical carcinogen, species and treatment procedure (route, dose, duration, etc.) for attempts to produce an animal model of prostatic adenocarcinoma.

Proposed Course: Similar studies have been and will be initiated with selected normal and radioactively labeled chemical carcinogens. This contract will require two or three years for completion.

Date Contract Initiated: June 15, 1972

Current Annual Level: $141,980

MICHIGAN, UNIVERSITY OF (NIH-NCI-72-3216)

Title: Isolation and Purification of Epidermal Chalone: A Tissue Specific Inhibitor of Cellular Proliferation

Contractor's Project Director: Dr. John Voorhees

Project Officer (NCI): Dr. Henry Hennings

Objectives: Chalones, the tissue-specific inhibitors of cell proliferation, have been found in several tissues, but have not yet been purified. The primary objective of this contract is the isolation and purification of epidermal chalone. Inhibitors isolated from the epidermis act in both the G1 and G2 phases of the cell cycle; the chemical nature of these factors will be determined.

Major Findings: Initially, the need existed to develop suitable assay systems other than the standard Bullough technique which is costly in time and materials. In this regard, the G-2 in vitro mouse ear mitotic inhibition assay is operational. Organ cultures from hairless mice were evaluated for incorporation of tritiated thymidine and ultimately rejected on the basis of poor correlation between liquid scintillation data and autoradiography, considerable epithelial necrosis, and an anticipated lack of precision of the assay with limited confidence in its relevance to physiology. An enzymatic assay has been developed for monitoring cell cultures. Epithelial cells from adult guinea pig ears are positive for histidase and negative for leucine aminopeptidase whereas dermally derived cells (presumably fibroblastic) have the opposite response. Histidase levels have also been found to differ in epithelial and malignant BALB/c cell cultures.

Significance to Biomedical Research and the Program of the Institute: The

Biological Models Segment has a program responsibility for investigating possible tissue-specific methods of blocking cancer development. Obviously, disturbances in the chalone mechanism of growth regulation could be involved in carcinogenesis. After purification of the active inhibitors from epidermis, the chalone level at different stages during epidermal carcinogenesis and the possible effect of exogenous chalone on epidermal carcinogenesis could be determined. The work performed under this contract will play an important role in future planning of the NCI program on organ-specific cancer control mechanisms.

Proposed Course: General emphasis will be placed on two major areas, cell culture development for assay and chalone purification. The in vivo assay will still be used to monitor chalone purification until the cell culture assay is clearly established as workable and relevant. The BALB/c malignant cell line and both a newborn mouse and guinea pig ear epidermal cell cultures will be further characterized for assay purposes. Putrescine and histamine will be studied for growth promotion. Cell lines will also require confirmation as to their epidermal origin; enzymatic markers will be used for this purpose. Chalone purification from epidermis of newborn rats by standard protein chemistry will continue using in vivo, in vitro and chemical assays, the latter using fucose and glucuronic acid as possible markers for this inhibitor. Correlation with the biological assay systems will be performed. The effect of isolated fractions during chalone purification on the rates of DNA synthesis and mitosis will be estimated.

Date Contract Initiated: April 1, 1972

Current Annual Level: $96,625

MICROBIOLOGICAL ASSOCIATES (NO1-CP-02199)

Title: Laboratory Service for Support in Carcinogenesis Bioassay and Related Activities

Contractor's Project Director: Dr. Russell Madison

Project Officer (NCI): Dr. Jerry Rice

Objectives: This contract provides for laboratory support services for the Carcinogenesis Program, NCI, in the field of experimental pathology. The project is intended to provide the following services: (1) perform bioassay studies according to protocols developed especially by NCI, including controlled feeding, parenteral administration, skin painting, intragastric intubation, intratracheal instillation and other techniques as required, using mice, hamsters, rats and other animal species as required; (2) perform animal treatments and maintain precise observation records as prescribed by individual NCI staff members who have initiated the protocols for different types of carcinogenesis studies; (3) conduct gross and histopathological examinations and evaluations of experimental animals according to NCI specifications, and to provide histological support for NCI in-house activities; and (4)

provide photographic services as required by the project and other NCI needs.

Major Findings: The contract facilities have been functioning at full capacity, with a rapid turnover among projects, of which approximately 18-22 are in progress at any given time. Protocols have been initiated by 11 different investigators within the Carcinogenesis Program and have involved parenteral injections, intratracheal and intragastric instillation methods, feeding and breeding studies. The current animal inventory includes approximately 2700 rats, 1000 mice, 500 hamsters and 140 guinea pigs. The light microscopy and histology laboratories completed approximately 26,000 stained slides, and the electron microscopy preparation laboratory produced approximately 1500 one micron slides and 20 ultra-thin grids. These slides were prepared from tissues of animals on experiments originating both in this facility and also in other programs of the Experimental Pathology Branch. Major areas of activity include the following: (1) intratracheal instillations of carcinogens and various diluents in hamsters and transplantation of tumors which develop, in support of studies carried out by the Lung Cancer Unit; (2) oral administration of carcinogens and modifiers to animals fed protein or vitamin deficient diets, or to immunosuppressed animals. This work was performed in support of studies carried out by the Carcinogen Screen Section, Experimental Pathology Branch and the Nucleic Acid Section, Chemistry Branch; (3) parenteral administration of compounds, both carcinogenic and non-carcinogenic for the exploration of various aspects of chemical carcinogenesis by the Bioassay Section, Experimental Pathology Branch; (4) studies to develop an animal model for pancreatic and prostatic carcinoma by the Biological Models Segment; (5) holding experiments for irradiated rats injected with cells grown and treated with chemical carcinogens in tissue culture; and (6) preparation of slides for light and electron microscopy.

Significance to Biomedical Research and the Program of the Institute: This contract provides an essential facility for the initiation and completion of long-term experiments involving large numbers of experimental animals. As an extension of NCI in-house research, the facility provides a means for rapid testing of new ideas in experimental pathology related to chemical carcinogenesis.

Proposed Course: This contract is being expanded by 23,000 square feet to be used for laboratories and animal facilities. Construction of a third floor on the existing building is presently in progress with completion scheduled for the fall of 1973. The scope of activities of this contract will then be broadened to include biochemical procedures and tissue culture techniques.

Date Contract Initiated: June 4, 1970

Current Annual Level: $733,419

MICROBIOLOGICAL ASSOCIATES (NIH-NCI-72-3300)

Title: Preparation of Cell Strains from Human and Animal Prostate

Contractor's Project Director: Dr. Monroe M. Vincent

Project Officer (NCI): Dr. Stuart Yuspa

Objectives: This project is designed to develop techniques for culturing epithelial cells of prostate tissue. The ultimate goal is to use these cultures for study of chemical carcinogenesis.

Major Findings: Standard tissue culture methods have resulted in predominantly fibroblast-like cultures. Clones of epithelial cells derived from these cultures have not developed into cultures which could be successfully carried as epithelial cell strains. A total of 23 subcultured lines (ranging up to 12 passages), derived from embryonic human, adult rat and adult dog prostates, have been stored as cryopreserved viable suspensions in nitrogen refrigerators.

Preliminary data of isozyme pattern indicate that some of the cell strains developed are identifiable as being derived from prostate. Attempts to utilize a histochemical method have not been successful; results suggest that a histochemical method, successful for frozen sections, is not capable of distinguishing prostate acid phosphatase in cultured cells.

Significance to Biomedical Research and the Program of the Institute: Although prostatic cancer is a leading cause of cancer deaths each year, little is known of the mechanism of carcinogenesis and few experimental models are available to study this. This project is designed to develop an *in vitro* model of functioning human and animal prostatic tissue and to use this for studying the cellular alterations during induced prostatic carcinogenesis.

Proposed Course: Initial studies will be directed toward establishing cell cultures of human and animal prostatic tissue and studying the morphological, biological, and biochemical behavior of these cells. In order to promote selective proliferation of functional epithelial cells (as against fibroblast-like connective tissue cells), hormones and related substances (such as glucocorticosteroids, thyroxine, insulin, chorionic gonadotropic hormone, testosterone and cyclic AMP) will be tested. Other environmental parameters such as pH, oxygen and carbon dioxide tensions, and temperature will also be investigated. Substances that may selectively inhibit proliferation of fibroblasts such as collagenase, hydroxy-L-proline, aminopropyl nitril, etc., will be examined.

Date Contract Initiated: June 28, 1970

Current Annual Level: $90,463

NEBRASKA, UNIVERSITY OF (at Lincoln)(NIH-NCI-72-3212)

Title: Chemical Carcinogen-induced Noduligenesis and Tumorigenesis in Whole Mouse Mammary Gland Organ Culture

Contractor's Project Director: Dr. M.R. Banerjee

Project Officer (NCI): Dr. Richard R. Bates

Objectives: This project utilizes the techniques of organ culture of whole mammary gland of mouse. (1) The susceptibility of mouse mammary epithelium to chemical carcinogen-induced neoplastic "transformation" in organ culture of whole mammary gland will be studied. Whole mammary glands in hormone supplemented organ culture will be treated with known carcinogenic hydrocarbons, during the periods of high and low DNA synthesis. The characteristics of the outgrowth produced by the treated cells after transplantation into gland-free mammary fat-pad in vivo will be measured as criteria of "transformation". (2) The mammary epithelium, hormonally induced to lobulo-alveolar development in organ culture will be treated with the chemical carcinogens at different periods of cell proliferative cycles (hence DNA replicative cycles). The lobulo-alveolar structures in the treated glands then will be allowed to regress in culture to a ductal condition similar to post-lactation mammary gland of weaned females. Occurrence of hyperplastic nodules and/or tumor-like lesions in the gland will be surveyed microscopically. Carcinogenicity of these "transformed" lesions will be tested in vivo by using the standard fat-pad transplantation procedure.

Major Findings: (1) A comparative study showed that of the 5 pairs of mammary gland the 2nd thoracic glands are most suitable for organ culture of whole mammary gland and these glands are now being used. (2) It was found that explants of the lobulo-alveolar mammary gland grown in organ culture produce outgrowths after transplantation into "gland free" mammary fat-pads of virgin females and these resembled the ductal outgrowth of normal mammary tissue of pregnancy. Thus the finding of the ability of organ cultured mammary tissue to produce outgrowths similar to normal mammary tissue of pregnancy is considered important because testing of neoplastic "transformation" will be done by fat-pad transplantation of carcinogen treated tissue. (3) The lobulo-alveolar structure of the mammary gland now can be maintained up to 14-18 days in culture. Furthermore, the fully developed gland after 5-7 days in culture will regress during subsequent incubation in hormonally depleted medium. The availability of this procedure of induction of lobulo-alveolar structures and their subsequent regression in long-term culture will now permit studies to determine whether detectable "transformation" can be induced entirely in the organ culture. (4) It was determined that during the five day culture period high levels of DNA synthesis in the mammary epithelium are evident on the 2nd and the 4th day and a 70% synchrony of cells in DNA synthesis can be obtained by FUdR treatment during the initial 18 hours incubation. (5) Toxicity levels of the two carcinogens, 3-methylcholanthrene (MCA) and 7,12 dimethylbenz(a)anthracene (DMBA) have been studied. Histologically 5 mcg/ml MCA and 1 mcg/ml DMBA showed little toxic effect.

Significance to Biomedical Research and the Program of the Institute: The Biological Models Segment has the goal of developing animal models which can be used to study the biology and pathogenesis of the major forms of human cancer. Factors which affect the susceptibility of various organs to chemical carcinogens and which allow progression of the neoplastic process are of interest. In addition, there is an urgent need to develop techniques by which the response of human tissues to chemical carcinogens can be studied; organ culture techniques are a promising approach. Thus, this project will study an organ of major importance to the Biological Models Segment and perform experiments which are related to major goals of the Segment. In addition,

development of such a biological system in organ culture may provide a rapid assay system for various environmental carcinogens.

Proposed Course: The cells cultured and synchronized by the procedures developed the first year will be exposed to a variety of carcinogenic agents. After in vitro proliferation, tissue will be examined for histopathologic changes and bioassayed by fat-pad transplantation. Additionally, carcinogen treated cultures during hormone induced lobulo-alveolar development will be induced to regress by hormone depletion or removal with subsequent examination for hyperplastic nodule development. It is anticipated that this project will require at least two additional years for completion.

Date Contract Initiated: April 1, 1972

Current Annual Level: $43,000

NEBRASKA, UNIVERSITY OF (Eppley Institute for Research on Cancer) (NIH-NCI-68-959)

Title: A Resource for Carcinogenesis Bioassays and Related Research

Contractor's Project Director: Dr. Philippe Shubik

Project Officer (NCI): Dr. Gio B. Gori

Contract narrative is reported under the Office of the Associate Scientific Director for Carcinogenesis

PAPANICOLAOU CANCER RESEARCH INSTITUTE (NIH-NCI-72-3288)

Title: Induction of Prostatic Adenocarcinoma in the Rat

Contractor's Project Director: Dr. Wilhelmina F. Dunning

Project Officer (NCI): Dr. Richard R. Bates

Objectives: To attempt induction of prostatic adenocarcinoma in an inbred A x C line 9935 Irish male and Copenhagen line 2331 male rats by the indirect application of 3-methylcholanthrene or 7,12-dimethylbenzanthracene combined with androgenic stimulation of the prostatic epithelium.

Major Findings: The initial experimental groups have been under treatment and observation for up to 200 days. Direct implantation performed under previous experiments required 329 days. However, evidence exists that the desired stimulation of the epithelium is being achieved. Animals that have died showed adenomatous hyperplasia of the prostatic epithelium and enlarged, dilated seminal vesicles.

Significance to Biomedical Research and the Program of the Institute:

Establishing a biological model of prostatic carcinogenesis is most important to the Program. Until such time that a reproducible, experimental protocol is available which characteristically induces adenocarcinoma of the prostate, the research necessary to enable an understanding of the cause and ultimate prevention of this disease will be markedly restricted. Model development is one of the major goals, and this project along with others is designed specifically to reach this goal.

Proposed Course: Animals will continue to be periodically observed for morphological alterations in the prostate and other major glands in an attempt to discern the influence of hormones on the carcinogenic process and derive insight into a suitable protocol for induction of prostatic adenocarcinoma.

Date Contract Initiated: June 16, 1972

Current Annual Level: $22,510

ST. LOUIS UNIVERSITY (NIH-NCI-72-3274)

Title: Synthetic Nitroso Derivatives as a Means of Concentrating Carcinogens in the Pancreas

Contractor's Project Director: Dr. Thomas Curphey

Project Officer (NCI): Dr. Richard A. Pledger

Objectives: The principal objective of this contract is the synthesis of N-nitroso derivatives of specific compounds known or suspected to have a high affinity for the pancreas. Certain derivatives will be labeled with tritium for tracer analysis.

Major Findings: Efforts have centered on the synthesis of potential carcinogens derived from neutral red and alpha amino acids. Neutral red was found to be quite impure and steps for purification have been developed. A nitrososarcosyl derivative of this dye has been synthesized and is currently undergoing biological evaluation under a separate contract.

Preparation of nitroso-containing amino acids has commenced with the successful synthesis of O-sarcosyl-L-serine in 70% overall yield without the contamination with the O-valeryl ester found in published syntheses. The formation of the by-product was avoided by acylation of carbobenzoxyserine benzyl ester with the symmetrical anhydride formed from carbobenzoxysarcosine and dicyclohexylcarbodiimide.

Significance to Biomedical Research and the Program of the Institute: Induction of pancreatic adenocarcinoma in animals is a prime goal of the National Cancer Institute. Such a successful protocol leading to reproducible tumor formation would permit the study of the factors responsible for this malignancy, the early cellular alterations during carcinogenesis and insight into ways to modify the induction process. The approach of using chemicals that are carcinogenic and preferentially bind or concentrate in the pancreas is not currently

feasible. Modification of chemicals known to concentrate in this organ with functional groups known to be carcinogenic may accomplish both tasks simultaneously.

Proposed Course: Certain compounds, such as puromycin and various amino acids which have an affinity for pancreatic tissue, along with further work on neutral red will be chemically modified. Available chemical groups for modification include -OH, -NH-, and -SH. Organically synthesized derivatives will be purified and submitted to the National Cancer Institute for testing.

Date Contract Initiated: June 28, 1972

Current Annual Level: $28,276

SOUTHWEST FOUNDATION FOR RESEARCH AND EDUCATION (NIH-NCI-72-3291)

Title: Gonadal Hormone Effects on the Prostate

Contractor's Project Director: Dr. Leonard Axelrod

Project Officer (NCI): Dr. Douglas H. Janss

Objectives: The goal of this project is to compare the endocrinology of the various lobes of the prostate in a variety of animal species in order to determine their similarities and differences from the human gland. These comparative studies will include the following: (1) determination of the testosterone and estrogen metabolites formed in the ventral and dorsolateral lobes of the rat prostate, the cranial, dorsal, medial and laterial lobes of the dog prostate and eventually the dorsal, medial and lateral lobes of the human prostate; (2) identification of the "active androgen" in these specific prostatic lobes; and (3) an evaluation of the effect of estrogen upon the metabolism of testosterone in these prostatic lobes. The results of this contract will be used for determining the species which will be used in carcinogenesis experiments.

Major Findings: Metabolic: Baseline data were developed for studies of prostate incubated under controlled and reproducible conditions of buffer composition, time, temperature, oxygenation and substrate concentration. It was found that testosterone is metabolized to a variety of C19 metabolites as a function of the age of the animal in both rat and dog species. The metabolism is quantitatively different in lateral and posterior lobes of the dog at a specific age. The major changes, both qualitatively and quantitatively, in the transformation of testosterone reflect the availability of enzymic activities as a function of age, particularly advanced age. Furthermore, new metabolites are found in the advanced age group which are not present in the controlled condition incubation of younger tissues. Estrogen (estradio-17B) inhibits select enzyme action in the metabolic transformation, particularly the 3B-ol dehydrogenase and 17Bol dehydrogenase in all groups.

Receptors: Application of a modified and refined sucrose gradient procedure

to rat ventral prostate extracts revealed two binding activities for 5a-dihydrotestosterone (5a-DHT). The high affinity, low capacity receptor had a sedimentation coefficient of 10.4S and a second persistent, lower affinity, higher capacity binding activity had a sedimentation coefficient of 3 to 4S. A highly reproducible, facile, and sensitive protocol was developed for measuring the binding parameters of the high affinity binding activity for 5a-DHT. The binding constants (in millions) for 5a-DHT, testosterone, and estradiol respectively were 740, 350 and 76 per mole. Binding constants for testosterone metabolites were from 1 to 3 orders of magnitude less than the value for 5a-DHT. A protocol for the measurement of high affinity transport and binding of cytosol receptor in highly purified ventral prostate nuclei was developed and applied to various cytosol receptor preparation for evaluation of their transport capability. These preliminary experiments indicate that the identify of the high affinity nuclear receptor extracted from purified nuclei was independent of the conformation of the cytosol receptor used as donor.

Significance to Biomedical Research and the Program of the Institute: It is estimated that 17,000 deaths will occur from adenocarcinoma of the prostate this year. There are no experimental models for this kind of cancer and very little has been done to develop such animal models suitable for detailed study of prostate carcinogenesis. This project is one of many initiated to utilize recently developed techniques and expertise in the study of this disease.

Proposed Course: This project is expected to continue at least three years to determine the exact nature of the changing metabolic patterns in each of the lobes of the two species as a function of age and hormone substrate, and to determine the changes in the high affinity receptor in each of the lobes on a comparative basis.

Date Contract Initiated: June 28, 1972

Current Annual Level: $111,892

STANFORD RESEARCH INSTITUTE (NIH-NCI-71-2166)

Title: Combined Effects of Chemical Carcinogens and Other Chemicals

Contractor's Project Director: Dr. David Jones

Project Officer (NCI): Dr. Richard Bates

Objectives: To investigate (1) the effect of mixtures of carcinogenic and noncarcinogenic chemicals on the induction of tumors when introduced into rodents by oral administration, (2) whether the interactions among groups of chemicals in acute and subacute toxicity experiments predict the effects of the same combinations in carcinogenicity experiments, and (3) the feasibility of conducting initial screening bioassays of chemicals in groups without thereby obscuring the carcinogenicity of any individual member of the groups.

Major Findings: The thirteen compounds selected for lifespan feeding, singly

and in various paired combinations, to Fischer 344 rats of both sexes have been acquired and tested for identity, purity and stability in diet. Preliminary acute (2-week) and subacute (8-week) studies have been completed for all but two compounds. Lead groups in the chronic studies have been on test for six months, so that primary data on lifespan distribution of tumors are not yet available.

Significance to Biomedical Research and the Program of the Institute: A major portion of the resources of the National Cancer Institute is devoted to testing environmental chemicals for carcinogenicity. In these tests, the experimental protocols generally attempt to expose animals to one chemical while minimizing exposure to other chemicals. There are two problems involved in this approach. First, in the human environment, men are exposed to multiple chemicals simultaneously and sequentially rather than to a single chemical. Second, resources are available to test only a small portion of the chemicals in our environment if they are tested singly. Newer chemicals are generated faster than the available resources are capable of testing each one by a protocol considered acceptable. This project is the beginning of a program that will investigate how mixtures of carcinogenic and non-carcinogenic chemicals interact to affect the induction of tumors and also to investigate the feasibility of conducting initial screening bioassays of chemicals in groups without thereby obscuring the carcinogenicity of any individual member of the group. The biological studies from this contract will be analyzed by mathematical statisticians working on a separate contract with the NCI to determine whether consistent mathematical models of interactions can be developed.

Proposed Course: It is estimated that this project will extend over four years. Acute and subacute studies of individual chemicals and pairs of chemicals are conducted in order to determine the dosage levels to be administered in the chronic carcinogenic studies. The results of all three studies will be analyzed statistically in order to determine what extent the interactions of two chemicals in carcinogenesis parallel the interaction seen in the short-term acute and subacute studies.

Date Contract Initiated: June 24, 1971

Current Annual Level: $761,594

TEMPLE UNIVERSITY (NO1-CP-33262)

Title: Biochemical and Morphological Components of Hepatic Carcinogenesis

Contractor's Project Director: Dr. Emmanuel Farber

Project Officer (NCI): Dr. Richard A. Pledger

Objectives: To develop a bioassay technique for rapid screening of potential hepatocarcinogens using the hyperplastic nodule as the end-point; to establish the relationship of nodular hyperplasia to the origin of hepatocellular neoplasms; to characterize biochemically and morphologically subcellular markers of the hyperplastic nodule and determine their occurrence in nodules produced

with diverse carcinogens; and obtain tissue in larger amounts for these studies. The test system should thus provide a means for rapid identification of hepatic carcinogens and offer insight into molecular mechanisms of pathogenesis.

Known or suspected hepato-carcinogens and hepato-toxins are fed to Wistar or Fischer strain rats in a basal diet of known composition and the production of hyperplastic nodules and/or malignant hepatomas are noted. Relationship of nodular proliferation to tumorigenesis are studied by histological observations at the light and electron microscope levels.

Major Findings: Standardized regimens for inducing hyperplastic nodules in the liver of animals have been obtained with 2-acetylaminofluorene (2-AAF), aflatoxin B1 and ethionine. The nodules are readily identified both on routine histologic examination as well as with special stains for glycogen. In contrast, alpha-naphthyl-isothiocyanate produces severe chronic liver disease without evident nodules. Unexpectedly, a normal bile acid, lithocholic acid, known to induce chronic liver disease, was found to induce severe liver hyperplasia that resembled that seen with the known carcinogens. By combining an acute injury to liver with short term feeding of the carcinogen, it has become possible to induce nodule-like formation within 2 to 3 weeks. This may enable the very rapid bioassay of chemicals for potential carcinogenic action using an end-point other than initiation.

Significance to Biomedical Research and the Program of the Institute: Hyperplastic nodules are a critical and potentially requisite, precancerous lesion which occurs prior to overt carcinomas. It is desirable to have as much biochemical and morphologic information as can be obtained on the events that characterize the early cellular response to carcinogens. These techniques may in the future become useful in screening procedures of suspected carcinogens.

Proposed Course: (a) To continue to define morphological and biochemical correlations in hepatic hyperplastic nodules produced by carcinogens, with the aim of elucidating common cellular and molecular changes caused by these carcinogens and (b) to develop a better scientific basis for a rapid bioassay procedure for carcinogens with end-points occurring later than the first initial interaction.

Date Contract Initiated: January 20, 1964

Current Annual Level: $42,467

TENNESSEE, UNIVERSITY OF (NIH-NCI-69-2077)

Title: Carcinogenic Studies of Polyurethanes

Contractor's Project Director: Dr. John Autian

Project Officer (NCI): Dr. Richard Bates

Objectives: Polyurethanes are widely used in the human environment and there is experimental data suggesting that some of these plastics may be carcinogenic.

However, polyurethanes are made with widely diverse chemical structures and none of the studies indicating possible carcinogenicity has been done with materials of known chemical structure. Thus, this contract was established to test the variety of chemically different polyurethanes of known structure by intraperitoneal implantation into rats and mice to ascertain what role the chemical structure may play in the induction of tumors.

Major Findings: A variety of chemically different polyurethanes were implanted into groups of rats and mice. Mortality in mice was very high in the early phases of the study, but even in the animals which survived over one year no tumors were observed. The rat studies resulted in local fibrosarcomas in most groups, but have failed to confirm the observation of Hueper that implanted polyurethanes can induce adenocarcinomas. It is still possible that these may develop after a longer latent period. However, if nothing but local sarcomas are seen, it will be difficult to determine whether these have resulted from the chemical or from the physical nature of the implants.

Significance to Biomedical Research and the Program of the Institute: The wide scale use of polyurethanes for various types of products and building materials has raised questions as to the potential hazards of these materials when particulates or powders are inhaled or injested by workers during their course of employment. Once absorbed by man these particles may degrade releasing carbamate or aromatic amine moieties or other chemical structures having possible carcinogenic activity. The data available suggested that the tumors may have been due to (1) chemical carcinogenesis, (2) solid state carcinogenesis, or (3) a combination of both types of carcinogenesis. Those acting by a chemical mechanism are likely to pose a much more serious problem to man than those acting by a solid state mechanism.

Proposed Course: This contract has fulfilled its objective and will not be renewed. Existing experiments are being completed and a final report will be submitted. Final conclusions could form a background against which future, and more definitive experiments could be designed, if such is warrented.

Date Contract Initiated: June 24, 1969

Current Annual Level: $64,000

TENNESSEE, UNIVERSITY OF (NIH-NCI-72-3282)

Title: Maintenance of Organ Explants from Rodent Pancreas

Contractor's Projector Director: Dr. Leonard Murrell

Project Officer (NCI): Dr. Stuart Yuspa

Objectives: The technique of organ culture is increasingly being used to bridge the gap between cell culture and *in vivo* conditions. The ability to maintain a differentiated tissue in culture permits direct histological and/or biochemical assessment of the effect(s) of chemical carcinogens on the tissue itself, and, simultaneously, evaluation of products released by

cultured explants. It is becoming apparent that each organ has specific culture requirements. In addition to the requirements common to all in vitro environments (temperature, humidity, etc) it is now recognized that many cultured organs require rigorous control (at specific levels) of pH, osmolality, hormonal milieu, and other system dependent factors, such as oxygen concentration. Therefore, any realistic approach to maintaining pancreas in culture must include detailed assessment of each of these conditions, as well as evaluation of their possible interactions. The present project attempts to elucidate some of the factors necessary to maintain rodent pancreas--and ultimately human pancreas--in organ culture. After development of appropriate culture conditions, the organ cultures will be used in studies of chemical carcinogenesis of the pancreas.

Major Findings: Classic organ culture methods are unsuitable for pancreatic acinar tissue. Therefore a perfusion culture system has been devised which automatically replaces culture medium at predetermined intervals. During the first seven months of this contract, perfusion cultures of pancreatic explants from 17-day post-fertilization rat fetuses, from neonatal rats, and from month old rats have been compared to static organ cultures of pancreatic explants from animals of the same ages. Such cultures, with a chemically defined culture medium and controlled composition gassing, suggest that: (1) pancreatic survival in organ culture is directly proportional to age at explantation; (2) amylase released from tissues into culture medium parallels, at least qualitatively, the viability of tissues as reflected by their histological structure; and (3) perfusion pancreatic cultures remain "viable" longer than control static cultures identical in treatment except for culture medium perfusion.

Significance to Biomedical Research and the Program of the Institute: Cancer of the pancreas results in over 18,000 deaths per year in the United States, and the incidence is rising. Among the various types of cancer causing death in the country, it ranks sixth. In spite of this, there has been little experimental work on cancer of the pancreas. There are no adequately developed animal models of this kind of cancer or adequate approaches to study in vitro transformation of pancreatic exocrine cells. This project is one of several initiated by the Biological Models Segment in order to develop animal models and experimental approaches to the study of the etiology and pathogenesis of this major form of human cancer.

Proposed Course: Current perfusion culture conditions preserve pancreatic structure and function for brief culture periods. To extend this viable period, exploration of the effects of the system dependent factors listed above will continue. Since it is probable that at least some of these factors will interact, statistical analyses designed to elucidate any interaction effects are included in the research plan. During the second year of this contract, it is hoped that organ culture conditions capable of maintaining pancreatic explants over periods suitable for testing potential carcinogenic stimuli will be defined.

Date Contract Initiated: June 29, 1972

Current Annual Level: $82,984

WEST VIRGINIA UNIVERSITY (NIH-NCI-72-3283)

Title: Relationship of Pituitary Hormones and Androgen on Prostate Metabolism

Contractor's Project Director: Dr. John A. Thomas

Project Officer (NCI): Dr. Douglas H. Janss

Objectives: The objective of this contract is the investigation of the influence of pituitary hormones on the morphology and biochemistry of various prostate lobes of several species of animals. The interaction of male sex steroids and non-gonadotropic hormones in vitro will be studied. Whether androgen-nongonadotropic hormone synergism observed in certain lobes of the prostate gland is due to prolactin-induced changes in the binding and/or the subcellular distribution of male sex hormone will also be determined. It will be established whether or not the levels of cyclic 3',5' AMP in the prostate gland are altered in the presence of either prolactin or somatotropin. Results from these studies may lead to a better understanding of some of the biochemical events involved in the normal and abnormal growth of the prostate gland.

Major Findings: Levels of sex accessory gland cAMP-H3 formed from adenosine-H3 are influenced by androgens. Castration generally reduced the levels of cyclic nucleotide while either testosterone or dihydrotestosterone enhanced its formation in vitro. Endogenous levels of cAMP did not differ among the various lobes (e.g. anterior, lateral, dorsal and middle) of the rat prostate gland. Varying doses of prolactin(ovine)(2,4 or 8 I.U.) exerted little effect on endogenous levels of cAMP in the different lobes of the rat prostate gland in vitro. However, the lateral lobes did exhibit some increases in the levels of cAMP following incubation with high doses of prolactin. Incubation of prostate glands (whole lobes, slices or homogenates) with radioactive testosterone resulted in the formation of significant amounts of dihydrotestosterone-H3. When rat prostate glands were incubated with prolactin, there generally was a significant reduction in the formation of dihydrotestosterone-H3, androstanedione-H3 and androstenedione-H3. Such reductions were usually more marked in the ventral and the dorsal lobes of the rat prostate gland, although some changes were also related to the particular age of the animal.

Significance to Biomedical Research and the Program of the Institute: It is estimated that there will be 35,000 new cases and 17,000 deaths from adenocarcinoma of the prostate this year. In spite of its role as a major cause of human deaths from cancer, there are no experimental models for this kind of cancer and very little work is being done to develop such models. It is generally accepted that secretions of the adenohypophysis are necessary for normal growth and metabolic integrity of accessory sex organs. This dependent relationship is indirect and is mediated through the testes. Evidence of direct organ effects of adenohypophysial secretions (prolactin or somatotropin) on accessory sex organ activity has not been as clearly accomplished as the demonstration of the indirect effects mediated through the gonads. This project is one of several developed by the Biological Models Segment in order to stimulate research on the etiology and pathogenesis of adenocarcinoma of the prostate. These projects are designed to select the most appropriate animals for experimentation, to develop tissue culture techniques utilizing

the prostate, and to use both in vivo and in vitro techniques for investigating the induction of adenocarcinoma of the prostate.

Proposed Course: The general approach will continue to be investigated. However, a greater emphasis will be given to in vivo experiments and to the effects that either prolactin or growth hormone exert on the subcellular distribution of radioactive androgens in different lobes of the prostate gland. The in vivo studies will be carried out principally in the different lobes of the rat prostate.

Date Contract Initiated: June 28, 1972

Current Annual Level: $74,371

WRIGHT STATE UNIVERSITY (NIH-NCI-72-3281)

Title: Cell Culture Development of Human and Guinea Pig Pancreatic Cells

Contractor's Project Director: Dr. Robert Hay

Project Officer (NCI): Dr. Stuart Yuspa

Objectives: To establish reproducible techniques for cultivation of pancreatic exocrine cells. These cultures will then be used for studies on chemical carcinogenesis.

Major Findings: Reproducible conditions have been defined for the primary isolation and maintenance of at least 3 distinct colonial cell groupings from human and guinea pig pancreas. Fibroblast clones are the most morphologically variable and have been classed for convenience as one type (F). Two epithelial cell types in culture (E1 and E2) have been recognized. E2 clones appear to develop from single cells, proliferate to a limited extent only, and have not yet been shown to yield products specific to exocrine cells. E1 cells are observed in colonial aggregates which are formed by gyration of the original cell suspensions. Such aggregates, which form a two dimensional colony on plating, release amylase for 96 to 120 hours after primary dissociation. Both E1 and E2 colony types have been maintained in vitro for over 30 and 100 days respectively. Subcultivation with retention of epithelial morphology has not yet been possible. The cells become rounded and more refractile, and eventually degenerate. No occurrence of spontaneous transformation with cells of either species has been noted.

Significance to Biomedical Research and the Program of the Institute: To develop an in vitro model system for the study of the events involved in the malignant transformation of pancreas would be a useful first step in understanding a disease responsible for 18,000 deaths a year. This project is part of an overall approach to find new methods to study major types of human malignant disease.

Proposed Course: Studies are in progress to determine the effects of various hormones, essential metabolites and secretogogues on protein and DNA synthesis

in cultured pancreatic cells. Concomitantly, more detailed investigations of the morphology of these epithelial colonies are to be initiated at both light and electron microscopic levels. The intent will be to maintain and characterize functional exocrine cells. Attempts will then be made to transform these into malignant pancreatic cells using chemical carcinogens. It is estimated that this project will last three years or more.

Date Contract Initiated: June 28, 1972

Current Annual Level: $50,153

SUMMARY REPORT

BIOLOGY AND IMMUNOLOGY SEGMENT

July 1, 1972 through June 30, 1973

The goals of the Biology and Immunology Segment are to apply knowledge in cell biology and immunology to the identification of carcinogenic agents and the prevention of cancers.

The program of this Segment includes two main areas of activity: an *in vitro* transformation program and an immunology program. The mission of this segment is to pursue those goals of the National Cancer Plan which for one or more reasons can best be done outside the National Cancer Institute. Contracts, therefore, are initiated at suitable sites such as universities, institutes, medical schools, commercial laboratories and foundations. The present categories of projects developed in this Segment are as follows:

(1) Evaluation of rapid methods for *in vitro* neoplastic transformation by chemicals and their applicability to screening environmental agents for carcinogenic potential;

(2) Identification and standardization of bacterial agents and fractions of these agents that induce immunological reaction against tumors;

(3) Characterization of tumor associated antigens that may be useful in the detection of cancer at a stage when interruption of the carcinogenic process might be possible;

(4) Participation of accessory factors of the immune system, such as complement, in the control of early cancer;

(5) Identification by immunologic methods of population groups exposed to known carcinogens;

(6) Influence of the immune system on carcinogenesis.

In Vitro Transformation Program

In vitro studies on the nature of interactions between chemicals and viruses are being done under contract at Biolabs, Inc., and Ohio State University Research Foundation. At Biolabs, Inc., the primary objective is to study *in vitro* assay systems which could be used for identification of potential carcinogens and to use these systems to evaluate the role of chemical carcinogens in promoting (enhancing) viral carcinogenesis.

Investigations have revealed that treatment of freshly isolated diploid fetal hamster cells with benzo(a)pyrene, 3-methylcholanthrene, 7-12,dimethylbenz(a)-anthracene, dibenz(a,c)anthracene or dibenz(a,h)anthracene enhances SA7 virus transformation by two-to eightfold when the chemical is added 18 hrs. prior to virus inoculation. The addition of benzo(a)pyrene, 3-methylcholanthrene or 7,12-dimethyl-benz(a)anthracene 4 hrs. after virus inoculation of the cells

in contrast results in a depression of transformation by as much as tenfold. The noncarcinogens phenanthrene, pyrene, perylene and benzo(e)pyrene do not enhance SA7 virus transformation. In comparison to the polycyclic hydrocarbon carcinogens, viral transformation was enhanced by the proximate carcinogens N-methyl-N'-nitro-N-nitrosoguanidine, or N-acetoxy-2-fluorenylacetamide or the alkylating agent methylmethanesulphonate when cells were treated 2 to 18 hrs. prior to as well as 5 hrs. after virus addition. Treatment of the cells either before or after virus addition with diethylnitrosoamine, dimethylnitrosoamine or 2-fluorenylacetamide, carcinogens inactive in standard mammalian cell in vitro systems presumably due to the requirement for metabolic activation, does not enhance viral transformation.

Increases in transformation frequency are directly related to chemical carcinogen; however, concentrations above those necessary to achieve maximal enhancement often reduce or completely inhibit viral transformation. The enhancement of transformation is not related to chemical selection of transformation-sensitive cells since the absolute number of foci per plate increases up to sixfold under conditions where little or no cell death occurs. Enhancement of viral transformation is also not attributable to the presence of chemically-induced foci as under the assay conditions foci of transformed cells are not observed using chemicals alone. The observations suggest that chemical carcinogens enhance viral transformation by interacting directly with the host cells rendering them more susceptible to transformation by virus.

At Ohio State University the objective is to evaluate human cell culture systems as in vitro assays for chemical carcinogenesis. This includes identification of potential carcinogens, evaluation of the role of hormones as inhibitors or promoters of in vitro carcinogenesis and investigation of the role of non-oncogenic and oncogenic viruses in promoting chemical carcinogenesis in human cells.

Investigations have demonstrated the formation of morphologically transformed foci of human embryonic lung fibroblasts subsequent to exposure to benzo(a)pyrene or N-methyl-N'-nitro-N-nitrosoguanidine in the presence of corticosteroids. Cell strains derived from transformed foci consisting of aggregates of multilayered fibroblasts or loosely intertwined crisscrossing fibroblasts have been heterotransplanted into the cheek pouches of thymectomized cortisone treated hamsters to evaluate the tumorigenicity of the cells.

Immunology Program

The isolation and purification of tumor suppressive molecules from BCG cell walls is the primary goal at the Rocky Mountain Laboratory, NIAID. Doctor Ribi has isolated a homogeneous material, termed P3, which in combination with the skeleton of the bacterial cell wall has tumor suppressive activity. P3 and cell wall skeleton, when tested individually, had minimal or no tumor suppressive action. This project, supported by interagency agreement between NCI and NIAID is also a source of BCG cell walls and cell wall fractions for persons interested in the effects of these materials on malignant disease.

At the Trudeau Institute work is under way to determine which BCG substrain has maximal tumor suppressive properties and to define conditions of BCG

preparations that preserve tumor suppressive activity. Four BCG substrains prepared under identical conditions were found to have equivalent tumor suppressive activity. The viability of BCG prepared at the Trudeau Institute (fresh frozen) is about 50-80%. The viability of several commercially available BCG preparations (freeze-dried) is about 1-2%. It is possible that dead BCG may have an adverse effect on BCG mediated tumor killing. This possibility is under investigation. This contract has functioned as a source of BCG for persons interested in the effects of BCG on malignant disease.

Another contract dealing with cell-mediated immunity, at the University of Illinois College of Medicine, has as its primary goal the determination of whether resistance to tumor growth can be transferred with cell-free extracts. Doctors Dray and Paque have shown that delayed hypersensitivity to soluble tumor antigens can be transferred in vitro. Inflammatory cells from the peritoneal cavities of normal guinea pigs were incubated with extracts of cells obtained from immunized animals. These "conditioned" normal cells responded as though they were obtained from an immunized animal.

Four contracts are devoted to studies of humoral tumor immunity. The significance of two fetal antigens is being studied during and after carcinogenesis: a-fetoglobulin and carcinoembryonic antigen (CEA). CEA arises in patients suffering from a wide variety of cancers of epithelial origin while a-fetoglobulin appears in animals and patients suffering from various forms of cancer of the liver. The goal of these contracts is to (1) define the chemical nature of these antigens, (2) devise better and refined tools for their detection, and (3) define their significance and relationship to the carcinogenic process. Although these contracts are in an early developmental stage, some progress has been made. For example, a rapid radioimmunoassay for human CEA is now being tested for its usefulness in monitoring CEA levels in large populations (Mallory Institute of Pathology). A radioimmunoassay for rat a-fetoblobulin is now operational and is being used to study the appearance of the molecule in sera of animals exposed to chemical carcinogens (University of California). This test is not available for guinea pigs and since guinea pigs serve as useful models for chemical carcinogenesis a test is being developed for a-fetoglobulins in this species (University of California). The effect of chemical carcinogens on the complement system is also under investigation (Children's Hospital, Boston). The second and fourth components are synthesized in macrophages: cells that seem to be of primary importance in the body's defense mechanism against cancer cells. It was found that the first sign of macrophage impairment in guinea pigs under treatment with diethylnitrosamine (DEN) is the loss of synthesis of the fourth component. This loss, however, can be found only if the chemical is administered to the animal; in vitro exposure does not result in C4 impairment. This finding suggests that DEN must undergo a metabolic change in the animal before this effect is measurable. The impairment of C4 synthesis is followed by the loss of other macrophage factors such as ability to adhere to surfaces. These exciting findings are now followed up under an expanded program.

Studies based on the fact that carcinogens are allergens and haptens are being done under four contracts. These studies have confirmed that certain polynuclear hydrocarbon carcinogens can sensitize guinea pigs so that a second exposure to the agent is recognized as an allergic reaction. Some progress has been

made (University of Texas and Becton-Dickinson) in finding suitable coupling agents for the carcinogens so that they become immunologically active in vivo. Antibodies are being made to several carcinogens by Dr. Van Vunakis at Brandeis University. In addition, rabbit antibodies have been made that react specifically with nicotine. Antibodies have also been made that react specifically with cotinine. Coded serum specimens from smokers and non smokers were tested by a radioimmunoassay using the rabbit antibodies to nicotine and cotinine. There was almost complete correlation between the presence of nicotine and cotinine in the sera and the fact that the specimen came from a smoker. These reagents should be useful in assessing the degree of smoking among individuals and as a means of testing the relative hazards of different cigarettes. At Case Western Reserve University animals are rendered immunologically tolerant to a chemical carcinogen and then tested for susceptibility to tumorigenesis by that carcinogen.

Three additional different projects are supported: One of them (Temple University) deals with a model for immunological studies of malignant melanoma in guinea pigs. This contract was started recently and melanomas are now being produced. At the University of Utah studies are being conducted to determine whether immunosuppressed mice have a lowered threshold of carcinogenesis. A phase of the guinea pig transplantable hepatoma studies dealing with the histopathology of tumor regression is being studied at the Oak Ridge National Laboratory.

AEC-NCI INTERAGENCY AGREEMENT (OAK RIDGE NATIONAL LABORATORY)(NCI-FS-64-13)

Title: NCI-AEC Carcinogenesis Program

Contractor's Project Director: Dr. Francis T. Kenney

Project Officer (NCI): Dr. Allen Heim

Contract narrative is reported under the Office of the Associate Scientific Director for Carcinogenesis.

BECTON, DICKINSON AND COMPANY RESEARCH CENTER (NIH-NCI-71-2168)

Title: Carcinogens as Allergens: Detection of Exposure to Carcinogens by Cell-Mediated Immunologic Reactions to the Carcinogens

Contractor's Project Director: Dr. Jerry J. Tulis

Project Officer (NCI): Dr. Herbert J. Rapp

Objectives: To determine whether exposure of experimental animals to known carcinogens via various routes will result in the elaboration of in vivo (delayed hypersensitivity) and in vitro (lymphocyte transformation or macrophage inhibition) immunologic reactions. The ultimate goal of the program is the development of in vitro screening procedures to determine the potential hazards to man from exposure to environmental carcinogens. Ancillary objectives include determinations of the retention, distribution, and clearance of carcinogens in aerogenically exposed animals.

Major Findings: Findings to date include the elaboration in experimental animals of delayed sensitivity responses upon subcutaneous or topical administration of benz[a]pyrene, dimethylbenzanthracene, benz[a]anthracene, or methylcholanthrene. Aerosol exposure of animals to these carcinogens has not yielded definitive evidence of induced delayed sensitivity. Preliminary data indicate that lack of sensitization via the aerosol route was not the result of immune tolerance. Progress has been made in the preparation and characterization of carcinogen-protein conjugates which act as allergens.

Significance to Biomedical Research and the Program of the Institute: The development of immunologic sensitivity in experimental animals upon exposure to known carcinogens as determined by in vivo and in vitro methods may provide insight to the environmental or occupational hazard and mechanism of action of carcinogens and provide information on environmental monitoring for these materials.

Proposed Course: Future work will include the screening of additional carcinogenic materials for elaboration of cell-mediated immunologic reactions, aerosol retention studies, tolerance and cross-reactivity studies, characterization of the in vivo binding of the hapten (carcinogen) with native protein, and monitoring for humoral responses in animals exposed to carcinogens.

Date Contract Initiated: June 22, 1971

Current Annual Level: $171,793

BIOLABS, INC. (NIH-NCI-71-2164)

Title: In Vitro Study of Interaction between Chemical and Viral Carcinogens

Contractor's Project Director: Dr. Bruce Casto

Project Officer (NCI): Dr. Joseph DiPaolo

Objectives: The specific objective of this contract is to study the process whereby chemical carcinogens promote viral tumorigenesis and to determine if oncogenic or non-oncogenic viruses may similarly promote tumorigenesis of known chemical carcinogens. This study is part of a program to develop in vitro screening methods for detection of potential carcinogenic hazards, either viral or chemical.

Major Findings: During the last year of this contract, several polycyclic hydrocarbons (carcinogenic and non-carcinogenic), 2-acetylaminofluorene (2-AAF) and its derivative acetoxy-AAF, methylmethanesulfonate (MMS), methylazoxymethanol acetate (MAM-Ac), N-nitrosodiethylamine (DEN), N-nitrosodimethylamine (DMN), and N-methyl-N-nitro-N-nitrosoguanidine (MNNG) were tested in hamster cells in combination with SA7. The polycyclic hydrocarbons, benzo(a)pyrene[BP], 3-methylcholanthrene (MC), dibenz(a,h)anthracene (1, 2, 5, 6-DBA), dibenz(a,c)anthracene (1,2,3,4-DBA), and 7,12-dimethylbenzanthracene (DMBA), which cause transformation of hamster cells in vitro (DiPaolo et at, 1969) increased the frequency of SA7 transformation of hamster embryo cells by 2- to 10- fold, but the non-carcinogens, phenanthrene (a), perylene (Pe) and pyrene (Py), failed to do so.

Enhancement of SA7 transformation was demonstrated with MC, BP and DMBA if cells were treated for 18 hr prior to virus addition; however, if these chemicals were added 4-5 hr post-inoculation, enhancement was not observed, or the transformation frequency was decreased by 5- to 10- fold.

An enhanced frequency of SA7-transformation was also observed when hamster cells were treated with MMS, MNNG, MAM-Ac, and acetoxy-AAF, but was not demonstrated with 2-AAF, DEN or DMN under the same conditions.

Direct evidence for DNA strand breaks was obtained by sedimentation of alkaline-denatured cell DNA through alkaline-sucrose gradients. The rationale for these studies was to determine if a direct correlation exists, for certain chemical carcinogens, between enhancement of viral transformation and the ability of a chemical to induce strand breaks in cell DNA. Single-strand breaks in cell DNA

were readily observed following 1-2 hr treatment of cells with compounds such as MMS, MNNG and acetoxy-AAF.

The effect of BA and ANF treatment of hamster cells on the enhancement of viral transformation of hamster cells by BP, DMBA and MC. Pretreatment of hamster cells for 24 hr with ANF: (a) inhibited the cytotoxicity of B(a)P, DMBA, and, to a lesser extent, MC; and, (b) inhibited enhancement of transformation by DMBA and B(a)P and had little effect on enhancement by MC. Simultaneous treatment of cells with ANF and the three carcinogens resulted in essentially the same effects as those outline above.

Some of the more interesting features of these experiments were: (a) in each case where the cytotoxicity was inhibited and the enhancement decreased (but not inhibited), the actual number of SA7 foci counted per plate greatly increased; (b) cells pretreated 24 hr with BA and subsequently challenged with DMBA (0.1 ug/ml) were essentially identical to cells not treated with BA in terms of cytotoxicity (1.5% and 1.4% of the cells surviving DMBA treatment respectively), but BA pretreated cells yielded 150 SA7 foci while no foci appeared in cells treated with DMBA alone; (c) the same phenomenon was found with BA and B(a)P, in that cells treated with BA and BP alone, but the number of SA7 foci was increased from 0 to 244 (simultaneous treatment with BA) or from 0 to 86 (24 hr pretreatment with BA); and (d) with 1.0 ug/ml of MC, simultaneous treatment with BA increased the actual number of foci from 19 (MC alone) to 122 (MC/BA) where only a 2-fold increase in survivors was found.

Significance to Biomedical Research and the Program of the Institute: In view of the various hypotheses concerning chemicals, viruses, and cancer, and due to the inherent difficulties in studies in the intact animal, it is advantageous to study the various interactions in a controlled *in vitro* system. This would establish not only the influence of oncogenic and non-oncogenic viruses in promoting chemical carcinogenesis but the role of chemical carcinogens in promoting viral tumorigenesis.

Proposed Course: The methods to be used in the proposed project have been developed in conjunction with the principal investigator in earlier studies of *in vitro* transformation. Diverse chemicals that are important to the human environment including pesticides, herbicides and industrial chemicals will be considered for their ability to influence viral transformation. The nature of the persistance and inhibition of enhancement by chemical carcinogens will be the subject of further studies. An attempt will be made to correlate the persistance of enhancement with DNA breaks, and where such breaks are not found an attempt will be made to find the bound chemical carcinogen. The factors influencing the quantitative transformation will be determined by using compounds known to influence the toxicity of carcinogens as well as by altering the type of cells at risk, the concentration of the chemical compound, and as the length of time the cells are exposed. An evaluation will also be made to determine if the phenomenon of enhancement observed will occur with other types of host cells and viruses. The uniqueness of this quantitative transformation is that transformation is enhanced when diploid cells are treated with chemical prior to the addition of virus; if virus is added subsequent to the chemical inhibition of transformation and/or enhancement of transformation occurs. Further studies will be carried out to determine

the nature of inhibition. Cells transformed by the combined action of chemical and virus will be characterized and compared to cells transformed by virus alone. Preliminary evidence indicates that the ability to grow in soft agar and tumorigenicity in animals, indicate that the chemical may contribute properties to the cells as well as stimulate viral transformation. Thus, the clones that have been transformed will be studied for antigenic changes (especially viral-specified antigens) and chromosome alteration with the hope that these parameters may be correlated with the altered tumorigenicity.

Date Contract Initiated: June 9, 1971

Current Annual Level: $186,375

BRANDEIS UNIVERSITY (NIH-NCI-72-3243)

Title: Production and Detection of Antibodies to Chemical Carcinogens and Other Small Molecules

Contractor's Project Director: Dr. Helen Van Vunakis

Project Officer (NCI): Dr. Herbert Rapp

Objectives: To obtain antibodies that react specifically with chemical carcinogens and other small molecules of interest in cancer research. The antibodies will be used as reagents to detect the presence of corresponding haptens in body fluids and tissues of animals and humans. Haptens attached to appropriate carriers will be used as antigens to detect presence of antibodies in the serum of animals and humans. These reagents will be tested for their suitability for the detection of exposure to environmental agents that may be related to cancer causation.

Major Findings: Sensitive and specific radioimmunoassays for nicotine and one of its major metabolites, cotinine, have been developed. Since nicotine is rapidly metabolized in the body, it was found in the sera of smokers only when blood for analysis was withdrawn minutes after they smoked. Concentrations of nicotine as high as 70 ng/ml have been found in such sera. The nicotine excretion in urine ranged up to 3 mgs/24 hr. sample. Whether nicotine in smokers' urine represents unaltered nicotine or a metabolite that has been reconverted to nicotine by a reductase is not clear at this time.

Cotinine has a half life of days and is present in the sera of smokers in concentrations as high as 600 ng/ml. Cotinine levels as high as 30 mgs/24 hr. sample are found in the urine of smokers. Cotinine is also present in the amniotic and spinal fluids of smokers. In cord venous and cord arterial blood, the cotinine levels were almost identical in samples from both circulatory systems, indicating that cotinine can pass from the circulation of the smoking mother into that of the fetus.

Results indicate that there is little or no correlation between the number of cigarettes smoked and the cotinine and nicotine levels in physiological fluids. The intake of nicotine is quite variable among smokers, since they inhale differently, take different numbers of puffs from a cigarette and may detoxify

nicotine in different ways depending upon the relative activities of the enzymes present in their tissues. Correlation of pathological conditions with levels of metabolite in various physiological fluids (rather than with smoking histories) might prove more informative in epidemiological studies.

Significance to Biomedical Research and the Program of the Institute: This work may provide a way to determine the degree to which individuals in different environments have been exposed to known chemical carcinogens. The immunologic approach does not require that the carcinogen be present in the individual at the time of testing. If the carcinogen or a related structure is present, it can be detected by reacting with the antibody. Demonstration that different groups are at risk may provide the impetus to remove certain agents from the environment.

Proposed Course: To proceed with the objectives as stated. Rabbits or other suitable animals will be immunized with the chemical substance of interest attached to a carrier that promotes the antigenic activity of the agent. Among the agents to be used are methylcholanthrene, dimethylbenzanthracene and nicotine. Others will also be tested. Radioimmunoassays will be tested.

Date Contract Initiated: April 20, 1972

Current Annual Level: $97,507

CALIFORNIA, UNIVERSITY OF (At La Jolla) (NIH-NCI-72-3258)

Title: Significance and Relationship of Fetoglobulins to the Induction of Hepatomas by Chemical Carcinogenesis

Contractor's Project Director: Dr. Stewart Sell

Project Officer (NCI): Dr. Tibor Borsos

Objectives: The objective of this contract is to develop a sensitive new method to study the relationship between the induction of primary hepatomas and the appearance and significance of fetoglobulins.

Major Findings: Rat alpha-fetoprotein, an oncofetal antigen associated with carcinoma of the liver, has been isolated from rat amniotic fluid. A sensitive radioimmunoassay has been developed. Rat alpha-fetoprotein is found in high levels in newborn and pregnancy sera but in only trace amounts in normal sera. High levels have been detected in association with rapidly growing transplantable Morris hepatomas, but values within normal limits have been detected in sera from rats bearing slow growing hepatomas. The half-life of alpha-fetoprotein is only 24 hours using radiolabelled alpha-fetoprotein indicating that the serum level should rapidly reflect changes in synthesis. Preliminary studies on sera from rats fed carcinogens indicate that alpha-fetoprotein serum levels may rise before frank neoplastic lesions are present.

Significance to Biomedical Research and the Program of the Institute: The primary goal of the Carcinogenesis Program is to prevent cancer in man by identifying carcinogenic compounds and formulations that may affect humans, as well

as by identifying the mechanism of action of known carcinogens. Both these approaches require extensive bioassay and other analytical technologies, as well as a continuing vigorous program for refinement and innovation of the same technologies.

This contract is suited to the goal-oriented program of the Biology and Immunology Segment of the Carcinogenesis Program. It applies immunochemical techniques to the detection and to the study of the significance of alpha-fetoglobulins as a consequence of chemical carcinogenesis. Furthermore, it has direct applicability to prospective and follow-up studies of cancer patients. This test may ultimately prove useful in monitoring workers who are exposed to carcinogens.

Proposed Course: Systematic studies on the effect of dietary carcinogens on alpha-fetoprotein levels in the rat are now feasible and will be the first objective during the second contract year. In addition, the kinetics of serum alpha-fetoprotein appearance in relationship to the growth of transplantable hepatomas will be determined. Guinea pig and human alpha-fetoprotein as well as well as rat alpha-macrofetoprotein will be isolated with the goal of developing radioimmunoassays for these proteins.

Date Contract Initiated: June 28, 1972

Current Annual Level: $132,436

CASE WESTERN RESERVE UNIVERSITY (NIH-NCI-72-3220)

Title: Specific Immunological Unresponsiveness to Chemical Carcinogens and Its Influence on Tumorigenesis

Contractor's Project Director: Dr. Jerome Pomeranz

Project Officer (NCI): Dr. Herbert Rapp

Objectives: To determine whether immunological responses to chemical carcinogens influence tumorigenesis qualitatively or quantitatively.

Major Findings: Optimal methods for regularly inducing contact sensitization to polycyclic aromatic hydrocarbons, in particular 7,12 dimethylbenz(a)anthracene, have been formulated and applied to guinea pigs and mice. In addition, procedures to render guinea pigs immunologically unresponsive to contact sensitization with these compounds by feeding or infusion have been devised. It has also been noted that in contrast to studies measuring humoral responses in mice, the administration of dimethylbenz[a]anthracene to guinea pigs did not alter their capacity to become contact sensitized to a simple chemical such as picryl chloride.

Significance to Biomedical Research and the Program of the Institute: The demonstration that guinea pigs and mice may be contact sensitized to carcinogens provides a means of measuring a parameter of cell mediated immunity to these compounds. The induction of contact tolerance to these chemicals permits

experiments in which the effect of absent cell mediated immunity to carcinogens may be studied. This project will attempt to correlate the presence or absence of specific immunity to a carcinogen with the compounds capacity to induce a malignant tumor.

Proposed Course: Guinea pigs that are immunologically unresponsive or contact sensitized to dimethylbenz[a]anthracene are available. However, methods for uniformly inducing tumors with this compound, which do not sensitize or tolerize the recipient, remain to be perfected. Once this has been accomplished, responsive or unresponsive animals may be evaluated for the effect of immunity to the carcinogen on their susceptibility to oncogenesis with the same compound.

Date Contract Initiated: February 15, 1972

Current Annual Level: $109,171

CHILDREN'S HOSPITAL MEDICAL CENTER (NIH-NCI-71-2278)

Title: Effects of Carcinogens on In Vitro Synthesis of Complement Components

Contractor's Project Director: Dr. Harvey Colten

Project Officer (NCI): Dr. Tibor Borsos

Objectives: The project has three major objectives: (1) to study the effect of chemical carcinogens directly on the synthesis of two immunologically active proteins, C2 and C4; (2) to study the early biochemical events resulting from the exposure of a cell to a carcinogen that inhibits or stimulates protein synthesis using C2 and C4 as biochemical markers; and (3) to develop a potential screening tool for the identification of possible carcinogens.

Major Findings: The following chemicals (in addition to those already reported) have been studied:

1-aminoanthracene
2-aminoanthracene
4-nitroquinoline-n-oxide
4-nitropyridine-n-oxide
7-12-dimethylbenzanthracene
Azoxybenzene
Methylazoymethanol acetate

Of all the agents tested thus far for their capacity to affect complement biosynthesis in vitro, 4-nitroquinoline-n-oxide was the most potent. This agent was effective at a concentration of 10^{-7}M. There was no effect of the agent on cell morphology or survival in vitro. Its non-carcinogenic analoque, 4-nitropyridine-n-oxide had no effect on complement biosynthesis at a concentration of 10^{-5}M. This agent and the other aromatic amines that were studied did inhibit complement biosynthesis and led to morphologic changes in the macrophage at higher concentrations (10^{-3}M, 10^{-4}M).

A hydrocarbon, dimethylbenzanthracene, and two azoxy compounds, azoxybenzene and methyzaoxymethanol acetate inhibited C2 and C4 biosynthesis in vitro. The minimal effective dose of these agents was approximately 10^{-3}M.

Peritoneal macrophages isolated from guinea pigs that were fed DEN for nine weeks synthesized C4 at a rate 20% of normal; the appearance of the cells and their capacity to synthesize C2 and total protein were unaffected. After 16-19 weeks of DEN feeding, chemotaxis, and other membrane dependent functions (glass adherence, "spreading") of the macrophages were depressed. Histological examination of the liver at 19 weeks revealed that these changes in macrophage function preceded the appearance of frank tumor. Incubation of normal peritoneal cells in the presence of DEN had no effect on their morphology or functions, suggesting the possibility that DEN is converted in vivo, but not in vitro to an inhibitor of macrophage function.

Significance to Biomedical Research and the Program of the Institute: The proposed contract would provide a relatively inexpensive, rapid way to examine the early events in carcinogenesis with particular emphasis on the effect of carcinogens on protein synthesis of immunologically active proteins.

The macrophage appears to play a key role in protection against and immunotherapy of malignancy. During the induction phase of chemical carcinogenesis the possibility is raised that in addition to an effect of the agent on the potential tumor cell, there is a concomitant effect of the agent on the immune capacity of the organism permitting establishment and growth of the aberrant cell type. It should be noted that such work could lead, therefore, to both a fundamental and practical understanding of the mechanisms of chemical carcinogenesis.

Proposed Course: The findings of several effects of diethylnitrosamine on macrophage function in addition to its effect on complement synthesis require expansion of future work to include the following:

a. To identify quantitative and qualitative changes in macrophage function associated with in vivo administration of chemical carcinogens.

b. To determine whether these alterations in macrophage function affect immunologic competence during induction of chemical carcinogenesis.

c. To determine, using non-carcinogenic hepatotoxins in vivo, whether changes in macrophage function and immune competence are specific for carcinogenic agents.

Date Contract Initiated: June 1, 1971

Current Annual Level: $40,262

FREDERICK CANCER RESEARCH CENTER (NIH-NCI-72-3294)

Task Order #10

Contract narrative is reported under the Office of the Associate Scientific Director for Carcinogenesis.

ILLINOIS, UNIVERSITY OF (NIH-NCI-72-3205)

Title: Transfer of Tumor Immunity with Cell-Free Extracts of Immune Lymphoid Cells

Contractor's Project Director: Dr. Sheldon Dray

Project Officer (NCI): Dr. Berton Zbar

Objectives: To determine whether tumor immunity can be transferred from an immunized donor to an unimmunized recipient with cell-free material and to isolate and characterize the active component in the cell-free material.

Major Findings: RNA-rich extracts prepared by the phenol method have been shown to confer specific immunologic reactivity to lymphoid cells of untreated animals as indicated by an *in vitro* assay for delayed hypersensitivity (i.e. cell-mediated immunity) which consists of measuring and comparing the migration of packed lymphoid cells from capillary tubes in the presence or absence of specific antigen. This was demonstrated using the specific soluble tumor antigens of two transplantable tumors, designated line-1 and line-10, which had been induced by the same carcinogen, diethylnitrosamine. Strain 2 guinea pigs were immunized to the line-1 and line-10 hepatomas and RNA-rich extracts were prepared from the lymphoid tissues by the phenol method. These RNA-rich extracts were then incubated with the peritoneal exudate cells (PEC) of untreated strain 2 guinea pigs. After, but not before, incubation with the RNA-rich extracts from line-1 or line-10 immunized animals, the PEC were specifically inhibited in their migration from capillary tubes by the line-1 or line-10 antigen, respectively. This indicated that cellular immunity to tumor antigens may be imparted to normal PEC by the RNA-rich extracts of lymphoid tissue.

Significance to Biomedical Research and the Program of the Institute: These results and those obtained previously using other antigens can form the basis for an immunotherapy model for the prevention of or abrogation of tumor growth by the use of these cell-free extracts.

Proposed Course: To determine whether such RNA-treated cells injected into guinea pigs can transfer tumor immunity as measured by the delayed hypersensitivity skin test and by resistance to tumor transplants. Also it is planned to inject such RNA-treated cells into guinea pigs to assess tumor regression. Other cell-free extracts will be used and the active component will be isolated and characterized.

Date Contract Initiated: February 1, 1972

Current Annual Level: $155,350

JOHNS HOPKINS UNIVERSITY (NIH-NCI-72-C-1074)

Title: Model Studies on Chemical Carcinogenesis

Contractor's Project Director: Dr. Paul Ts'o

Project Officer (NCI): Dr. Joseph DiPaolo

Objectives: Under the joint sponsorship of the National Cancer Institute and the Atomic Energy Commission, Johns Hopkins University organized a conference on "Model Studies on Chemical Carcinogenesis". The purpose of this conference was to bring together scientists and scholars in the field of chemical carcinogenesis and related sciences to exchange and examine information and ideas.

The topics discussed can be summarized into the following five areas: (1) recent advances in chemical carcinogenesis with special emphases on polycyclic hydrocarbons; correlation of the physico-chemical, organic, and biochemical studies to the basic mechanism of this abnormal biological process; (2) interrelations of chemical, physical, and viral carcinogenesis; (3) recent advances in cell transformation and cell mutagenesis; (4) prospects in anti-carcinogenesis; such as the nucleic acid repair process and the inhibition of metabolism of carcinogens; and (5) recent advances in cancer biology, especially in the area of immunology as it relates to the problem of carcinogenesis and possible therapy.

Major Findings: The Conference developed a useful exchange of information which will be published.

Significance to Biomedical Research and the Program of the Institute: For Government agencies, concerned organizations and individual scientific and medical workers, the resulting critical and comprehensive report provided views on the current position and future prospects in this field.

Proposed Course: The proceedings of this conference will be published in full.

Date Contract Initiated: June 23, 1972

Current Annual Level: $19,000

MALLORY INSTITUTE OF PATHOLOGY FOUNDATION (NIH-NCI-71-2275)

Title: Detection of Carcinoembryonic Antigens in Humans

Contractor's Project Director: Dr. Norman Zamcheck

Project Officer (NCI): Dr. Tibor Borsos

Objectives: This project is designed to develop reagents and methods that are suited for the detection of carcinoembryonic antigens (CEA) in serum.

Major Findings: (1) Agarose Electrophoresis of CEA - Counterimmunoelectrophoretic (CEP) techniques on rehydratable agarose strips were developed for the detection of CEA. The sensitivity of the CEP technique was not significantly

increased by (a) the addition of polymeric anions or agarose additives used in the preparation of the rehydratable agarose strips. (b) Prerun of the CEA samples. (c) Electrophoretic reversal of test reactants, or (d) concentration of CEA by conditions operating under the Kohlrausch regulating function, conductivity shifts, and discontinuous gel concentrations.

In order to increase the sensitivity of this system a new radioimmune assay for CEA was developed in which anti-CEA antibodies coupled to affinity chromatography beads "captured" CEA and radio-labelled CEA during electrophoresis.

(2) Antiserum Production - Rabbits were injected at birth with normal human plasma. Subsequently, when mature they were immunized with CEA prepared from metastases of colonic cancer. The anti-CEA antisera did not need absorption with extracts of normal liver and colon to remove antibodies to them and were interchangeable with goat anti-CEA antisera for use in assay systems for the detection of clinically significant levels of CEA. (This does not preclude the possibility that much higher concentrations of normal tissue extracts might react with the antisera.) The exact specificity of these and other anti-CEA antisera remains to be determined.

In order to test further the specificity of the rabbit antiserum, we isolated the gamma golobulin antibodies from the goat and rabbit anti-CEA antisera and tested them by immunoelectrophoresis for antibodies to (a) normal human serum, (b) CEA prepared at Boston, Nutley, and Montreal, and (c) PCA extracts of cirrhotic liver. These tests yielded multiple precipitin lines with the CEA antigens indicating that the CEAs from Boston, Montreal, and Nutley were either not homogenous or else contained "fragments" of CEA. In addition, the rabbit anti-CEA antiserum did not react with PCA extracts of cirrhotic liver, thus indicating either qualitative or quantitative differences from the goat anti-CEA antisera.

Significance to Biomedical Research and the Program of the Institute: There is interest in developing screening techniques that permit identification of populations at risk to cancer. Present evidence indicates that colon cancer in man is accompanied by the appearance in serum of a carcino-embryonic antigen. The identification of an antigen characteristic of colon cancer will help elucidate the etiology of colon cancer and will yield information regarding control of this form of cancer.

Proposed Course: The rapid screening method will be further developed; its specificity, sensitivity and clinical usefulness will be studied and compared with standard radioimmunoassays for CEA. Serum and plasma from patients with neoplastic and non-neoplastic diseases will be tested.

Date Contract Initiated: June 1, 1971

Current Annual Level: $83,028

OHIO STATE UNIVERSITY RESEARCH FOUNDATION (NIH-NCI-72-2047)

Title: In Vitro Study of the Nature of Interaction between Chemical and Viral Carcinogens

Contractor's Project Director: Dr. David Yohn

Project Officer (NCI): Dr. Joseph DiPaolo

Objectives: To determine the dose response of carcinogens on Syrian hamster cells and human embryonic cells of lung or other appropriate origin which together with oncogenic and/or non-oncogenic viruses enhance in vitro transformation. The ability of steroid-hormones to regulate or modify these processes will be examined.

Major Findings: Morphologic transformation of human embryonic lung cells including WI-26 cells, has been obtained with Benzo[a]pyrene (BP) and methylcholanthrene (MCA). Enhancement of BP transformation has been obtained with cortisone acetate and hydrocortisone acetate. Thus far the transformed cells have not produced tumors in immunologically suppressed hamsters.

Significance to Biomedical Research and the Program of the Institute: In view of the various hypotheses concerning chemicals, viruses, and cancer, and due to the inherent difficulties in studies in the intact animal, it is advantageous to study the various interactions in a controlled in vitro system. It is important to use both human and hamster cells to establish the influence of oncogenic and non-oncogenic viruses in promoting chemical carcinogenesis and the role of chemical carcinogens in promoting viral carcinogenesis. It is critically important to know whether hormones augment or can be used to control carcinogenesis in vitro by chemicals and/or viruses.

Proposed Course: To determine the cytotoxicity curve of carcinogens and of analogs of the carcinogens that do not initiate transformation events. To induce hyperplastic events (cell proliferation) in WI-38 and/or WI-26 human embryonic lung female and male primary lines, respectively, with cortisone acetate or other hormones. To induce transformation in human embryonic lung female and male primary lines, respectively, with cortisone acetate or other hormones. To induce transformation in human embryonic lung cells initiated de novo with RNA and DNA containing viruses with and without chemical carcinogens.

Date Contract Initiated: December 20, 1971

Current Annual Level: $93,964

SCRIPPS CLINIC AND RESEARCH FOUNDATION (NIH-NCI-72-2046)

Title: Isolation and Chemical Characterization of Soluble Human Tumor (CEA) Specific Antigens

Contractor's Project Director: Dr. Ralph Reisfeld

Project Officer (NCI): Dr. Tibor Borsos

Objectives: The aim of this contract is to obtain human tumor specific antigens in a highly purified and chemically well-characterized form. This includes the development and application of new, large-scale purification

methods and the thorough chemical and biological characterization of carcinoembryonic antigens (CEA) of colon cancer.

Major Findings: Efforts to date have concentrated on obtaining reagents. Some preliminary experiments have been carried out to test possible isolation procedures. Mild peptic digest of rabbit IgG has shown the feasibility of obtaining small peptides and increasing their immunogenicity by cross-linking them with glutaraldehyde for the purpose of inducing an immune response specific for a selected segment of a molecule. When applied to CEA this may lead to an antiserum less ambiguous than those currently available as well as a highly specific immunoadsorbent.

An enzymatic method of iodination has been worked out which is currently being used for the high specific activity iodination of CEA for the double antibody assay and will ultimately be used in an attempt to iodinate cell-surface CEA to investigate the relationship between the CEA antigen at this level and isolated CEA. This method, employing Sepharose-bound bovine lactoperoxidase, employs much more gentle conditions for the iodination reaction than do the other techniques commonly used.

Large quantities of antisera have been obtained from two goats immunized with soluble CEA antigens. These antisera are being used in the production of immunoadsorbent columns as well as in the CEA assay.

Two assay procedures are currently being used in this laboratory: the double antibody method and the counterimmunoelectrophoresis method.

Antigenic material has been isolated by the PCA method from water extracts of tumor tissue obtained for comparison purposes. This material is currently being evaluated. Several tissue culture lines established from primary tumors and metastases of patients with colonic adenocarcinomata are carried and were found to release CEA antigen in their culture media. One of the lines is being converted to suspension culture and may prove to be a good source of antigen.

Significance to Biomedical Research and the Program of the Institute: This contract would permit the immunochemical and chemical characterization of CEA of human tumors and would contribute to the understanding of the development and possible etiology of this cancer. In addition highly purified antigen of this tumor would greatly facilitate the development of refined second generation tools for the detection of human colon cancer.

Proposed Course: Highly purified soluble CEA will be isolated from colonic adenocarcinoma and from sera from the tumor-bearing patients obtained under contract from the University of California at San Diego (NIH-NCI-72-3258) and from the Mallory Institute of Pathology Foundation (NIH-NCI-71-2276). Since the CEA antigen seems to be readily extracted from tissue in water or low salt concentrations, procedures for isolation will be concentrated on rather than procedures for extraction. Antisera will be formed against CEA in goats and rabbits against: (1) CEA positive tissue culture cells, (2) CEA isolated by the methods described above and (3) proteolytic digestion products of CEA. Attempts will also be made to isolate CEA from cultured cells and/or from the media from such cultures.

Date Contract Initiated: December 1, 1971

Current Annual Level: $73,778

IVAN SORVALL, INC. (AI-32513)

Title: Fractionation of BCG Cell Walls

Contractor's Project Director: Dr. Edgar Ribi

Project Officer (NCI): Dr. Berton Zbar

Objectives: The objective is to fractionate BCG cell walls for the purpose of isolating, purifying and identifying tumor suppressive substances.

Major Findings: A homogeneous material termed P3 has been isolated which in combination with fractions of the bacterial cell wall has tumor suppressive activity. Cell wall fraction and P3 when tested individually had minimal or no tumor suppressive action.

Significance to Biomedical Research and the Program of the Institute: A major goal of the program is to find ways to prevent or treat cancer. Use of BCG offers a reasonable approach to achieving this goal since it is known that BCG can interfere with the development of cancer in animals challenged with a chemical carcinogen and it has also been shown that established metastatic cancer in animals can be cured by intralesional injection of living BCG or BCG cell walls attached to oil droplets.

Proposed Course: To continue to isolate and test cell wall fractions and to serve as a source of these fractions for other interested investigators.

Date Contract Initiated: January 9, 1973

Current Annual Level: $39,676

TEMPLE UNIVERSITY (NIH-NCI-73-3200)

Title: Induction of Malignant Melanoma in Guinea Pigs

Contractor's Project Director: Dr. Wallace Clark

Project Officer (NCI): Dr. Herbert Rapp

Objectives: The objective of the project is the induction in guinea pigs of malignant melanoma which is histogenetically analogous with the disease as it occurs in human skin.

Major Findings: It has been shown that malignant melanomas in man develop via a biphasic or monophasic growth pattern. Those tumors which develop following biphasic growth comprise about 75-80% of primary human cutaneous malignant

melanomas seen in the Northeastern United States. These tumors are of two types: malignant melanoma of the superficial spreading type and malignant melanoma of the lentigo maligna type (Hutchinson's melanotic freckle type). These tumors, while clearly different from each other, show an initial centrifugal growth phase characterized by the intraepidermal spread of melanocytes and, in the case of malignant melanoma of the superficial spreading type, associated centrifugal spread of melanocytes in the upper part of the papillary dermis.

Animal models commonly used for the study of melanoma include a variety of mouse and hamster tumors, the majority of which have arisen as dermal neoplasms. The mouse, rat and hamster do not have an active intraepidermal melanocytic system, but have melanocytes in the dermis and in hair bulbs and generally, the kinds of tumors that arise are not histogenetically similar to human melanomas. Furthermore, the animal tumors are carried as transplantable tumors and there is no way of predictably inducing malignant melanomas in animals, which resemble the human lesions.

It has been shown that painting guinea pig skin with DMBA will produce a low yield of tumors that are similar to the human tumors. Studies in a guinea pig called the Weiser-Maple guinea pig which has a uniform color coat have shown that the ears contain large numbers of melanocytes as does the general body epidermis. The normal intra-epidermal melanocytic system is similar to human epidermal melanocytes. This animal may, therefore, be painted in multiple sites with the hopes of producing a relatively high yield of a tumor similar to the human disease.

A variety of experiments designed to produce a significant number of these tumors in the Weiser-Maple guinea pig is being carried out. These experiments include direct painting of both ears and both flanks with DMBA; painting in a similar fashion followed by irradiation with ultraviolet light and painting followed by immunosuppression from the beginning and, later, immunosuppression when biologically malignant tumors appear.

Significance to Biomedical Research and the Program of the Institute: The significance of the project is to produce a tumor which is similar to the human disease, including a biphasic growth pattern progressing to biological malignancy. If primary tumors with significant potential for biologic malignancy evolve, the guinea pig may be studied in a variety of ways, including immunotherapy and chemotherapy, that have the possibility of direct applicability to man. An attempt to manipulate the evolution of a primary tumor that will progress to full biological malignancy through immunologic means may block tumor progression and may illuminate the nature and behavior of malignant melanoma in man.

Proposed Course: The formal experiments now underway have already shown in the experimental animals that there is hyperpigmentation, probable increase in the number of melanocytes, and atypical hyperplasia of the stratified squamous epithelium.

The method of painting, accelerating with light, and altering by immunologic methods will be continued as outlined above.

Date Contract Initiated: October 1, 1972

Current Annual Level: $99,612

TEXAS, UNIVERSITY OF (NIH-NCI-72-3210)

Title: Development of In Vitro Methods for the Detection of Cell-Mediated Immunologic Reactivity to Chemical Carcinogens

Contractor's Project Director: Dr. Daniel E. Thor

Project Officer (NCI): Dr. Herbert Rapp

Objectives: To determine the feasibility of devising in vitro immunologic tests for the detection of prior exposure to known chemical carcinogens.

Major Findings: Maximun guinea pig sensitization to two carcinogens, DMBA and 3-MCA has been obtained with a modified split-adjuvant technique.

Preliminary data shows that (1) direct addition of carcinogens in a variety of solvents are either cytotoxic to sensitized lymphocytes and peritoneal exudate cells (PEC's) or they can not, as haptens trigger a cell-mediated-immune response in vitro, (2) carcinogen-albumin carrier complexes are water soluable and can inhibit sensitized guinea pig PEC'S in a migration inhibition assay (MIF) for CMI, and (3) these carcinogen-carrier complexes are not reactive or toxic with normal, nonsensitive guinea pig PEC's .

A microtechnique for the assay of migration inhibition factor has been devised to screen carcinogen-carrier complexes. Both direct and indirect MIF assays have been positive in highly carcinogen sensitive guinea pigs utilizing the agarose droplet technique.

Significance to Biomedical Research and the Program of the Institute: If successful, these studies will make it possible to determine whether human individuals have been exposed to a variety of agents known to be carcinogenic in animals and suspected of being carcinogenic in man. The development of an immunogenic carcinogen-carrier complex with specificity might also be very useful in surveillance studies. Significant differences among various populations in their immunologic reactivity to known carcinogens might make it possible to correlate the degree of reactivity with the subsequent development of cancer. Correlations of this kind might provide the impetus to remove the agents from the environment.

Proposed Course: Utilizing guinea pigs highly sensitized to chemical carcinogens, experiments are in progress to evaluate specificity in vitro and in vivo. Although MIF data appears to differentiate sensitized and nonsensitized animals, blast transformation may still be feasible if high background nucleotide incorporation can be minimized or eliminated. Major efforts will be made to improve mole ratios of carcinogen-carrier complexes and evaluate the most efficient macromolecular carrier.

Date Contract Initiated: February 16, 1972

Current Annual Level: $87,034

TRUDEAU INSTITUTE, INC. (NIH-NCI-72-3221)

Title: Tumor Inhibition of Mycobacteria: Standardization of Mycobacteria Preparations

Contractor's Project Director: Dr. George Mackaness

Project Officer (NCI): Dr. Berton Zbar

Objectives: To determine optimal conditions for the use of mycobacteria as inhibitors of tumor cell growth.

Major Findings: Lyophilized vaccines, as normally supplied commercially, have been found to contain a low percentage of viable bacilli. Viability ranged from 8% to less than 2% in the four preparations tested. The lyophilized product also contains large amounts of soluble antigen. There is a distinct possibility that this could interfere with tumor suppressive activity and have other undesirable side effects. As an alternative, it was found possible to harvest BCG from actively growing cultures and store them at -70 degrees C without loss of viability or change in biological activity. In the interests of more precise dosage, and also to avoid dead or disintegrating bacilli in the inoculum, freezing is recommended as the method for preserving BCG during storage and transportation.

Strain differences have been demonstrated in the vigor of the immune responses provoked by BCG in regional lymph nodes. When tested by direct inoculation of line ten tumors in Strain 2 guinea pigs, the same strains showed correlated differences in tumor suppressive activity. On the basis of these two criteria, the Pasteur strain of BCG appears to be the most potent, and Glaxo the least potent of the strains now being used as tumor suppressive agents. It was also observed with this same tumor model that growth enhancement may occur if tumors are infiltrated with a sub-effective dose of BCG. This suggests that care must be taken in the selection of strains and the estimation of dosage.

In related studies, it was found that BCG promotes the formation of specifically sensitized lymphocytes. Observations have been made in two systems employing sheep red blood cells (SRBC) and a mouse mastocytoma (MA). The cell-mediated immune response that can be obtained by injecting SRBC or irradiated MA is greatly amplified by a prior injection of BCG. Enhancement occurs only if the SRBC or MA are introduced into the lymphoid tissues (nodes or spleen) which are under active stimulation by BCG. The effect becomes greater as the response to BCG develops. In the case of SRBC it is seen as an increase in the level and duration of delayed-type hypersensitivity; the response to irradiated MA is manifest as specific immunity to tumor cell challenge. Optimal dosage and the best interval between the injection of BCG and irradiated MA has been determined for one strain of BCG only.

The mode of action of BCG as an immunopotentiating agent for the induction of cell-mediated immunity appears to depend upon an unexplained capacity of BCG

to prevent the negative feedback which normally interrupts the induction of T cells; and results in a more intense and more protracted response on the part of T cells.

Significance to Biomedical Research and the Program of the Institute: This contract will determine optimal conditions for the use of mycobacteria as inhibitors of tumor cell growth. This knowledge will indirectly add to understanding immune factors that affect oncogenesis.

Proposed Course: The contractor will supply data comparing the different mycobacterial strains with respect to (1) inhibition of tumor growth in vivo, (2) development of specific tumor immunity in vivo and in vitro, and (3) safety of administration.

Date Contract Initiated: February 24, 1972

Current Annual Level: $175,128

UTAH, UNIVERSITY OF (NIH-NCI-71-2272)

Title: Carcinogenesis Bioassay Resource for Determining the Effect of Chronic Immunosuppression on Physical and Chemical Carcinogenesis

Contractor's Project Director: Dr. Ernst J. Eichwald

Project Officer (NCI): Dr. Curtis C. Harris

Objectives: The primary objective is to determine in a carefully defined experimental situation whether or not certain immunosuppressive agents in common clinical use (Imuran, Methotrexate, Cytoxan, Prednisolone, and ALG) have carcinogenic potential, either alone, or in combination with known carcinogenic agents. Further, it is hoped to be able to provide additional information on the relationship between immunosuppression and tumor formation by correlating the carcinogenic potential and immunosuppressive capacity of these agents.

Major Findings: To date the studies have concentrated on (1) determining threshold doses for skin carcinogenesis in C3Hf mice by (a) chronic UV exposure and (b) chronic topical benzo[a]pyrene application; and (2) determining doses and regimens of the immunosuppressive agents which are both effective and non-toxic when applied chronically. Due to the extremely long-term nature of the carcinogen studies, these are still in progress and will extend well into the third year. Doses and regimens of methotrexate and prednisolone have been found which permit long-term survival of chronically treated mice and prolong the survival of H-2 incompatible skin grafts. With cytotoxan and imuran, no chronic regimens which are both compatible with survival and immunosuppressive by the criterion of allograft rejection have been found. For these two agents, maximum tolerated doses are being determined which can be employed in long-term experiments in combination with carcinogen treatment.

Two findings, peripheral to the objectives have arisen from studies of UV

carcinogenesis. First, preliminary data suggest that skin allograft survival is prolonged on UV-treated animals. If this phenomenon can be confirmed and shown to have an immunological basis, this might provide further evidence of the importance of the immune response in carcinogenesis. Second, it has been observed that the latent period for UV carcinogenesis at a particular dose level in C3Hf mice is considerably shorter in males than in females. The basis for this observation is unknown.

Significance to Biomedical Research and the Program of the Institute: The primary impetus for this investigation is the clinical observation that patients receiving chronic immunosuppressive treatment seem to exhibit an increased incidence of malignant disease. These studies are designed to answer to the following questions in a carefully defined experimental situation: (1) Are certain immunosuppressive agents in common clinical use carcinogens or co-carcinogens, and (2) is there a correlation between the immunosuppressive capacity of these agents and their ability to induce or promote carcinogenesis?

Identifying agents which are carcinogenic and/or establishing a causal or contributory role of immunosuppression in carcinogenesis would support the National Cancer Institute's objective of "determination of cause". This might also aid in (1) identifying the population at risk, (2) suggesting a means of preventing cancer by more judicious use of immunosuppressive agents, and (3) providing a sound theoretical basis for the treatment of cancer by means of immunotherapy.

Proposed Course: Studies on carcinogens and immunosuppressive agents will be completed. Some information from studies in progress on the effects of methotrexate on carcinogenesis will be available also. Similar experiments with other immunosuppressive agents are scheduled to begin within the year, but these will require 18 to 24 months for completion.

Date Contract Initiated: May 7, 1971

Current Annual Level: $180,370

SUMMARY REPORT

CARCINOGEN METABOLISM AND TOXICOLOGY SEGMENT

July 1, 1972 through June 30, 1973

The Carcinogen Metabolism and Toxicology Segment, formally constituted in the spring of 1973, has as its primary goal the identification of those components of complex systems which function as environmental carcinogens and the development of analytical methodology for their detection and quantitation. Metabolic and toxicological studies of environmental carcinogens in selected animal systems are to be conducted in an attempt to provide further knowledge of the metabolic pathways involved in the carcinogenic process, and an explanation for varying responses of different animal species and man to chemical carcinogens. The Segment will also investigate the mechanisms of chemical carcinogenesis in selected in vitro systems. The goal of the Segment is to identify means to block the metabolic pathways leading to "activated" carcinogens.

In addition to the Segment Director, Dr. Elizabeth Weisburger, and Manager, Dr. James Sontag, there is an Advisory Group which functions as a technical review committee of on-going contracts, evaluates proposals for new contracts, and recommends priority areas. Members of the Advisory Group include several from outside the Federal Government and represent all of the pertinent scientific areas relating to the activities of the Segment.

At the time of its establishment, through a reorganization of the previous Bioassay and Information and Resources Segments, 5 on-going contracts were transferred to the Carcinogen Metabolism and Toxicology Segment. They are reported under those Segments. Some of these contracts are designed to study the possible carcinogenicity of fungal products and effect of diet on chemical carcinogenesis. Other contracts are concerned with the occurrence of N-nitroso compounds in the environment and development of short-term bioassays for detection of chemical carcinogens.

It is anticipated that during this fiscal year 4 new contracts will be awarded in the area of nitrosamine research. In addition, 3 contracts are expected to be awarded to determine the usefulness of mutagenicity as a prescreen for chemical carcinogens.

CALIFORNIA, UNIVERSITY OF (SAN DIEGO) (NIH-NCI-70-2206)

Title: Pulmonary Tumors in Mice for Carcinogenic and Co-carcinogenic Bioassay

Contractor's Project Directors: Dr. Michael B. Shimkin
Dr. A. J. Kniazeff

Project Officer (NCI): Dr. Elizabeth K. Weisburger

Objectives: The objectives develop along the following two lines: (1) Bioassay - Testing of chemicals for carcinogenic activity using the strain A mouse pulmonary-tumor-induction technique developed by Shimkin and Andervont in 1940. Strain A mice are characterized by having lungs that are very susceptible to developing primary adenomatous tumors. The time of appearance and number of tumors is remarkably affected by a wide variety of exogenous chemical carcinogens. This biological system has been used during 30 years by several investigators for the bioassay of a broad variety of chemicals; e.g., alkylating agents, carbamates, aziridins, polycyclic hydrocarbons and aflatoxin. The bioassay is sensitive, quantitative, and has been reproducible over the many years of use. The test can normally be completed in six months. A variety of chemicals have been proposed for testing (food additives from the GRAS list, organohalide chemicals, cigarette smoke condensates, nitrosamines and related compounds). Compounds already known to be positive or negative for carcinogenic activity will be retested in this system to determine to which degree the results parallel those of conventional bioassays of long-term duration. Depending on the correlation of results, the usefulness of this test as a quick and inexpensive screening method will be determined.

(2) Co-carcinogenicity Investigations - The interaction of viruses and chemicals in carcinogenesis is being studied using two systems: (a) Evaluation of virus-chemical interaction on the production of pulmonary tumors in strain A mice. Strain A mice are pretreated with either Moloney leukemia or sarcoma viruses followed by administration of the chemical carcinogens, 3-methylcholanthrene or urethan. The nature of possible virus-chemical interaction is being evaluated by quantitation of the developed lung adenomas.

(b) Determination of the sensitivity of feline cell cultures to transformation with chemical carcinogens after pretreatment with feline leukemia virus (FLV). Feline whole embryo cells are treated with FLV followed by exposure to various dilutions of the chemical carcinogens,

methylcholanthrene and dimethylbenzanthracene. Replicate cultures are treated in parallel with the chemicals only. The cell populations are then monitored for transformation by such criteria as morphological change, growth in soft agar, karyology, growth rate, and tumorigenicity in the hamster cheek pouch. In this manner, virus-chemical interaction in feline cell transformation is being investigated, along with the determination of the suitability of feline cells to serve as substrates for the testing of a broad variety of chemicals for carcinogenic activity.

Major Findings: (1) Bioassay - During the past two years, the strain A mouse pulmonary tumor system has been applied to the bioassay of 41 food additives, 20 chemotherapeutic drugs and 15 organohalide chemicals. The chemotherapeutic drugs are either being employed or evaluated for use in cancer chemotherapy. The organohalide chemicals are mostly industrial intermediates. The tumor response of chemically-treated mice was compared to that of the appropriate vehicle controls with the following results: (a) Food additives - Cinnamyl anthranilate was carcinogenic; all others were negative at the dose levels employed.

(b) Chemotherapeutic drugs - Thio-TEPA, isophosphamide, uracil mustard, estradiol mustard, B-deoxythioguanisine, phenesterin, dapsone, dibenzyline, 1-propanol 3,3-iminodimethanesulfonate hydrochloride, 3-5-azacytidine and pyrimethamine, were carcinogenic; all others were negative.

(c) Organohalide chemicals - Bioassays are complete on all but two chemicals; so far, 2-bromobutane, 1-bromo-2-methylpropane, 1-iodopropane, 2-iodobutane, and 1-iodobutane are carcinogenic and the others negative.

(2) Co-Carcinogenicity Investigations - (a) Virus-chemical interaction in the development of pulmonary tumors in strain A mice - The results of preliminary studies indicate that pretreatment of strain A mice with Moloney sarcoma virus causes a 30-50% reduction in the pulmonary adenoma response to both 3-methylcholanthrene and urethan. Repeat studies are currently underway to confirm these results and to determine whether the decrease in tumor response is due to immunological factors or to non-specific reactions such as sickness, fever, etc.

(b) Feline cells in monolayer culture showed pronounced resistance to high levels of chemical carcinogens in the early stages of chemical treatment. No deleterious effect on cells was observed at 50 and 100 ug/ml levels of methylcholanthrene. Some reduction in the rate of cell replication was, however, noted in cultures after 21 days of cultivation. Cells treated with 10 and 20 ug/ml of methylcholanthrene showed no deleterious effects after 75 days in culture.

These results demonstrate the high resistance of feline cells in monolayer culture to chemical carcinogens.

3T4 feline cells obtained from Naval Biomedical Laboratory, Oakland, when treated with feline leukemia, methylcholanthrene and the combinations of the two carcinogens, initiated changes in cell populations

suggestive of transformation. Tumorigenesis studies in cats and in hamsters are in progress to evaluate these observations.

Significance to Biomedical Research and the Program of the Institute: This project will integrate into the activities of the Carcinogenesis Program by attempting to develop a rapid and reliable bioassay of chemicals for carcinogenic activity. Research in this area is of high priority due to the vast expense and time required to screen chemicals by the presently used animal bioassays. This research project will also provide timely extension of the existing bioassay capabilities.

Proposed Course: To continue work in the areas outlined above.

Date Contract Initiated: June 22, 1970

Current Annual Level: $142,052

MASSACHUSETTS INSTITUTE OF TECHNOLOGY (NIH-NCI-70-2180)

Title: Environmental Occurrence of N-Nitroso Compounds

Contractor's Project Directors: Dr. Steven Tannenbaum
Dr. Michael Archer

Project Officer (NCI): Dr. Elizabeth Weisburger

Objectives: The interaction of nitrite with substituted nitrogen compounds can lead to a series of compounds containing the N-nitroso residue. Reports of the presence of nitrosamines in several foods and beverages raises the question of their significance in the etiology of human cancer. The contractor is therefore developing analytical procedures for nitrosamines in foods and beverages. Since nitrite has been shown to be fairly ubiquitous in the environment, it is important to know which conditions favor its reaction with a specific nitrogen compound. Therefore, studies are also being conducted to define the reaction kinetics in foods and model systems and identify reaction promoters and retardants.

Major Findings: A number of commercial meat products and one fish product have been examined for volatile nitrosamines. A new extraction and clean-up procedure has been developed for examination of alcoholic beverages for volatile nitrosamines, including nitrosomorpholine. The nitrosation kinetics of secondary amines and amino acids has been described and the rate of reaction studied in two-phase systems, frozen systems, ascorbic acid solutions, and milk. These investigations have demonstrated that there are compounds which enhance and inhibit nitrosation reactions. Studies have also continued on nitrite in human saliva and methodology has been developed for measurement of a number of important variables related to salivary nitrite, including microorganisms.

Significance to Biomedical Research and the Program of the Institute: Since N-nitrosamines have proved to be multipotential carcinogens in all animal species tested, they may represent significant hazards to man as environmental contaminants contributing to the "spontaneous" cancer incidence. This project will aid in determining the total extent to which nitrosamines may be present in the environment and the conditions favoring their formation during processing and storage. Thus measures to prevent this formation could be instituted.

Proposed Course: The project will continue along the same line, attempting to refine analytical techniques for nitrosamines and determining conditions favoring their formation. Additional emphasis will be placed on analysis of foods and beverages from geographical regions having a high incidence of cancer. Studies will also continue on control of levels of salivary nitrite.

Date Contract Initiated: June 11, 1970

Current Annual Level: $97,685

MASSACHUSETTS INSTITUTE OF TECHNOLOGY (NIH-NCI-73-3217)

Title: Toxicity and Carcinogenicity Associated with Fungal Growth on Foodstuffs

Contractor's Project Directors: Dr. Gerald Wogan
Dr. George Buchi
Dr. Arnold Demain

Project Officer (NCI): Dr. Elizabeth Weisburger

Objectives: To investigate the biochemical, toxic and carcinogenic effects of aflatoxins, their metabolites and analogs; to determine the metabolic fate of aflatoxins in various species; to synthesize aflatoxin metabolites; and to isolate, characterize and investigate the toxicity and carcinogenicity of other mycotoxins produced by molds isolated from foods or food raw materials.

Major Findings: Carcinogenesis evaluations on dose-response relationships of aflatoxin B_1 and on comparative potency of B_1 and M_1 (hydroxy-B_1) in rats are being prepared for publication. Studies on toxicity and carcinogenicity of P_1 are in progress. Metabolites of aflatoxin B_1 are being isolated from mouse urine for structure elucidation. Metabolism by microsomal preparations from livers of animal species and man is being studied in vitro. A new mycotoxin from A. clavato-nanico has just been identified as a compound of novel structure, and has been named cytochalasin G to indicate its relationship to previously known compounds. Fermentation techniques are being established for production of that toxin as well as for initial studies on approximately 30 additional toxin-producing fungi isolated from Asian foodstuffs.

Significance to Biomedical Research and the Program of the Institute: Evidence is accumulating that chemical products arising from microbial spoilage (particularly involving mold-damage) of foods or food raw materials can appear in the human food supply and may be associated with the etiology of liver cancer in several areas of Asia and Africa. The scope of the problem in terms of numbers of compounds and/or molds that may be involved has not yet been accurately defined, but is potentially great. This project, which is related to other NCI contracts on naturally-occurring carcinogens, is designed to produce relevant information on these questions.

Proposed Course: Experiments will continue along the lines suggested by the objectives outlined above. Studies on the comparable metabolism of aflatoxins may help to delineate the mechanisms involved in rather wide species differences in response to these compounds. Similarly, studies on the biochemical effects, toxicity and carcinogenicity of analogs and metabolites may provide evidence on active forms of the agent, and on sites of action. Identification of new mycotoxins from other molds isolated from food samples will provide further definition of the scope and character of the potential hazards to man represented by these toxins.

Date Contract Initiated: June 25, 1962

Current Annual Level: $191,348

MASSACHUSETTS INSTITUTE OF TECHNOLOGY (NIH-NCI-73-3238)

Title: Interactions Between Diet and Chemical Carcinogenesis: A Bioassay System

Contractor's Project Director: Dr. Paul M. Newberne

Project Officer (NCI): Dr. Lionel Poirier

Objectives: To determine whether a diet marginally deficient in lipotropes presents an increased risk of cancer induction to man; also to confirm and extend previous observations on the antagonism between dietary methyl donors and chemical carcinogens.

Major Findings: Initial studies of two carcinogens, diethylnitrosamine (DEN) and dibutylnitrosamine (DBN) are completed as are preliminary studies to establish an effective dose of dimethylhydrazine (DMH); studies of DMH, dimethylnitrosamine, aflatoxin B_1 and aflatoxin G_1 carcinogenesis are in progress.

Both DEN and DBN were more effective carcinogens for the liver in lipotrope-deficient rats than in normal rats. DEN, fed in the diet, decreased the life span of lipotrope-deficient rats, particularly of those with hepatocarcinoma, and hastened the appearance of tumors although the ultimate incidence was high in both groups. Fifty percent of deficient rats were dead with hepatocarcinoma at the time the tumor first appeared in rats fed control diet. Esophageal tumors were found in a slightly greater incidence in deficient rats.

DBN, given subcutaneously, induced hepatocarcinoma in much greater incidence in deficient than in normal rats. The incidence of other tumors was slightly but not significantly decreased.

From the results of these two studies, it is clear that lipotrope deficiency enhances the susceptibility of the liver to carcinogenesis by nitrosamines, as was found earlier with aflatoxin B_1, even when the carcinogen is given by injection as DBN was in this study. There is as yet no definite effect of diet on cancer induction in other organs. The study of DEN is being repeated using a lower dose which may increase the differences between the two groups. The carcinogens currently under study will add information on renal and colon carcinoma. DMH and aflatoxin G_1 were more toxic to deficient rats during administration.

Significance to Biomedical Research and the Program of the Institute: The findings obtained in these experiments may be of considerable practical and theoretical significance. If the low-lipotrope diets change tumor yield or distribution with a number of carcinogens, changes in diet patterns of certain segments of the human population might be warranted as would changes in diet fed to experimental animals used to test drugs, food additives, environmental pollutants, etc., for carcinogenicity. Further, extensive examination of the role of methyl donors and related compounds in chemical carcinogenesis would be expected to yield information leading to preventive, retardative or therapeutic measures against human cancer.

In the major findings to date, the most important is the demonstration that nitrosamines, like aflatoxin B_1, are more effectively carcinogenic for the liver in lipotrope-deficient rats than in normal rats. This bears on the attempts of NCI to find ways to prevent human disease in two ways. First, the induction of hepatocarcinoma in rats is one of the major assays for substances potentially carcinogenic for man. The enhanced sensitivity of test animals fed lipotrope-deficient diet can be used both to shorten the time required for assay and to investigate compounds whose carcinogenic activity may be weak. Second, hepatocarcinoma in the U.S. is strongly correlated with cirrhosis; human cirrhosis in turn is associated with alcoholism and its consequent malnutrition. Thus, the basis for the association between hepatocarcinoma and cirrhosis may be enhanced susceptibility of the nutritionally-damaged liver to environmental carcinogens. Identification of other organs in which malnutrition enhances chemical carcinogenesis may explain and suggest remedies for other epidemiologic observations in people.

Proposed Course: To increase the number of carcinogenic compounds administered to rats receiving a normal or marginally lipotrope-deficient diet.

Date Contract Initiated: February 1, 1972

Current Annual Level: $83,125

TEMPLE UNIVERSITY (NIH-NCI-65-102º)

Title: A Search for Carcinogens among Mycotoxins

Contractor's Project Director: Dr. Fritz Blank

Project Officer (NCI): Dr. Elizabeth Weisburger

Objectives: This contract is aimed at identifying possible carcinogens from fungi pathogenic to man. The original screening program included various extracts and fractions from 17 fungal species. Presently, efforts are being concentrated on the carcinogenic activity of Candida parapsilosis and Candida albicans. These species are the most often isolated pathogenic fungi from clinical specimens as well as having the highest carcinogenicity in the preliminary screening program.

Major Findings: Mycelia of fungi are produced and lyophilized by Dr. F. Blank at Temple University. Extraction of the mycelia (hexane, chloroform and hot and cold methanol) and fractionation of these extracts for bioassay is performed by Dr. G. Just at McGill University. Bioassays are done by Dr. S.J. Mann at Temple University. In CFW female mice, injections of high doses of hot and cold methanol extracts of Candida parapsilosis gave an increase in the frequency of sarcomas at the site of injection. In Strain A female mice, injections of the hot methanol extract gave a significant increase in the frequency of lung tumors. A scheme for the separation and chemical identification of the major fractions of Candida parapsilosis has been developed.

Significance to Biomedical Research and the Program of the Institute: This study deals with potential carcinogenic fungal metabolites which may be of importance to man through contract or their presence in food or other commodities. Such metabolites may be responsible for some of the "spontaneous" cancer incidence in the population.

Proposed Course: The largest number of sarcomas was induced by certain extracts of Candida parapsilosis. When repeated, fewer sarcomas were obtained as a result of changed growth conditions. The original method of growing the fungus was in small Erlenmeyer flasks whereas the later production was done in a fermentor to increase fungal yields. Presently, Candida parapsilosis is being grown in Erlenmeyer flasks on shakers according to the original conditions. Extraction of the shaker grown mycelia as well as fermentor grown mycelia is being fractionated into extracts that duplicate the original extracts. The various extracts are being bioassayed in CFW female mice for sarcomas and in Strain A female mice for lung tumors. The co-carcinogenicity of the Candida parapsilosis extracts is being investigated.

Date Contract Initiated: June 12, 1965

Current Annual Level: $89,961

SUMMARY REPORT

CHEMISTRY AND MOLECULAR CARCINOGENESIS SEGMENT

July 1, 1972 through June 30, 1973

The objective of the Chemistry and Molecular Carcinogenesis Segment is to study molecular changes in cells as they relate to carcinogenesis. With this in mind, during the past year the Segment has formulated a program to develop an increased understanding of chemical carcinogenesis. Five major areas were selected for major emphasis. These were (a) in vitro carcinogenesis, (b) genetics of somatic cells, (c) biochemistry of chromatin and DNA, (d) studies of the cell surface membrane, (e) studies on microsomal membranes and their associated enzymes, especially hydroxylases.

1. In Vitro Carcinogenesis. The use of in vitro carcinogenesis systems for studying mechanisms was set to be the goal of highest importance. Should such systems be available to investigators generally, they could be used in the study of the other items in our program since cells in various stages of malignancy would be available for comparison. More importantly, a reduction to the single cell level represents the lowest organizational level for studies of malignancy. Because the importance of this subject appears to transcend the interests of our Segment, an administrative decision has been made to pursue this subject further at the level of the whole Carcinogenesis Program.

Dr. Sachs (Weizmann Institute) reports advances in understanding the chromosomal control of malignant transformation. His studies support construction of a model which suggests that a certain balance of chromosomes is found in transformed cells. Alteration in this balance may produce reversion to a normal phenotype. These studies offer the possibility of gaining increased understanding of the genetic biochemical control of specific phenotypes.

2. Genetics of Somatic Cells. The three contracts in this area have produced new varieties of mutant cells and some which have been transformed to malignant, apparently as the result of a mutation. Dr. Basilico (New York University), using hamster cells, has found over 26 low reversion rate mutants. Particularly valuable are mutants derived from cells already mutant in hypoxanthine-guanosine phosphoribosyl transferase (HGPRT), i.e., double mutants, none of which seem to be defective in DNA, RNA or protein synthesis. Also, a mutant has been isolated which is defective in processing of large molecular weight precursor to 28S ribosomal RNA, while 18S and other RNA's are synthesized normally. This mutant will be extremely valuable for the manipulation of ribosomes in studies on carcinogenesis.

Dr. Siminovitch (Ontario Cancer Institute) has isolated several mutants which have alterations in plasma membrane functions. Several of these have been isolated and, to a limited extent, characterized. The sodium-

potassium-ATPase activity of one mutant was shown to be different in its responsiveness to ouabain, a specific inhibitor or this enzyme. Also, mutants resistant to the lectins concanavalin A and phytohemagglutinin have been isolated, and these membrane-altered cells exhibit a change in responsiveness to a variety of other substances which interact with cell membranes. Since several favored hypotheses about the nature of the cancer cell concentrate upon changes in the cell membrane, it is likely that these mutants will find application in cancer studies.

In studies performed by Dr. di Mayorca (University of Illinois, College of Medicine), chemical carcinogens induced temperature-conditional changes in cells resulting in the ability of these cells to grow and divide under conditions in which the growth and division of normal cells is restrained. A model explaining the molecular mechanism of chemical carcinogenesis has been presented which is accessible to experimentation. It is possible that extension of these experiments to a variety of carcinogens will reveal the molecular basis of carcinogenesis, and also provide a much-needed bioassay system for identifying chemical carcinogens.

3. Binding of Non-Histone Proteins to DNA. The histones have been recognized as possible regulating proteins, and are being studied in many laboratories. Recently, other proteins have been found attached to DNA or in the nucleus, and a similar function has been suggested for these. Two contractors in this area are studying such proteins and attempts at isolation and characterization have been started. Dr. Hnilica (University of Texas) has found a specific non-histone nuclear protein fraction in rat liver chromatin. This fraction shows 4 major bands on analysis by polyacrylamide gel electrophoresis and binds strongly to homologous DNA. Large scale production of these proteins and amino acid analyses are currently under investigation.

Dr. Roth (University of Connecticut) has found various methods for making DNA-cellulose. Successful preparations will then be used to chromatograph protein fractions and separate out those showing affinity for homologous DNA. Simultaneously, four techniques for the initial bulk isolation of non-histone nuclear proteins have been explored to date. Two of these techniques show some promise and the proteins obtained from them (using rat liver as the source) are currently being further characterized as to their variety and affinity for DNA.

4. Studies on Surface Membranes. The role of the surface membrane in controlling the passage of molecules into and out of the cell may be of decisive importance in the development of malignancy. Extensive studies in this area are beginning to lay the foundation for more detailed investigation of the role of the membrane in carcinogenesis. Several contracts to be initiated will study transport proteins in the membrane. There has been one contract with Professor Friesen (McGill University) on prolactin and its role in the membranes of mammary tissue. His laboratory has successfully isolated prolactin in high yield from frozen pituitaries. He is using the purified material to establish the amino acid sequence, and to characterize the binding proteins in mammary tissue. In addition, he is refining methodology for prolactin assay in serum using purified prolactin as an antigen in a radio-immune assay.

5. Microsomal Enzymes. The heme moiety of P450 is a focal point for the catalytic activity for hydroxylating enzymes located in the microsomal membranes. Dr. Levin (Johns Hopkins) has studied the transport of heme synthesized in the mitochondria. He found that heme synthesized in mitochondria is transferred to the microsomes and has identified soluble substances in the cytosol which function in this transfer. The study affords insight into the complexity involved in the assembly of this important microsomal enzyme.

Studies from Dr. Calvin's laboratory (AEC-University of California at Berkeley) indicate possible mechanisms for the activity of benzopyrene. They have found that position number 6 is highly reactive and may be an important molecular site.

Dr. Sachs (Weizmann Institute) has studied the activity of the hydroxylase in several human tissues and found that cultured cells derived from human fetuses metabolize polycyclic hydrocarbons. Epithelial cells metabolize BP more extensively than fibroblast cells. They have made the beginnings of a study on the genetics of this enzyme system.

OTHER: Two contracts to establish the distribution of haptoglobin genotype in patients with cancer of the pancreas have passed the half-way mark. Both Dr. Altschuler (Home for Jewish Aged/Philadelphia Geriatic Center) and Dr. Anderson (University of Texas) find a haptoglobin gene 1 frequency equal to 0.44 in the patient series. Control populations have lower gene frequency (Hp^1=.34-.48). Whether this data has statistical significance may be known at the end of the last contract year.

Dr. Calvin (AEC-University of California at Berkeley) has shown that rifampicin suppresses the development of mammary tumors caused by the potent carcinogen, DMBA. These studies, incomplete at present, suggest possible chemical intervention in chemical carcinogenesis.

A contract with Polysciences has been in force for approximately one year to develop new gels, better suited for electrophoretic separation of large molecules. The most promising materials so far studied are based on polyethylene glycol diacrylate. Using this material, pore sizes are larger and gel structure more open. Full characterization of this material and its use as an electrophoretic anti-convection substrate will be determined in the future.

AEC-NCI INTERAGENCY AGREEMENT (UNIVERSITY OF CALIFORNIA AT BERKELEY) (NCI-FS-71-58)

Title: Molecular Processes Involved in the Carcinogenic Action of Polycyclic Aromatic Hydrocarbons

Contractor's Project Director: Dr. Melvin Calvin

Project Officers (NCI): Dr. Harry V. Gelboin
Dr. Andrew C. Peacock

Objectives: To define the chemically reactive position of polycyclic hydrocarbons and to determine the interaction of the reactive intermediate with cell macromolecules and the nature of interaction with oncogenic viruses.

Major Findings: A great deal of progress has been made in setting up methodology both for the intrinsic chemistry of carcinogenic hydrocarbons as well as the specific chemistry related to cellular constituents such as the DNA and protein components. Work has begun on the interaction of carcinogenic hydrocarbons not only with cells in tissue culture but with the oncogenic viruses in them. The first of the purely chemical results has been published, in which the activation of one position of a carcinogenic hydrocarbon results in reaction for another. Preliminary results on the effects of the hydrocarbon on the cell membrane have also been observed. A publication has been prepared describing the inhibition of carcinogenesis by dimethylbenzanthracene using certain rifamycin derivatives, known to inhibit reverse transcriptase.

Significance to Biomedical Research and the Program of the Institute: The widespread distribution of polycyclic hydrocarbons as a result of the combustion of fossil fuel, both in moving and in stationary power plants, as well as the combustion of agricultural wastes, makes them important environmental carcinogens. Their presence as a principal component of tobacco smoke whose carcinogenicity has been clearly demonstrated epidemiologically makes it even more critical to know their mode of action -- the role of enzymes in the activation as well as in the binding of the carcinogen, the cellular components and the components of oncogenic viruses. A first step has already been taken in helping to understand how these hydrocarbons may become active. Furthermore, the possible detection of their first stage of activity either by enzyme induction, such as has been demonstrated in animals as well as in tissue cultures for the microsomal oxidases, or by the induction of other enzymes which may be considered to be characteristic of the cancerous, such as reverse transcriptase, makes their being diagnostic for the beginning of the condition

a realistic one. An indication that there may be methods for effective prevention using a specific enzyme inhibitor has appeared in the demonstration that certain rifamycin derivatives given by suitable means can indeed slow down the appearance of hydrocarbon induced mammary tumors. The possibility of an effective treatment is always before us, particularly in terms of the cell membrane changes.

Proposed Course: Biophysical studies, including such techniques as mass spectroscopy and electron-spin resonance, will be applied to provide information on changes in cell structure following transformation in culture with chemical carcinogens. In addition, more detailed studies will be carried out to identify the chemical products of the interaction of polycyclic hydrocarbons with cells in tissue culture, as well as with the contained intracellular oncogenic viruses.

Date Contract Initiated: April 12, 1971

Current Annual Level: $185,851

AEC-NCI INTERAGENCY AGREEMENT, OAK RIDGE NATIONAL LABORATORY (NCI-FS-64-13)

Title: NCI-AEC Carcinogenesis Program

Contractor's Project Director: Dr. Francis T. Kenney

Project Officer (NCI): Dr. Allen H. Heim

Contract narrative is reported under the Office of the Associate Scientific Director for Carcinogenesis.

CONNECTICUT, UNIVERSITY OF (AT STORRS) (NIH-NCI-72-3268)

Title: Development of New Methods for Isolating Non-Histone Proteins With Affinity for Homologous DNA

Contractor's Project Director: Dr. Jay S. Roth

Project Officer (NCI): Dr. C. Wesley Dingman

Objectives: To develop and explore new techniques for isolating from mammalian cells non-histone proteins which show a high affinity for DNA, particularly homologous DNA since such proteins have the a priori possibility of being important in the regulation of genetic transcription.

Major Findings: Various methods for making DNA-cellulose are being explored. Successful preparations will then be used to chromatograph protein fractions and separate out those showing affinity for homologous DNA. Simultaneously, four techniques for the initial bulk

isolation of non-histone nuclear proteins have been explored to date. Two of these techniques show some promise and the proteins obtained from them (using rat liver as the source) are currently being further characterized as to their variety and affinity for DNA.

Significance to Biomedical Research and the Program of the Institute: The regulation of both DNA replication and DNA transcription involves proteins which show a specific affinity for homologous DNA. In many cases this affinity for DNA probably involves specific nucleotide sequences in the DNA. The future understanding of the control of DNA replication and transcription, both in normal and malignant cells, depend upon the ability to isolate and characterize those proteins involved in these processes. This project, if successful, should be of great help to those investigators interested in exploring the possibility that some aspects of cellular behavior, including malignancy, can be explained by alterations in the kind or amount of proteins which function by virtue of their specific binding to DNA.

Proposed Course: Different subcellular fractions will be tried as sources for these proteins and different chromatographic techniques will be applied in attempts to find the best possible means for isolating and fractionating the largest possible set of cell-specific, DNA-binding, non-histone proteins.

Date Contract Initiated: June 29, 1972.

Current Annual Level: $57,338

HOME FOR THE JEWISH AGED (NIH-NCI-71-2269)

Title: Study of Serum Haptoglobin Types in Patients with Carcinoma of the Pancreas

Contractor's Project Director: Dr. Henry Altschuler

Project Officer (NCI): Dr. Andrew C. Peacock

Objectives: The objectives are (1) to identify individuals who because of their genetic makeup may be liable to an increased risk of acquiring malignant disease of the pancreas; (2) to determine whether genetic characterisitcs believed to have significance with respect to leukemia and probably breast and lung cancer are significant in cancer of the pancreas.

Major Findings: Serum samples have been collected from individuals with diagnosis of cancer of the pancreas and other individuals, both healthy and with various diseases, to constitute control groups. The serum haptoglobin types are determined for each serum and a determination made whether the group with pancreatic cancer differs from the control groups with respect to the distribution of haptoglobin genes.

In addition, the ABO blood group distribution of the individuals belonging to these various groups is determined.

Significance to Biomedical Research and the Program of the Institute: There is an increasing need to define differences between individuals who are susceptible to some form of malignant disease and those who are not. The project may be helpful in this regard by establishing a possible marker characteristic of a very frequent and increasing form of cancer in man, for which very few etiological clues currently exist.

Proposed Course: With the increasing cooperation of a number of hospitals and physicians, it is expected that additional blood samples from patients with carcinoma of the pancreas can be collected. Collection and determination of haptoglobin types and ABO blood group distribution will be continued. The data will be tabulated for computer analysis on both the haptoglobin type and ABO blood group distribution.

Date Contract Initiated: April 29, 1971.

Current Annual Level: $28,988

ILLINOIS, UNIVERSITY OF (NIH-NCI-72-3303)

Title: Temperature Sensitive Mutants in In Vitro Carcinogenesis

Contractor's Project Director: Dr. Giampiero di Mayorca

Project Officer (NCI): Dr. John Bader

Objectives: To define a system for the study of chemical carcinogenesis in cell culture; to identify cells responsive to a variety of known carcinogens; to quantitate the transformation observed; to analyse the biochemical defects responsible for transformation.

Major Findings: Temperature dependence of the transformed phenotype of cells transformed by two different nitrosamines and of a spontaneously transformed clone has been demonstrated. In addition, BHK_{21} cells were transformed by brief exposure to different concentrations by benzo[a]-pyrene(BP). All the BP transformed clones seem to exhibit the same kind of conditional expression of the transformed phenotype found for the DMN and NMU transformed clones. When grown at 32°C their phenotype is normal as judged by the inability of plating in soft agar; when grown at 38.5°C their phenotype is transformed, they acquired the ability of plating in soft agar.

Infection of DMN and NMU clones (grown at 32° phenotypically normal) by hamster sarcoma virus or polyma virus resulted in transformation with the same frequency as that observed for the parental normal BHK_{21} cell.

An in vitro assay for transformation of three additional mammalian cell lines, 3T3 Swisse, (mouse), F111 (rat), and Fanconi anemia (men) by

the agar suspension technique has been developed.

Significance to Biomedical Research and the Program of the Institute: An exploitable system for the determination and analysis of chemical carcinogens is essential to progress in understanding carcinogenesis with the ultimate view to preventing and/or curing tumors induced by chemicals.

Proposed Course: Some cells exposed to chemical carcinogenesis have been observed by this investigator to behave as tumor cells at one temperature but as normal cells at a lower temperature. The possibility that chemical carcinogens generally produce temperature-dependent defects will be examined, and attempts to define the specific biochemical change responsible for tumorigenesis will be made.

Date Contract Initiated: June 20, 1972

Current Annual Level: $39,765

JOHNS HOPKINS UNIVERSITY (NIH-NCI-71-2169)

Title: Studies on the Regulation of the Heme Moiety of P-450 in Relationship to Carcinogen Metabolizing Activity

Contractor's Project Directors: Dr. Ephraim Y. Levin
Dr. Peter R. Adams

Project Officer (NCI): Dr. Harry V. Gelboin

Objectives: To determine the mechanism of regulation of the synthesis and degradation of the heme moiety of P-450, and to examine this regulation with respect to carcinogen metabolism.

Major Findings: In vitro studies of microsomal heme synthesis from labelled precursors have shown the presence of a component in liver cell sap which transfers mitochondrially-synthesized heme to microsomes. An assay method has been developed for this component, showing it to be active at low concentrations. Bovine serum albumin is relatively inactive at comparable protein concentrations.

Significance to Biomedical Research and the Program of the Institute: The heme transfer component may be instrumental in regulating the level of microsomal heme and, through this, the carcinogen metabolizing activity of the cell. These aspects of cancer development should then be accessible to direct study.

Proposed Course: To determine the properties of the heme transfer component, and the effect of carcinogens on its role in microsomal heme synthesis.

Date Contract Initiated: April 30, 1971

Current Annual Level: $41,183

McGILL UNIVERSITY (NIH-NCI-72-3295)

Title: Isolation, Purification, and Characterization of Human Prolactin

Contractor's Project Director: Dr. Henry Friesen

Project Officers (NCI): Dr. Andrew Peacock
Dr. Marie Green

Objectives: (1) To study methods for the purification of human prolactin from (a) the pituitary and (b) amniotic fluid; (2) to provide small amounts of human prolactin and antiserum to human prolactin; (3) to develop radioreceptor assays for prolactin.

Major Findings: Human prolactin has been purified from a side fraction obtained during the course of purification of human growth hormone from acetone dried human pituitary glands. Methods have been developed for the purification of human prolactin from frozen human pituitary glands obtained at post mortem. The yield of prolactin from this source is considerably greater than from acetone dried glands. Approximately 50 ug prolactin pituitary was obtained.

A total of 50 mgs human prolactin has been obtained so far. The purity of the human prolactin has been validated by bioreceptor- and radioimmunoassay as well as electrophoretically and by amino acid sequence analysis.

Pilot studies have been carried out on the purification of human prolactin from amniotic fluid. Methods are being explored for the handling of 25-50 litre batches of amniotic fluid.

Antibodies to human prolactin have been generated by immunizing 6 guinea pigs and 6 rabbits with human prolactin. Some of the antisera obtained have proved to be satisfactory for the development of radioimmunoassays.

Specific membrane receptors for prolactin have been identified in mammary tissue of rabbits and rats. These membrane receptors have been utilized for the development of a specific and sensitive radioreceptor assay for prolactin and other lactogenic hormones.

Significance to Biomedical Research and the Program of the Institute: It is now feasible for the first time to examine serum prolactin concentrations in normal subjects as well as in patients with breast cancer under basal conditions as well as after provocative and inhibitory tests. These studies may reveal significant differences in the secretion of human prolactin between normal and breast cancer patients.

The development of a radioreceptor assay for prolactin has several important features. It permits rapid bioassays of prolactin preparations obtained from many different species. It makes structure function studies of prolactin feasible. The successful development of methods for identifying prolactin receptors in mammary tissue of animals should prove helpful in

the search for and quantitation of prolactin receptors in human breast tissue both normal and cancerous.

Proposed Course: The purification of human prolactin from pituitary glands will be continued in order to obtain sufficient material for the complete amino acid sequence analysis, for structure-function studies, for radioimmunoassays, and to have material on hand to provide human prolactin to other investigators around the world for assays.

Date Contract Initiated: July 30, 1972

Current Annual Level: $38,723

NEW YORK UNIVERSITY (NIH-NCI-71-2183)

Title: The Isolation, Propagation, and Storage of Mutant Vertebrate Cells with Specific Biochemical Lesions

Contractor's Project Director: Dr. Claudio Basilico

Project Officer (NCI): Dr. John P. Bader

Objectives: The objective of this contract is the isolation and characterization of ts mutants of somatic animal cells. Conditional lethal temperature sensitive mutations have proven to be extremely useful for a variety of studies with microorganisms, and should likewise be helpful in studies with somatic animal cells. The defect of ts mutants is usually attributable to a protein which is non-functional at high temperature, but can function normally at low temperature. Since this kind of mutation is not associated with a particular gene, a wide spectrum of cell functions can thus be studied. Thus, ts mutants should be very useful to further our understanding of the eukaryotic cell and its regulatory mechanisms. Such knowledge is also basic to our elucidating the neoplastic process.

Major Findings: A number of ts mutants of the Syrian hamster cell line BHK 21/13 have been isolated, and preliminary characterization begun. About 40 ts mutants of BHK have been isolated, including several derived from an hypoxanthine-guanine-phosphorybosil transferase deficient BHK variant. These last cells, therefore, carry two mutations and are invaluable for genetic analysis. These mutants exhibit low reversion and low leakiness, and as such are very suited for biochemical and genetic studies. In most of them, treatment with Nitrosoguanidine increases the frequency of reversion five to tenfold, suggesting that these mutations are in single genes, possibly originating from simple base substitutions. The ts mutations studied are recessive, as shown by experiments of somatic cell hybridization.

More detailed analysis of one of these mutants reveals an inhibition of production of 28S rRNA to the extent of 95%, the impairment taking place at the 32S stage.

Significance to Biomedical Research and the Program of the Institute: Since altered cell membranes may be directly responsible for conversion of cells to malignant forms, such mutants will be extremely valuable in studies on carcinogenesis. This laboratory has isolated temperature sensitive mutants, one of which is defective in the processing of ribosomal RNA from a precursor. This single mutant allows the examination of a number of physiological processes related to RNA metabolism and their role in carcinogenesis.

Proposed Course: The proposed course aims at: (a) the isolation of additional ts mutants, particularly from cells already carrying other genetic markers; (b) the improvement of the selection and mutagenization procedures for the purpose of obtaining specific classes of ts mutants (DNA synthesis, protein synthesis, etc.); (c) the further characterization of the mutants already obtained, both from the biochemical and genetic point of view; (d) the use of some of the mutants for specific experiments aimed at the investigation of the relationship between ribosomal RNA synthesis and cell division, or the study of the host cell functions required for Adenovirus multiplication, or Polyma neoplastic transformation.

Date Contract Initiated: June 26, 1971

Current Annual Level: $78,325

ONTARIO CANCER INSTITUTE (NIH-NCI-72-2051)

Title: The Isolation, Propagation and Storage of Mutant Vertebrate Cells with Specific Biochemical Lesions

Contractor's Project Director: Dr. Louis Siminovitch

Project Officer (NCI): Dr. John Bader

Objectives: (1) Isolation of cell lines carrying mutations affecting key cell functions such as DNA replication or mitosis, or key cell structures such as the plasma membrane. (2) Biochemical and physiological characterization of the mutant lines. (3) Examination of the properties of hybrid cell lines obtained by fusion of cells of one mutant line with other normal or mutant lines, for complementation analysis and possible mapping. (4) Extension of the techniques, as they are developed, to a variety of cell types chosen not only on the basis of their suitability for studies of the regulation of cell multiplication and differentiation, but also for other purposes, such as the detection of environmental carcinogens and mutagens.

Major Findings: Conditional lethal temperature-sensitive mutants of Chinese hamster ovary cells have been selected. All isolates have been characterized in a preliminary way and one isolate has been studied intensively. In this mutant, protein synthesis is defective at the non-permissive temperature, most probably due to a temperature-sensitive leucine synthetase. A method to isolate specific temperature-sensitive

mutants for protein synthesis has been developed.

Mutants resistant to inhibitors such as ouabain, α-amanitin, colchicine, concanavalin A (conA) and phytohaemagglutinin (PHA) have been selected. The ouabain mutation is probably co-dominant and its frequency of occurrence has been measured. The mutation to α-amanitin probably involved the structural gene for RNA polymerase, since mutants have normal enzyme levels with altered sensitivity to the drug. Colchicine-resistant mutants seem to involve an alteration in the cell membrane. Such mutants show collateral resistance to other unrelated drugs, such as actiomycin D. ConA-resistant mutants also seem to show associated defects, such as temperature sensitivity. The PHA mutants are in the process of being characterized.

Significance to Biomedical Research and the Program of the Institute: Cancer may be regarded as a disease in which cells are genetically altered such that their regulation of cellular multiplication and differentiation is defective. Temperature-sensitive mutations affecting key functions involved in cell growth, and in particular DNA replication and mitosis are of specific interest in respect to obtaining an understanding of the breakdown of regulation during carcinogenesis. Suitably characterized cell lines are useful not only for studies of cellular regulatory processes, but for other purposes as well. One example is the use of cell culture systems for the detection of environmental carcinogens or mutagens. In addition, studies on the genetic basis of drug resistance are of special interest in respect to chemotherapy. For example, our finding that mutation to resistance to colchicine and to conA, engenders collateral changes in sensitivity to other drugs must be considered in any consideration of multiple drug trials.

Proposed Course: To continue with the studies as planned for the next two years.

Date Contract Initiated: January 1, 1972

Current Annual Level: $64,936

POLYSCIENCES, INC. (NIH-NCI-72-3245)

Title: Optimizing Electrophoretic Separation of Proteins and Nucleic Acids with New Hydrogels

Contractor's Project Director: Dr. B. D. Halpern

Project Officer (NCI): Dr. Andrew Peacock

Objectives: To develop hydrogels specifically suitable for gel electrophoresis. Such gels will permit greater elutibility of substances from the gels, larger pore size for study of large molecules and incorporation of specific reagents to produce desirable associative effects to improve the resolution.

Major Findings: Several new hydrogel systems have been developed, based on α,ωpolyoxyethylene glycol diacrylates. Preliminary results indicate that these gels have more available large pores than acrylamide-MBA gels as indicated by the electrophoretic mobilities of ribosomal RNA (16S+ 23S RNA) and high molecular weight DNA's, especially with gels made of polyethylene glycol 20,000 diacrylate. Preliminary studies of electrophoresis of DNA indicate that DNA molecules of $>10^6$MW, which are otherwise difficult to separate, could be separated by use of trace amounts of a cationic monomer incorporated in the gels, making use of selective charge effects. Further work on the electrophoretic separation of high molecular weight RNA and DNA is in progress, using the above parameters of large pore size and charge effects. Several new reversible gels have been developed based on new reversible crosslinkers-gels solubilized by suitably mild chemical treatment, and thermally reversible monomer system. The application of these gels for electrophoresis and recovery of samples is under investigation.

Significance to Biomedical Research and the Program of the Institute: Important elements in cellular control reside in the macromolecules particularly DNA and RNA in the cell. This project will promote the isolation, characterization and study of such molecules.

Proposed Course: Specific types of gels will be developed and studies for the electrophoretic separation of high molecular weight RNA's (ribosomal and viral) and DNA's, related to the problems of carcinogenesis studies.

Date Contract Initiated: May 17, 1972

Current Annual Level: $54,051

TEXAS, UNIVERSITY OF (M.D. ANDERSON HOSPITAL AND TUMOR INSTITUTE) (NIH-NCI-71-2268)

Title: Study of Serum Haptoglobin Types in Patients with Carcinoma of the Pancreas

Contractor's Project Director: Dr. David E. Anderson

Project Officer (NCI): Dr. Andrew Peacock

Objectives: The objective is to identify persons who, because of their genetic constitution, may have an increased risk for developing cancer of the pancreas.

Major Findings: Serum samples are collected from patients with the diagnosis of cancer of the pancreas and from patients with diagnoses of other neoplasms. Samples are also collected from healthy controls. Serum haptoglobin type is determined for each cancer patient and control, and the various types of cancer patients are then compared to the controls with regard to the distribution of the haptoglobin type and the frequency of the haptoglobin 1 gene.

The contractor has found 52 patients with a provisional diagnosis of cancer of the pancreas, 32 of whom were suitable for study and were typed and analyzed, 10 have not yet been typed and/or analyzed, and 10 did not fulfill the requirements of the study. The collection mechanism has been modified to increase the number of patients the third year. Also typed and analyzed are 1,613 patients with other cancers and 288 healthy controls. The results to date indicate an excess of haptoglobin type 1-1 and haptoglobin 1 gene in patients with cancer of the pancreas compared with controls, but the sample size is still to small to demonstrate a statistically difference. An unexpected finding is the occurrence of pancreatic cancer on a familial basis in some cases.

Significance to Biomedical Research and the Program of the Institute: This project may be helpful in establishing a possible marker characteristic for a very frequent form of cancer in man for which very few etiological clues presently exist. The project is attempting to determine whether genetic characteristics believed to have significance with respect to leukemia are significant in cancer of the pancreas.

Proposed Course: Approximately 50 samples of serum from patients with cancer of the pancreas are anticipated during the coming year. Data from previous studies (on breast and leukemia) suggest that about 100 samples may be required to show the expected differences. The racial and age distribution in the control group will depend on the characteristics found in the cancer group. An attempt will be made to add additional controls, including older persons (65-90 years) of various ethnic groups (i.e., from denominational old age homes) and from persons hospitalized for causes other than pancreatic cancer. Approximately 500-700 such serums will be studied.

Date Contract Initiated: May 19, 1971

Current Annual Level: $24,654

TEXAS, UNIVERSITY OF (M.D. ANDERSON HOSPITAL AND TUMOR INSTITUTE (NIH-NCI-72-3269)

Title: Non-Histone, DNA Binding, Proteins From Normal Rat Liver and Chemically Induced Rat Hepatomas

Contractor's Project Director: Dr. Lubomir S. Hnilica

Project Officer (NCI): Dr. C. Wesley Dingman

Objectives: The objective of this project is the isolation of significant amounts of non-histone chromosomal proteins from normal rat liver and from rat hepatomas.

Major Findings: A non-histone protein fraction represented by 3 major polypeptide bands in acrylamide gel electrophoresis (in the presence of 0.1% sodium dodecyl sulfate) was isolated from rat liver chromatin. This

protein fraction binds strongly to homologous DNA. Its binding to rat DNA is stronger than that of the histones. When complexed to homologous DNA, this fraction elicits tissue-specific antibody formation in rabbits and the tissue-specificity of rat liver chromatin detectable by micro-complement fixation is determined by these proteins. By immunochemical criteria, the DNA binding protein fraction in hepatoma is different from the corresponding proteins in liver.

Significance to Biomedical Research and the Program of the Institute: One of the major goals of the Carcinogenesis Program is the elucidation of the mechanism of action of known carcinogens. One hypothesis for malignant transformation is that carcinogens alter the regulation of genetic transcription, either by a direct effect on particular genes or by an alteration of those proteins which function in regulation of gene transcription. The purpose of this contract is to fractionate and characterize, by present techniques, that set of proteins which are now thought to act as regulators of genetic function and then to determine whether or not members of this set are altered in amount of kind during the induction of hepatomas in the rat by chemicals. Knowledge of these biochemical processes would permit the study of ways to influence or otherwise modify significant steps in the malignant transformation process.

Proposed Course: The proteins, once isolated, are to be fractionated and examined for species which show qualitative or quantitative differences between normal liver and tumor. Such species will then be further characterized as to their physical properties, enzymatic properties, and the specificity of their derivation from normal or malignant hepatic parenchymal cells.

Date Contract Initiated: June 29, 1972

Current Annual Level: $59,451

WEIZMANN INSTITUTE OF SCIENCE (NIH-NCI-70-2217)

Title: The Role of the Enzyme Aryl Hydrocarbon Hydroxylases and its Induction in Polycyclic Hydrocarbon Carcinogenesis

Contractor's Project Director: Dr. Leo Sachs

Project Officer (NCI): Dr. Harry V. Gelboin

Objectives: (1) To study the effect of polycyclic hydrocarbon activated metabolites on cytotoxicity, mutagenicity, and transformation of human cell cultures; (2) To study the chromosomal control of chemical carcinogens with refined methods of chromosome identification; (3) To study the chromosomal control of the microsomal carcinogen metabolizing enzyme system.

Major Findings: Cells can be transformed by the chemical carcinogen, dimethylnitrosamine, which will revert under certain circumstances to relatively normal states or at least to a loss of malignancy. A chromosomal

balance at these various states has been examined and found that certain chromosomal groups seem to be identified with the expression and suppression of malignancy by cells transformed by polyoma virus. Thus, the data suggest that viral and nonviral carcinogens induce malignancy by inducing similar chromosomal rearrangements. In other aspects of the study the stability of the reverted state has been analyzed and the data has been obtained on the incidence of reversion as well as rereversion to the malignant state. The most recent aspects have show that when cells revert to the malignant state they contain a high frequency of segregance which lose this property rapidly. The different degrees of stability appear related to differences in the balance of chromosomally located factors that determine the expression and suppression and transformed properties.

Significance to Biomedical Research and the Program of the Institute: Polycyclic hydrocarbons represented major contaminants in the environment and are serious threats to the human organism in a highly industrialized society. The enzyme system dealing with these agents is aryl hydrocarbon hydroxylase, and our findings indicate that the enzyme system is responsible for the activation of the hydrocarbon to a toxic and probably a carcinogenic form. The observation that transformed cells can revert back to normal properties is an exciting and important finding that may enable an understanding of conditions favoring a reversion of carcinogen transformed cells back to a normal state.

Proposed Course: To further clarify the relationship between the presence of the enzyme, the metabolism of carcinogenic hydrocarbons, and malignant cell transformation. Inhibitors of the enzyme, such as 7,8-benzoflavone, will be tested for their effect on cell transformation induced by carcinogenic hydrocarbons other than benzo(a)pyrene. The revertants produced from transformed cells without detectable enzymes will be tested for their enzyme activity in order to determine whether the suppression of malignancy results in the re-appearance of the enzyme in the reverted cells. Revertants from cells transformed by carcinogenic hydrocarbons and other chemical carcinogens will also be tested for their susceptibility to the cytotoxic and cell transforming activity of carcinogenic hydrocarbons. This contract has now added an investigator who has had extensive experience in the metabolism of polycyclic hydrocarbons in cell culture. He will look at the profile of metabolites under different conditions of enzyme level and in the normal, malignant, reverted and rereverted state.

Date Contract Initiated: May 26, 1970

Current Annual Level: $75,400

SUMMARY REPORT

COLON CANCER SEGMENT

July 1, 1972 through June 30, 1973

The goal of the contract program of the Colon Cancer Segment is to identify the environmental carcinogen(s) responsible for colon cancer in the U. S. environment. Its methods are to supplement the Demography Area's questionnaire studies on Japanese (who show a sharp increase in incidence upon migration from Japan to the United States), and to develop other laboratory and epidemiologic studies that will amalgamate the migrant program and the several relevant Carcinogenesis Area investigative programs into a coordinated whole. Close liaison is maintained with the National Program for Large Bowel Cancer.

Large bowel cancer is, except for skin cancer, the most common cancer afflicting the U. S. population. Only lung cancer causes more cancer deaths. The NCI migrant program has helped establish that bowel cancer is caused by an environmental factor and promises to be the first epidemiological investigation to throw suspicion upon particular diet items as causal factors. Moreover, the migrant cohorts are optimal for testing hypotheses advanced by others. The Segment hopes to mobilize the resources and diverse scientific talents necessary to follow up the findings of the migrant epidemiologic studies and to provide related base-line information.

Following animal analogies, most hypotheses on the cause of human bowel cancer assume that the carcinogen is produced in the large bowel by bacteria acting on some fecal precursor, either ingested or, like bile, secreted preferentially by people on particular diets. The ongoing analysis by the Biometry Branch, NCI, of case-control data from Hawaiian Japanese does show specific differences of diet for the colon cancer patients. One possibility is that there is a particular type of microorganism in cancer patients that has produced a specific potent carcinogen. To determine this, the Segment is characterizing, for the first time, the specific flora of tumor-bearing Hawaiian Japanese and suitable controls through a Demography contract with Virginia Polytechnic Institute, (NIH-NCI-71-2427). (A paralled study of Californian Japanese is being terminated, despite a productive first year, because of lack of funds.) The program plan is to transfer the strains of bacteria most characteristic of tumor-bearing patients to the NCI Frederick Cancer Research Center where they can be studied to see if they produce carcinogens and, if so, under what circumstances and from what substrate precursors.

With the complexity of fecal flora (as many as 600 species per person) and the difficulty in handling these anaerobes, many of which have never been metabolically characterized, the Segment would like to be able to examine feces directly for carcinogenic substances. There is no good way of doing this at present. At the Oak Ridge National Laboratory, Dr. Lijinsky is undertaking a broad study of nitrosamines, probably the most ubiquitous potential human carcinogens in relation to gastrointestinal cancer. A major objective will be to find how to obtain hard evidence for a role of a nitrosamine in human large bowel cancer.

The other possibility is that a very general bacterial reaction produces a carcinogen from some specific fecal component. The American Health Foundation contract is devoted primarily to the search for such general reactions and to the testing of common fecal constituents not now known to be carcinogens for both general carcinogenicity and for carcinogenic effects on large bowel mucosa.

Major findings in the contract program during the past year include the following: In studies of humans on different diets, the American Health Foundation group has shown that those on the typical American "high-risk" diet have much higher β glucuronidase activity than do people on "low-risk" diets. Since many carcinogens and other toxic chemicals are rendered harmless through biliary excretion as glucuronides, this enzyme could be a major unmasker of carcinogens in the feces. Also, Americans consuming a western-type diet excreted high levels of bile acids as well as increased amounts of microbially degraded acid and neutral steroids compared to other groups. In vitro incubation studies also indicate that acid and neutral steroids were more extensively degraded to various metabolites by the anaerobes isolated from Americans on Western diet compared to vegetarians. These metabolites might well be carcinogens and are being tested as they become available in adequate amounts.

Many N-nitroso compounds are potent carcinogens for animals and this group of compounds represents a significant potential carcinogenic hazard for man. The possible formation of nitroso compounds in vivo from nitrite and secondary or tertiary amino compounds is the most important aspect. The investigations underway might establish the significance of formation of nitrosamines from amines ingested by man as drugs, food components and agricultural chemicals. At Oak Ridge, Lijinsky has shown that the combination of a secondary amine, heptamethyleneimine, and one tertiary amine, aminopyrine (pyramidon), with nitrite, by concurrent administration in drinking water to rats, gave rise to a high incidence of malignant tumors of, respectively, lung (squamous carcinoma) and liver (hemangioendothelioma). In the case of both amines, the tumors produced were those arising after feeding of the respective nitrosamines to rats. It can be deduced that the amines reacted with nitrite in the gastrointestinal tract to form significant quantities of nitrosamines. It has also been shown that nitrosamines are formed by interaction of several tertiary amines and alkylamides with nitrous acid. Measurable amounts of dialkylnitrosamines were formed at concentrations of a few milligrams per milliliter of amines and nitrite. Compounds examined include drugs, natural components of food, pesticides and herbicides. The kinetics of these nitrosations of secondary and tertiary amines show a large steric retarding effect in the presence of methyl groups α to the nitrogen atom.

In the Memorial Hospital contract, Lipkin, running parallel studies in mice and polyposis patients, has not only shown his model system to have enzymatic analogies to early changes in humans but has definitely shown that, in people with the genic lesion that leads to polyposis, a failure to repress DNA synthesis precedes any visible abnormal change. This is the first step anyone has taken to defining the defect produced by this dominant gene mutation.

Other parts of the contract program are either still in the phase of developing resources and techniques or just beginning to apply these techniques to

the human problem. The two epidemiology contracts, designed to confirm and elaborate on Haenszel's diet studies, are still at the stage of questionnaire development. The American Health Foundation has chosen 1,-2 dimethylhydrazine in rats from among many chemicals and species as the standard animal model, is now testing the system in germ-free animals, has established an intrarectal assay system, and has begun testing of bile metabolites, especially those characteristic of high risk individuals, but is not yet in a position to present definitive results.

The proposed course of the segment program is to seek more and more specific information about the diet factors, to characterize patients with bowel tumors more and more specifically as to flora and digestive metabolism, and to learn in model systems what these characteristics mean in terms of colon carcinogenesis.

CONTRACT NARRATIVES

COLON CANCER SEGMENT

July 1, 1972 through June 30, 1973

AEC-NCI INTERAGENCY AGREEMENT (OAK RIDGE NATIONAL LABORATORY) (NIH-NCI-72-204)

Title: Role of Nitrosamines in Carcinogenesis

Contractor's Project Director: Dr. W. Lijinsky

Project Officers (NCI): Dr. John Cooper
Dr. John W. Berg

Objectives: To investigate the possible relevance of nitrosamines to human cancer. This will involve the following: (1) Surveying the distribution of nitrosamines and nitrosatable amines in the environment, (2) developing new analytical methods for amines, (3) investigating the formation of nitrosamines from amines and nitrite in chemical systems and in vivo and, (4) examination of the structural characteristics of nitrosamines related to organ specific carcinogenicity.

Major Findings: (1) The testing of one secondary amine, heptamethyleneimine, and one tertiary amine, aminopyrine (pyramidon), together with nitrite, by concurrent administration in drinking water to rats, has given rise to a high incidence of malignant tumors of, respectively, lung (squamous carcinoma) and liver (hemangioendothelioma). In the case of both amines, the tumors produced were those arising after feeding of the respective nitrosamines to rats. It can be deduced that the amines reacted with nitrite in the gastrointestinal tract to form significant quantities of nitrosamines. None of the control animals fed nitrite or either amine alone has died with a tumor. (2) The formation of nitrosamines by interaction of several tertiary amines and alkylamides with nitrous acid in purely chemical systems has been continued. Measurable amounts of dialkylnitrosamines were formed at concentrations of a few milligrams per milliliter of amines and nitrite. Compounds examined include drugs, natural components of food, pesticides and herbicides. (3) The kinetics of nitrosation of secondary and tertiary amines have been examined, particularly the bearing of chemical structure on the rate of formation of nitrosamines. There is a large steric retarding effect in the presence of methyl groups α to the nitrogen atom. The reaction of tertiary amines is, in general, much slower than that of secondary amines. (4) Methods have been developed for the isolation, separation and identification of secondary and tertiary amines in food and other mixtures, such as tobacco smoke.

Significance to Biomedical Research and the Program of the Institute: Many N-nitroso compounds are potent carcinogens for animals and this group of compounds represents a significant potential carcinogenic hazard for man. The possible formation of nitroso compounds in vivo from nitrite and secondary or tertiary amino compounds is the most important aspect. The investigations underway might establish the significance of formation of nitrosamines from

amines ingested by man as drugs, food components and agricultural chemicals and help explain some of the cancer incidence in the human population.

Proposed Course: To pursue the objectives stated above.

Date Contract Initiated: June 30, 1972

Current Annual Level: $310,000

AMERICAN HEALTH FOUNDATION (NIH-NCI-71-2310)

Title: Experimental Large Bowel Carcinogenesis

Contractor's Project Director: Dr. Ernest L. Wynder

Project Officer (NCI): Dr. John W. Berg

Objectives: To establish suitable animal models of colon carcinogenesis in bacteriologically defined and controlled systems; to determine the effect of dietary factors as modifiers of colon carcinogenesis; to determine the carcinogenicity of selected bile metabolites; to carry out parallel studies in animal and man relating the role of diet, in general, and acid and neutral steroids and tryptophan metabolites, in particular, in colon carcinogenesis; to isolate pure strains of the major groups of intestinal microflora from high and low risk populations, and study their enzymatic capability to degrade acid and neutral steroids into potentially carcinogenic compounds.

Major Findings: Colon tumors were induced by 1,2-dimethylhydrazine (DMH) in rats, but this process was influenced little by the fat level in the diet. DMH treatment in rats caused a slight change in fecal acid and neutral steroid excrition. Diet and DMH treatment showed effect on cecal microflora and cecal β-glucuronidase activity in rats. Studies in man indicate that the fecal microflora of Americans consuming a mixed Western diet showed increased ability to hydrolyze glucuronide conjugates as compared to those of American vegetarians, Seventh-Day Adventists, Japanese and Chinese. Americans consuming a Western-type diet excreted high levels of bile acids as well as increased amounts of microbially degraded acid and neutral steroids, compared to other groups. *In vitro* incubation studies also indicate that acid and neutral steroids were more extensively degraded to various metabolites by the anaerobes isolated from Americans on Western diet compared to vegetarians. Anaerobes isolated from American on Western diet showed a higher hydroxycholanyl-dehydrogenase and -dehydroxylase activity than those from vegetarians.

Significance to Biomedical Research and the Program of the Institute: Colon cancer constitutes one of the major types of cancer in the United States and in the Western World. The etiology of colon cancer, and factors or mechanisms related thereto are unknown. The current long-term effort is part of a key program to link animal experimentation and the study of human cancer causation. The project also serves as a flexible, skilled, well-equipped multi-disciplinary central resource for the Colon Cancer Segment as regards wide-ranging, mainly non-bacteriological laboratory investigations.

The information and new developments provided by this active research program are designed to yield ultimately an understanding of the contribution of exogenous and endogenous factors, such as diets, intestinal microflora, bile constituents, to the etiology of colon cancer in man. Evaluation of the main elements in this complex puzzle may eventuate in the formulation of a rationale and sound set of preventive approaches.

Proposed Course: (1) To study DMH carcinogenesis and MNNG carcinogenesis in germfree and defined flora rats; (2) to continue studying fecal chemistry and microflora in populations of epidemiologic interest with regard to colon cancer, and in patients with colon cancer and with polyps; (3) to study the effect of high meat, high animal fat diet, in relation to appropriate controls, on the chemical components and microflora of feces; (4) to investigate the effect of different meat diets on chemical components of feces in germfree and control rats; (5) to test the carcinogenicity of bile metabolites in the mouse skin induction-promotion system, as well as in germfree and conventional rats by intrarectal infusion; (6) to study the ability of pure strains of fecal microflora, isolated from groups of populations on different diets, to degrade or metabolize acid and neutral steroids and other bioactive compounds into potentially carcinogenic compounds; (7) to develop information on the metabolism of nitrosomyoglobin and other nitrosated chemicals in germfree and conventional rats.

Date Contract Initiated: June 30, 1971

Current Annual Level: $349,243

FREDERICK CANCER RESEARCH CENTER (NIH-NCI-72-3294

Task Order #7

Contract narrative is reported under the Office of the Associate Scientific Director for Carcinogenesis.

GEORGIA, MEDICAL COLLEGE OF (NIH-NCI-72-3280

Title: Epidemiologic Study of Colon Cancer Among Blacks and Whites

Contractor's Project Director: Dr. Warren Gullen

Project Officer (NCI): Dr. Margaret Howell

Objectives: To explore through case control studies whether or not dietary factors are associated with cancer of the colon and, if so, to identify specific dietary components which have implications for prevention of the disease.

Major Findings: No major findings are available since the contract is in a developmental phase involving the construction and pretesting of a diet questionnaire.

Significance to Biomedical Research and the Program of the Institute: Epidemiological studies of migrants, particularly the Japanese in Hawaii and migrants from rural to urban areas in this country, have indicated an increased risk of colon cancer associated with the new place of residence. Such studies point to environmental factors in the etiology of colon cancer, of which diet is the most likely. This study will be one of few undertaken thus far to explore the association of diet and colon cancer in Americans living in the continental United States.

Proposed Course: In a group at relatively low risk for colon cancer, Blacks in Georgia, colon cancer cases will be compared on dietary and demographic data with control cases. For contrast, a case control study will also be performed on a group at comparatively higher risk, Whites in Georgia. Cases, about 150 of each group, will be identified in hospitals in Atlanta. The control for a case will be the next patient admitted to the same hospital, matched for age, race, and sex and without medical problems suspected of being associated with colon cancer.

Date Contract Initiated: June 30, 1972

Current Annual Level: $13,964

KAISER FOUNDATION RESEARCH INSTITUTE (NIH-NCI-73-3215

Title: Epidemiologic Study of Colon Cancer Among Blacks

Contractor's Project Director: Dr. Gary Friedman

Project Officer (NCI): Dr. Margaret Howell

Objectives: To search for dietary factors which may be associated with cancer of the large bowel and to identify specific dietary components which have implications for prevention of the disease.

Major Findings: This is a new contract, and no findings are available at this time.

Significance to Biomedical Research and the Program of the Institute: Research on Japanese migrants to Hawaii has clearly implicated dietary factors in the etiology of cancer of the large bowel. The present study is one of few undertaken to explore the association between diet and the disease in Americans living in the continental United States. If the Hawaiian findings are confirmed, the results will permit greater specificity in the laboratory testing of chemical and bacteriologic agents in the disease process.

Proposed Course: Cases of cancer of the large bowel among a group of relatively low risk (Blacks) will be identified in hospitals in five San Francisco Bay Area counties. The estimated 195 cases will be compared with age-sex-race matched controls (three per case) on diet history and relevant demographic information.

Date Contract Initiated: December 1, 1972.

Current Annual Level: $45,480

MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES (NIH-NCI-72-2041)

Title: Regulatory Control of Cell Proliferation in Colonic Tissue (in Familial Polyposis)

Contractor's Project Director: Dr. Martin Lipkin

Project Officer (NCI): Dr. Lioniel Poirier

Objectives: To determine the enzymatic and/or kinetic changes that characterize the earliest stages of the pathological bowel mucosa of familial polyposis and so to determine the basic molecular lesion initiating carcinogenesis in this condition inherited through a dominant gene.

Major Findings: (1) Inheritance of a Trait Leading to Prolonged DNA Synthesis in Colon Polyposis Families - In familial polyposis, prior to the appearance of polyps, colonic epithelial cells lose the ability to repress DNA synthesis, and a sequential expression of proliferative and differentiation specific defects develop as neoplasms form. Individuals in the general population with isolated polyps have shown these abnormalities, but their expression in familial polyposis is more widespread and occurs at an earlier age.

(2) Thymidylate Synthetase and Thymidine Kinase Activities in Intestinal Mucosa - Thymidylate synthetase activity paralleled thymidine kinase activity. The highest activity occurred in crypt cells of jejunum with a sharply decreasing gradient in both enzymes as cells migrated onto villi. The findings indicate that activities of the two enzymes involved in DNA synthesis are highest in proliferating cells. These findings contrast with previous data on enzymes involved in other areas of nucleic acid metabolism that increase in normally maturing intestinal cells. The latter include thymidine phosphorylase, adenosine deaminase and adenine-, and hypoxanthine phosphoribosyltransferases, all of which increase in villus compared to crypt cells.

(3) Induction of Colonic Neoplasms in Mice with 1,2-Dimethylhydrazine - In order to provide an animal model of colon polyposis and carcinoma to accompany our studies in man, both polyps and carcinomas were induced in mice by a chemical carcinogen, 1,2-dimethylhydrazine (DMH). The findings have indicated interesting similarities between the development of neoplasms in familial polyposis and in mice given a chemical carcinogen.

Of particular interest were the following observations that apply to both Familial Polyposis and chemical carcinogenesis in colons of mice: (1) chemically induced carcinomas develop in flat colonic mucosa as well as in polyps; (2) before the appearance of morphological evidence of colon carcinoma there is, in both human and animal colon cells, a loss of the ability to repress DNA synthesis during migration of epithelial cells to the colonic mucosal surface; (3) the activity of a differentiation-specific enzyme hypoxanthine

phosphoribosyltransferase is decreased in neoplastic colon cells in man and in intestinal cells of rodents.

Significance to Biomedical Research and the Program of the Institute: There is every reason to believe that the adenoma-cancer transition is the same in multiple polyposis as it is in colon cancer generally. It seems quite possible that the earlier stages of tumorigenesis might also be the same. If so, the polyposis syndrome, caused by a single gene mutation, would be a most productive place to look for the earliest possible marks of carcinogenesis. Also, by identifying the underlying molecular alteration, we could be a great deal closer to determining what type of environmental factor we are looking for. It is submitted that genetic defects of this type have been the most fruitful source of our understanding of the molecular pathology underlying human disease and that our study of colon cancer should take advantage of the existence of this appropriate "experiment in nature."

Proposed Course: The comparative ratios of thymidylate synthetase and thymidine kinase, as well as adenine and hypoxanthine phosphoribosyltransferase, will be studied in polyps removed from patients with familial polyposis. In addition, the thymidylate synthetase and thymidine kinase ratios will be studied in the familial polyposis cells in order to indicate whether differences in specific metabolic input pathways leading to DNA synthesis may be present in the surface cells that have been observed to incorporate thymidine ^{3}H. The findings will clarify which abnormalities in nucleic acid intermediary metabolism exist in the familial polyposis cells during the proliferative and differentiation specific phases of their life cycle.

Date Contract Initiated: October 15, 1971

Current Annual Level: $115,400

OXFORD UNIVERSITY (NIH-NCI-72-3215)

Title: Determination of Disease Linkages

Contractor's Project Director: Dr. John Baldwin

Project Officer (NCI): Dr. John W. Berg

Objectives: To investigate hypotheses that certain diseases (such as coronary heart disease) are caused by the same factors as specified bowel tumors, and will therefore be linked in individuals in a general population more frequently than would be expected by chance.

Major Findings: Project was delayed six months because of lack of personnel and no data are available.

Significance to Biomedical Research and the Program of the Institute: The association of colon cancer and other diseases in populations has been the subject of much discussion. Determination of the links in individuals would provide invaluable clues to etiology and, as well, provide markers of high

risk for detection programs. While we continue to seek this kind of information from U. S. sources, all are small and/or highly selective populations, and the data are not readily accessible to say the least. Major segments of later parts of the program will be based on these epidemiologic findings.

Proposed Course: Existing computer programs will determine which diseases are associated with bowel tumors by determining which preceded or succeeded bowel tumors more often than would have been expected from the incidence in the general population of the region. Statistically significant associations will be subjected to case review to verify diagnoses and to rule out artifactual associations. The project officer will serve as principal investigator with responsibility for scientific input (Dr. Baldwin serves as the Administrative head only).

Date Contract Initiated: April 9, 1972

Current Annual Level: $11,000

SUMMARY REPORT

INFORMATION AND RESOURCES SEGMENT

July 1, 1972 through June 30, 1973

The Information and Resources Segment (I&RS) has two major areas of responsibility within the Carcinogenesis contract program. The first of these requires it to provide support to other segments by assuming responsibility for the direction and management of contracts which are essential for the continued functioning of that segment's activities but for which no particular expertise is available within that segment's working group. An example of such an activity lies in the responsibility by this Segment for the contract under which particulate materials are prepared for use as a resource in contracts conducted within the Lung Cancer Segment. Such support is essential since the sorts of knowledge and skills required to provide continuing review and direction to the overall Lung Cancer Program are quite different from those which are needed to direct and manage a program in the area of fine particle technology. It is in just such areas that the chemical expertise available in the I&RS working group can best support the overall program.

The second function of the Segment lies in the anticipation, planning, and provision for services which meet the resource needs of the total Carcinogenesis Program as distinct from the needs of specific segments. Such activities are exemplified by the production of the journal, Carcinogenesis Abstracts, and its distribution to that part of the scientific community having an interest in the problems with which we are concerned. The resources which this Segment provides to the overall program seem to fall into three broad general categories of animals, chemicals, and information. The amount of activity devoted to the information portion of our program is by far the greatest of the three.

Doctors J.A. Cooper and M. Litwack served as Segment Director and Manager, respectively. They are supported by a working group composed of NCI personnel having expertise in specific areas of concern. This staff group includes Drs. D.A. Kaufman, N.P. Page, and T. Cameron in the area of animal resources; Drs. L. Keefer and E.K. Weisburger in the area of chemical resources; and Dr. S. Siegel and Mr. T. Kuch to provide expertise in the area of information sciences. In addition, a Segment Advisory Group has been formed which includes six members of the scientific community external to NCI. Since even such an expanded working group will contain a very limited range of expertise, it will be necessary for us to continue to draw on other members of the scientific community on an ad hoc basis to assist in the evaluation of individual proposals, in the conduct of site visits, and in the long-range planning of program resource needs. Such a program of involvement of scientists from the community as a whole is now under way and, in fact, in this fiscal year nineteen persons have been utilized on such an ad hoc basis, fifteen of them being from outside of the NCI.

In March 1973 a segment reorganization occurred. This has resulted in two specific effects: (1) the formation of a new segment which has been named

the Carcinogen Metabolism and Toxicology Segment, and (2) the bringing together of all bioassay and bioassay support contracts into a single segment known as the Bioassay Operations Segment. In accomplishing this reorganization five contracts were transferred from the Information and Resources Segment to the Bioassay Operations Segment since they were completely dedicated to the support of bioassay activities. In addition, those program activities which were primarily oriented towards studies of nitrosamines and mycotoxins have been removed from our area of direct responsibility and constitute the nucleus of newly formed Carcinogen Metabolism and Toxicology Segment. These transfers have resulted in some reduction of the level of overall segment activities during Fiscal Year 1973. It is projected that the Segment's activities at the end of this year will include some eighteen contracts and a total budget of approximately 1.8 million dollars. Slightly more than half of these funds will be expended in the area of information services; the greater part of the remainder will be devoted to chemistry resources with a somewhat lower level of activity in the animal resources area.

Chemical Resource Activities:

In order to establish a quantitative basis for the comparison of carcinogenic effects observed in various laboratories, it is vital that standard reference materials be available. Such materials must be of high purity and be well characterized. A source of such materials has been developed in collaboration with Starks Associates, Inc. The contractor is acquiring, purifying, and characterizing specified compounds and repackaging them in smaller quantities for storage and distribution to investigators in the cancer research community. Approximately 50 such materials are presently available and more are being added on a continuing basis. A program has also been initiated at the Frederick Cancer Research Center to provide additional synthetic and analytical support to the Carcinogenesis Program. This program interfaces closely with the activity just described and permits the application of very sophisticated analytical techniques where necessary in the characterization process as well as providing a resource where extremely potent carcinogenic substances may be synthesized and purified.

The Illinois Institute of Technology Research Institute provides support in meeting a specific resource need of the Lung Cancer Program. This group provides highly purified and characterized powders and emulsions for use in intratracheal instillation studies. The emulsions are composed of carcinogenic materials (or their non-carcinogenic analogs) in adequeous systems, are of high purity, and have known size distributions. The powders are prepared from carbon and a variety of metal oxides and have defined size distribution, shape, surface area, and porosity. The powders are provided to investigators either alone or after coating with benzo[a]pyrene. An additional contract program will be implemented during this year with the Illinois Institute of Technology Research Institute to investigate the stability of benzo[a]pyrene when adsorbed on the surface of these particles.

The collaborative program with Ash Stevens, Inc., continues to provide interested investigators with purine and pyrimidine nucleotides specifically tailored to function as selective irreversible inhibitors of nuclear RNAase. This aspect of the program is attempting the development of a selective drug probe enabling investigators to control the destruction of messenger RNA in the cell nucleus. In addition, a number of these products have been shown to function as inhibitors of the reverse transcriptase of oncogenic viruses and leukemia cells.

Efforts to develop and apply specific analytical methods to the analysis of volatile nitrosamines in cigarette smoke condensates have been made at the Southwest Research Institute. These activities have now reached a successful conclusion and have been made available to the analytical program of the Tobacco Research Segment. The activity is being phased out during the current support period.

Information Resource Activities:

Activities of the Segment in this area are designed to meet a variety of information needs on the part of the scientist or science administrator working in the general area of carcinogenesis research.

A continuing need exists to make more easily available, on a timely basis, the relevant information being generated in those disciplines which impact on the Carcinogenesis Program Area. This requirement is being met by the journal, Carcinogenesis Abstracts, which is produced for us under contract with the Franklin Research Institute. The magnitude of this activity is increasing continuously as the overall level of activity in cancer related research rises.

Information which may offer leads as to the carcinogenic potential of specific molecules or classes of molecules is an ever present need in our program area. This results in a requirement for scanning of the pertinent literature to collect references to any long-term testing activities which may appear. This activity is being carried out on a continuing basis under a contract with the John I. Thompson Company and results in the production of a series of published volumes entitled Survey of Compounds Which Have Been Tested for Carcinogenic Activity PHS-149. During the past year this series has been brought up-to-date and volumes now in press will result in complete coverage of the literature through 1971.

To make the information in these volumes more readily accessible cumulative indexes have been prepared which permit entry from a variety of starting points. In addition to the usual parameters, these indexes include CAS registry numbers, formal nomenclature, synonymns, molecular formulas, and Wiswesser Line Notation (WLN). The line notation indexes, together with a cumulative permuted index have been generated through collaboration with information scientists at Frederick Cancer Research Center and the Institute for Scientific Information. It is anticipated that these indexes will be converted to a machine readable form during this year so that searches can be conducted more readily on a number of parameters simultaneously. In addition, it should be noted that the addition of WLN

to the files will permit for the first time substructure searching to be accomplished; while the addition of CAS registry numbers permits our files to be linked with a variety of other data bases which are currently available.

In an attempt to maintain a state of current awareness of the substances which might be undergoing long-term testing throughout the world, we are providing partial support to an activity being conducted by the International Agency for Research on Cancer (IARC). This activity provides a mechanism for the establishment of an international registry of compounds undergoing long-term toxicity testing.

The growing concern with our abilities to predict the carcinogenic potential of molecules based on reports of other biologic effects has caused the Segment to furnish partial support for the Environmental Mutagen Information Center (EMIC) located at the Oak Ridge National Laboratory. This program functions in the mutagenesis area in a fashion similar to our literature related activities in the carcinogenesis area. Elements of chemical information are being entered into the EMIC data base which permit direct linkage to the index portions of PHS 149.

Several activities are underway which are designed to develop mechanisms which will assist scientists in making decisions as to which chemicals should receive priority in our testing or investigational programs to best utiize the limited resources available.

In collaboration with the International Agency for Research on Cancer we are assisting in the production of a monograph series which provides a balanced evaluation of the available data on individual chemicals as to their carcinogenic risk to man. The first volume of this series is now available and two additional volumes are in press. In addition, we have underway in collaboration with the Stanford Research Institute a developmental program having as its objective the collection, analysis, and systematization of information on the chemical description, production, and human exposure of chemicals which have significant contact with man. This contractor is now in the process of refining their ability to identify and quantitate specific population exposure levels based on their knowledge of chemical production and distribution.

Animal Resources:

In the past well-characterized specific pathogen free (SPF) have been provided for the bioassay program by means of a transfer of funds to the Division of Cancer Treatment (DCT) contracts (Charles River Laboratories and Battelle Memorial Institute). At times these sources have not been able to meet the demands of the bioassay program. For this reason the animal farm at Frederick Cancer Research Center (FCRC) was immediately activated upon award of the contract to operate the Center. Production colonies of several mouse strains, the Fischer/344 rat, Hartley Strains 2 and 13 guinea pigs, and New Zealand rabbits are now capable of supplying all FCRC needs and are distributing animals to other NCI activities.

The development of Strains 2 and 13 guinea pig breeding colonies at FCRC, based upon a nucleus of breeders supplied by the in-house NIH colony, has been most successful and fortuitous. Soon after the establishment of this resource, the parent colonies at the NIH Reservation were stricken by an epidemic of Salmonellosis. The newly activated FCRC colonies were able, in part, to supply the NIH requirements and maintain essential research at a minimal level. By the end of Fiscal Year 1973 these colonies will be in full operation and producing adequate numbers of pigs for the intra- and extramural programs.

The Segment is also responsible for the development of new animal models for cancer research. The wild European hamster (Cricetus cricetus L.) has been observed to develop bronchogenic epidermoid cancer similar to that seen in man. Attempts by the Medizinische Hochschule to domesticate and breed these animals under laboratory conditions has been quite successful. The colony is now in the F_3 generation with laboratory-raised animals having lost the aggressiveness and poor breeding characteristics of wild-caught animals. Studies with several carcinogens have confirmed their susceptibility to epidermoid lung cancer. The Segment is considering the development of other animal models. Proposals are being evaluated in two species of marmosets and small marsupials. Marmosets appear to develop intestinal cancer similar to man. The small marsupials may be useful for cancer studies as they can be easily treated with carcinogens in a highly undeveloped stage.

A contract with the Massachusetts General Hospital, in collaboration with the Angell Memorial Animal Hospital will provide monographs on tumors of various organs in the dog and cat with a comparison of tumors of man. The first two monographs on the respiratory system and thyroid gland are now in final stages of preparation.

The Segment is responsible for providing information on animals and animal care to the Carcinogenesis Program. Current standards of animal care are not addressed to the long-term holding of animals or many host-environment considerations. In an effort to develop such guidelines the Segment sponsored a workshop on this subject in April 1973. The Institute of Laboratory Animal Resources (NAS/NRC) will publish the proceedings and develop standards for long-term animal care under contract with the Segment.

CONTRACT NARRATIVES

INFORMATION AND RESOURCES SEGMENT

July 1, 1972 through June 30, 1973

AEC-NCI INTERAGENCY AGREEMENT (Oak Ridge National Laboratory) (NIH-NCI-72-203)

Title: Environmental Mutagen Information Center (EMIC)

Contractor's Project Director: J.S. Wassom

Project Officer (NCI): Dr. Sidney Siegel

Objectives: The objective of this project is to create and maintain a data base of chemical mutagenesis information concerned with testing of chemicals in one of the many available mutagenic assay systems and with pertinent data which can be used to further the understanding of mutagenic activity of some chemical agents which can then be processed and disseminated to the scientific community.

Major Findings: EMIC screens the world scientific literature for information on the mutagenicity of chemicals and enters this information into a computerized data base. To make this data easily available bibliographies are compiled, distributed at periodic intervals, and published primarily in the form of articles reporting experimental results, meeting abstracts, review articles, symposium proceedings, brief notes in scientific newsletters or bulletins, letters to the editor, feature articles, book chapters, editorial commentary, and book reviews. Utilizing an abstraction technique called data tabulates, key data from publications are extracted and entered into the computer. EMIC at the beginning of this fiscal year had 8,000 computerized citations in its data base. Of these, approximately 1,200 have been processed through the tabular abstraction program. The remainder are on file as bibliographic entries with accompanying Agent/Organism keywords and Chemical Abstract Registry Numbers.

Significance to Biomedical Research and the Program of the Institute: Some chemicals present in the environment may, along with radiation, effect the genetic stability of living systems. As the potential genetic danger of the chemicals became generally recognized, the need to collect information in assessing the mutagenic potential of environmental compounds became apparent. EMIC, a part of the Environmental Information System (EIS) located at the Oak Ridge National Laboratory, was organized in 1969 to undertake this task of data management. The proximity of the other participating centers, the Forest Information Center, the Ecological Sciences Information Center, the Toxic Materials Information Center, and the Toxicology Information Response Center, makes it possible to utilize each other's data banks.

Proposed Course: As the result of increasing interest in the correlation between mutagenesis and carcinogenesis, it is highly desirable that such a

data base concerned with biologic response to exogenous chemicals be maintained on a continuing basis.

Date Contract Initiated: June 30, 1972

Current Annual Level: $52,000

ASH STEVENS, INC. (NIH-NCI-72-3293)

Title: Synthesis of Purine and Pyrimidine Nucleotides

Contractor's Project Director: Dr. Calvin Stevens

Project Officer (NCI): Dr. Michael Sporn

Objectives: The general aim of this contract is to design and make available a new type of pharmacological agent or agents which will be useful in investigating the manner in which chemical and viral carcinogens affect the metabolism of newly synthesized and messenger RNA in the cell nucleus, as well as for the investigation of a variety of other problems in chemical and viral carcinogenesis. The specific aim of the contract is to synthesize an organic chemical which will irreversibly inactivate the nuclear enzyme which destroys newly synthesized and messenger RNA in the nucleus itself; the chemical agent must be selective and biologically active. An additional aim of this contract which has been added since its inception is that compounds which have been synthesized under this contract be assayed for possible inhibition of the RNA-dependent-DNA polymerase (reverse transcriptase) of oncogenic viruses and leukemic cells.

Major Findings: Chemical synthesis work in the past year has emphasized two major areas: (1) higher molecular-weight nucleotides, i.e., dimers and tetramers and (2) 2'-and 3'-O-alkylribonucleotides. Six tetrathymidylates were prepared as candidate active-site-directed enzyme inhibitors, representing a new first in nucleic acid synthesis. Currently, these are being modified to increase their potency and the synthesis of hexamer analogs has been initiated. The chemical synthesis developed in this program make available larger quantities of pure oligothymidylates for enzymatic assays and biological evaluation than is possible by biochemical preparative techniques.

In calendar year 1971, this project reported the synthesis and testing of 2'-O-methylated uridine mononucleotide derivatives against both the exoribonuclease and the reverse transcriptase. Based on the encouraging assay results, this project made available some nine 2'- and 3'O-alkyl-nucleosides for testing in various biochemical systems: Cm, Um, Im, Ce, Ue, Cm(3'), Um(3'), and Gm and Am as 2',3'-mixtures. Certain of these nucleosides are now being converted into nucleotide enzyme inhibitors. Overall, a total of 36 compounds, including replicates of earlier preparations, were submitted in the past year. Reverse transcriptase assays, carried out by Drs. Schrecker and Gallo (NCI), have been reported.

Significance to Biomedical Research and the Program of the Institute: Within the past few years, there has accumulated an important body of data which show that in many different vertebrate cell types, most of the RNA that is made in the cell nucleus is destroyed in the nucleus itself very shortly after synthesis. Published data indicate that as much as 90% of the newly synthesized RNA is destroyed in the nucleus itself, and that its half-life may be only a few minutes.

This entire field is still virtually untouched with respect to the problem of carcinogenesis. It is well established that chemical carcinogens have immediate and profound effects on the metabolism of RNA in the cell nucleus. The development of a selective drug probe, which would enable the investigator to stop the destruction of messenger RNA in the cell nucleus (without interfering with its synthesis), would open up a wide variety of experimental work in the field of chemical or viral carcinogenesis.

Morover, the synthesis and testing of potential inhibitors of reverse transcriptase, a field in which this contract has already yielded successful results, is an area of high priority and relevance in other aspects of the NCI total program.

Proposed Course: To continue to develop new synthetic nucleotide analogs that will have greater biological potency and selectivity. To continue to test these nucleotide analogs in carcinogenesis test systems, such as their effects on malignant transformation of cells by Rous sarcoma virus, as well as for their effects on the RNA-dependent-DNA polymerase (reverse transcriptase) of oncogenic viruses and transformed cells.

Date Contract Initiated: June 21, 1972

Current Annual Level: $149,197

FRANKLIN RESEARCH INSTITUTE, LABS (NO1-CP-33309)

Title: Preparation of Carcinogenesis Abstracts Vol. XI

Contractor's Project Director: Dr. Bruce H. Kleinstein

Project Officer (NCI): Dr. Sidney Siegel

Objectives: To provide an up-to-date abstract service for carcinogenesis researchers throughout the world in a format designed to provide under one cover scientific work in the various disciplines which relate to cancer etiology.

Major Findings: This contractor has performed well and delivered the required product on time.

Significance to Biomedical Research and the Program of the Institute: The multidiscipline approach used in the study of carcinogenesis needs an information exchange medium which gathers the relevant articles under one

cover and once done allows easy intellectual cross fertilization among the disciplines to produce a more rapid evaluation of information in the study of carcinogenesis.

Proposed Course: To continue the publication for an indefinite period of time and to include all relevant material in each issue instead of a set number of abstracts and/or citations.

Date Contract Initiated: February 1, 1973

Current Annual Level: $99,691

FREDERICK CANCER RESEARCH CENTER (NIH-NCI-72-3294)

Task Order # 9

Contract narrative is reported under the Office of the Associate Scientific Director for Carcinogenesis.

FREDERICK CANCER RESEARCH CENTER (NIH-NCI-72-3294)

Task Order # 12

Contract narrative is reported under the Office of the Associate Scientific Director for Carcinogenesis.

IIT RESEARCH INSTITUTE (NIH-NCI-70-2245)

Title: Production and Characterization of Particulate Materials for Studies in Respiratory Carcinogenesis

Contractor's Project Director: Mr. Reg Davies

Project Officer (NCI): Dr. John Cooper

Objectives: To do research into both the methodology and efficiency of coating particles with selected polynuclear hydrocarbons and to store them in a manner to prevent deterioration in quality. This project's objectives are to specifically prepare and characterize the following materials: (1) powder fractions, differing in size, composition and surface area; (2) powder fractions coated with selected polynuclear hydrocarbon; (3) dispersions of coated particles in selected vehicles; and (4) stable emulsions of selected polynuclear hydrocarbon carcinogens in selected vehicles.

Characterization of the samples includes the following determinations: (a) purity of the sample as determined by emission spectroscopy; (b) the actual particle size distribution of the sample; (c) information on the conformation

of particles in the sample as determined using the scanning electron microscope; and (d) a surface area measurement performed on the sample by inert gas absorption.

Major Findings:

1. Particle Preparation - Gram quantities of carbon, ferric oxide, alumina, nickel oxide, and cobalt oxide have been prepared in four size ranges with 90% of the particles by mass falling within the specified limits of 0.5-1.0 microns, 2-5 microns, 5-10 microns, and 15-30 microns. In addition, all four fractions of ferric oxide were prepared with 90% of the particles by number falling with the specified limits. The cascade sedimentation method was used. Fractions of these materials are in use in respiratory studies.

2. Particle Coating - Particles of carbon, ferric oxide, and alumina, including both the frequency and mass fractionated ferric oxide particles, have been coated with benzo[a]pyrene (BP) and have been supplied to various organizations for animal studies. Particles of hematite have been coated with pyrene for other animal experiments. In addition, particles of nickel oxide and cobalt oxide and surface area modified samples of hematite and alumina have been successfully coated with BP so these materials are now also available for animal experiments. Over 80 coating preparations have been made to date, and on each sample photomicrograph characterization for free BP and the degree of agglomeration has been made. The various organizations to which these materials have been supplied include the National Cancer Institute, Massachusetts Institute of Technology, Oak Ridge National Laboratory, Tampa VA Hospital, and IIT Research Institute's Life Sciences Division.

   Normal coatings of BP on carrier dusts are done on a 1:1 by weight basis. Maximum weight ratios of BP to carrier dust were determined for 1/2-1 micron carbon, 2-5 micron hematite, and 0-5 micron hematite. BP preparations of these three carrier dusts can now be supplied with higher BP ratios.

   The period of time the coated particle has remained usable has increased as a result of a study involving storage temperature. BP needle formation has been greatly reduced with the storage environment maintained at -70° C.

3. Dispersion of Particles in Liquids - Due to the sedimentation instability of BP-coated particles in gelatin-saline vehicles during transport, all coated particles have been supplied as dry powders. In all cases, samples of control dust have been submitted at the same time.

4. Preparation of Emulsions - In some programs, BP was administered as a ball-milled preparation (emulsion) in the presence of gelatin and saline. Various BP loadings were prepared as requested, approximately 50 preparations have been made. All of these preparations have been characterized with respect to the BP content and particle size distribution.

Significance to Biomedical Research and the Program of the Institute: This effort is intended to produce the particulates required for the execution of other contracts in the Carcinogenesis Program. In addition, this effort will provide investigative and production support for such contracts, in the areas of fine particle technology and stable emulsion preparation, characterization, storage and thus can supply a closely controlled species of both coated and uncoated particles.

Proposed Course: It is intended that this contract be continued as a production and service resource for related contract activities in this area. It is anticipated that the number of preparations made for the various Carcinogenesis programs will remain the same in fiscal year 1974.

The workscope for the coming support period will include: (1) The preparation and characterization of the 20 powder fractions will continue. (2) The preparation of coated fractions of particles will continue. Here, it is expected that other carcinogens will be introduced. (3) The preparation and characterization of stable BP emulsions will continue. (4) The surface area modification of the 5-2 micron fractions of all materials and the coating of these modified fractions with BP will continue. (5) Investigation of the coating mechanism and attempts to define the reasons for variation in coating efficiency will continue. (6) BP solubilization rate studies will continue. (7) Characterization of carrier dust particles according to density and hardness will be performed.

Date Contract Initiated: June 10, 1971

Current Annual Level: $98,565.

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER (NIH-NCI-70-2076)

Title: Program on the Evaluation of Carcinogenic Risk of Chemicals to Man

Contractor's Project Directors: Dr. Lorenzo Tomatis
Dr. Claus Agthe

Project Officers (NCI): Dr. John Cooper
Dr. Sidney Siegel

Objectives: To assist in the establishment of a data base containing a balanced evaluation of data on individual carcinogens made by an independent group of international experts on the carcinogenic risk of chemicals to man and the final goal is to identify chemical carcinogens to which man is exposed to allow the detection and quantification of risk.

Major Findings: The International Agency for Research on Cancer (IARC) has collected relevant data for the preparation of draft monographs. These draft monographs are either discussed in small meetings or circulated by mail to experts for comment during the course of their preparation. NCI Staff attended the 1970, 1971, and 1972 IARC Working Groups on the "Evaluation of Carcinogenic Risk of Chemicals to Man." The first monograph on the Evaluation

of Carcinogenic Risk of Chemicals to Man was published in 1972. Volume 2, including monographs on 7 groups of closely related substances, and Volume 3, including monographs on 17 polycyclic aromatic hydrocarbons and heterocyclic compounds, are in press.

Significance to Biomedical Research and the Program of the Institute: (1) The IARC represents a unique resource as a virtue of its easy communication with nations which sometimes could not otherwise freely exchange views. It is most likely that only such an international organization could establish either the registry of chemicals under test or the expert committees desirable for the production of the proposed monographs. (2) This activity will assist NCI in carrying out the responsibilities stated in the National Cancer Act of 1971 to "collect, analyze, and disseminate all data useful in the prevention, diagnosis, and treatment of cancer including the establishment of an international cancer research data bank to collect, catalogue, store, and disseminate, insofar as feasible, the results of cancer research undertaken in any country for the use of any person involved in cancer research in any country."

Proposed Course: In an attempt to meet the specified objectives, data sheets will be prepared on the individual compounds to be considered. The data sheets will contain the essential data needed for the evaluation of carcinogenicity tests in a condensed and uniform format for easy comparison. The data sheets will be utilized by the individual expert (or experts) designated to prepare monographs on the specific chemical(s) in question. These draft monographs will either be discussed in small meetings or circulated by mail for comment during the course of their preparation. When the final version of the draft monograph is completed it will be made available to all members of the committee who are invited to participate in discussion and revision when the document is considered in the annual meeting. The possibility will be explored of generalizing this data base in the future so that other types of input, such as experimental data, could be accommodated. We recognize the desirability of making any such data base as compatible as possible with existing resources.

Date Contract Initiated: June 30, 1972

Current Annual Level: $110,000

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER (NIH-NCI-70-2076)

Title: Etiology of Esophageal Cancer in the Caspian Littoral of Iran

Contractor's Project Director: Dr. J. Kmet

Project Officers (NCI): Dr. U. Saffiotti
Dr. M. Schneiderman

Objectives: The purpose of this study is to attempt to identify the etiologic factors associated with the striking variation to esophageal cancer incidence along the Caspian Littoral of Iran. In this region the incidence changes by

thirtyfold in women and by sixfold in men in areas having physical separations of less than 300 miles. Case control studies have indicated that alcohol and tobacco smoking are unlikely to be of etiological importance in this area. Accordingly it is proposed to develop a population based survey rather than a case history study. A sample of the populations divided according to cancer incidence will be studied in respect to (a) food and drink habits, (b) occupation, (c) personal habits, (d) deficiency and other disease patterns, and (e) a limited number of genetic markers.

It is believed that a study of these characteristics may help us to identify those factors which are relevant to the geographic variation in incidence.

Major Findings: As this is a new contract, no major findings can be reported at this time.

Significance to Biomedical Research and the Program of the Institute: Esophageal cancer is probably the best example available to us of a tumor which would appear to be causally related to environmental factors. If those factors can be identified and removed from the human exposure situation, we would have made a major advance in the prevention of a specific human cancer. In addition to providing an attractive opportunity to apply epidemiologic methodology to a tumor type having a high probability of being induced by an environmental agent, an increasing concern with esophageal cancer has occurred as a result of its high incidence in the black male population of this country and its apparently increasing incidence in this population group.

Proposed Course: It is not likely that any single national or international organization could successfully complete a study in depth of the environmental factors which may be related to esophageal cancer incidence in this part of the world. As a result this study will be conducted through a collaborative effort involving the Institute of Public Health Research, Tehran; the Food and Nutrition Institute, Tehran; the International Agency for Research on Cancer (IARC) Regional Center, Tehran; and the IARC headquarters Unit in Lyon. Financial support for the activity will be derived from the IARC, the Government of Iran, the British Government, and the National Cancer Institute. It is felt that this problem can be attacked effectively only through the mechanism of a collaborative program coordinated by an agency at the international level.

It is anticipated that the studies planned will be completed during the current support period.

Date Contract Initiated: November 14, 1972

Current Annual Level: $90,000 (14 months)

MASSACHUSETTS GENERAL HOSPITAL (NIH-NCI-71-2128)

Title: Atlas on Comparative Morphology and Classification of Spontaneous Neoplasms in Dog, Cat, and Man

Contractor's Project Director: Dr. Alan Schiller

Project Officer (NCI): Dr. Norbert Page

Objectives: To create a series of monographs on spontaneous and induced tumors encountered in the dog and cat, together with information on their histopathology, epidemiology, and etiology, with emphasis on comparative aspects of similar neoplasms of man.

Major Findings: During the second year of this contract there were three major achievements: (1) The autopsy and hospital populations have been obtained and tabulated to allow for correlations of frequency, distribution, and occurrence of specific neoplasms; (2) Manuscripts on respiratory tract and thyroid neoplasms are nearing completion while the study of tumors of the central nervous, bone, hematopoietic, and gastrointestinal systems has been initiated; (3) Fine structure (electron microscopy) of a number of spontaneous dog and cat neoplasms has been studied.

Significance to Biomedical Research and the Program of the Institute: The projected use of these books would be to provide (1) a consolidated morphological and biological description and illustration of spontaneous neoplasms in the dog and cat with an accompanying bibliography and standardized nomenclature, (2) a description of new spontaneous and experimentally produced canine and feline tumors heretofore unreported, and (3) comparative study with human neoplasms for use in selection of animal models for research.

Proposed Course: This is a cooperative project between human pathologists at the Massachusetts General Hospital and veterinary pathologists at the Angell Memorial Animal Hospital. During each year the major emphasis will be on performing the histological examinations and obtaining data from animal case histories. Each monograph will deal with a specific organ system or organ depending upon the quantity of data available.

Date Contract Initiated: May 1, 1971

Current Annual Level: $55,000

MEDIZINISCHE HOCHSCHULE HANNOVER (NIH-NCI-71-2148)

Title: Development of a Lung Tumour Model with Large Wild Hamsters (*Cricetus cricetus L.*)

Contractor's Project Director: Professor Dr. Ulrich Mohr

Project Officer (NCI): Dr. Thomas Cameron

Objectives: This contract was initiated to explore the possible usefulness of the European hamster (*Cricetus cricetus L.*) as an animal model for the induction of respiratory tract tumours morphologically similar to tumours found in man. The specific aspects are to (1) capture the wild hamsters and establish a breeding colony, (2) determine the incidence of spontaneous

tumours especially bronchogenic carcinomas, and (3) test for sensitivity to induction of lung cancer by known carcinogens.

Major Findings: Preliminary findings indicated that this species of hamster had keratinising bronchogenic squamous cell carcinomas if they were obtained from an industrialized area of West Germany. It was not clear whether this species was highly sensitive to cancer of the lung induced by environmental pollutants or had a high natural spontaneous incidence.

Hamsters obtained from a rural area have been adapted to the laboratory and have been bred. An F_3 generation is now being raised for further development of a breeding colony as well as an inbred colony.

Eight known carcinogens are being tested in the wild caught animals. LD50's and tolerated doses have been determined and are being administered in chronic studies. A brief communication was published describing the findings in DEN treated animals.

Significance to Biomedical Research and the Program of the Institute: Regardless of the causative factors of the squamous cell carcinoma, having available laboratory animals with this characteristic would be invaluable to cancer research. Other characteristics which would make this animal superior to the Syrian golden hamster are its larger size and its longer lifespan. At present there is no satisfactory animal model for study of the natural pathogenesis of squamous cell carcinoma.

While this type of tumour can be induced in the Syrian golden hamster via intratracheal instillation of chemical carcinogens, they do not spontaneously develop these tumours. Consequently, it is necessary to have reservations in extrapolating data obtained with the Syrian hamster to man. In addition, the use of an animal that is subject to the spontaneous occurrence of squamous cell carcinoma, might be more suitable to studies of lung cancer using a natural exposure method, i.e., inhalation of aerosols.

The program is of direct and intimate relevance to the lung cancer program. If this species can be bred and maintained as a laboratory animal, it would be a valuable animal for the bioassay programs as well as to study mechanisms and pathogenesis of spontaneous lung cancer.

Proposed Course: It is expected that this project will be completed in June, 1974 (three years). Initially it was necessary to capture and adapt this wild species to laboratory conditions and develop a breeding program. The natural occurrence and incidence of diseases, especially lung tumours has to be determined.

The effort to obtain hamsters from an industrialized area has not been successful but will be continued during the next year. The chronic testing of known carcinogens by the intracheal instillation method will be continued. As the project progresses,studies on inhalation of carcinogens will be conducted as determined by NCI program needs.

Date Contract Initiated: June 22, 1971

Current Annual Level: $50,000

MIAMI, UNIVERSITY OF (NIH-NCI-71-2274)

Title: Search for Possible Plant Causes of Esophageal Cancer

Contractor's Project Director: Julia F. Morton

Project Officer (NCI): Dr. John Cooper

Objectives: This project is designed to identify potential carcinogens which may be responsible for the high incidence of esophageal carcinoma in humans in certain parts of the world and thus to help explain why the distribution of esophageal cancer is irregular and often high in one region or portion of a country while adjacent regions have a low incidence. The activity is designed to identify leads which can be further defined by large-scale epidemiologic studies.

Major Findings: On Curacao, in the Netherlands Antilles, there is an incidence of 20-23 new cases of esophageal cancer annually in a population of 140,000. Previous investigations by this contractor have revealed widespread use of bush teas by the general population including those developing esophageal cancer. Several of these teas have been shown (by members of the National Cancer Institute staff) to induce sarcomas in rats, especially Krameria ixina, Melochia tomentosa and Acacia villosa. An effort was made to discover a factor common to Curacao and to other portions of the world where the incidence of esophageal cancer is high. A search of the Morton Collectanea revealed the use of tannin-containing food and beverages in many of the high-risk areas. In order to test the seeming tannin-cancer correlation, an investigation has been conducted in coastal South Carolina. Eight adjacent southeastern counties have emerged as high-risk areas for esophageal cancer. In two counties, the female rate equals the male; in one county, it is higher. Low income residents of these counties have a remarkably high tannin (and related phenol) intake through local plant remedies, "bush teas", homemade wine, chewing tobacco, and orally-taken snuff. Furthermore, an extra risk for males appears to lie in the swallowing of airborne particles by sawmill and fertilizer-factory workers, cement mixers, and exposure to hot asphalt and creosote.

Significance to Biomedical Research and the Program of the Institute: The aims of this project are in direct line with the basic objectives of the Carcinogenesis Program, e.g., to identify potential carcinogens in man's environment and to supply these materials for phytochemical study and bioassay. Finally, to stimulate steps to eliminate the hazards and lower the incidence of the disease.

Proposed Course: This project will terminate at the end of the current contract period, and that the Contractor will assist in a large-scale

epidemiological survey in South Carolina, based on the preliminary evidence gathered under this contract.

Date Contract Initiated: June 30, 1971

Current Annual Level: $58,174

SOUTHWEST RESEARCH INSTITUTE (NIH-NCI-72-2065)

Title: Development and Application of Analytical Methods of Volatile Nitrosamines in Complex Mixtures

Contractor's Project Director: Dr. Donald E. Johnson

Project Officer (NCI): Dr. John Cooper

Objectives: The objectives of this contract are to apply analytical methods developed during the program to the analysis of N-nitrosamines in the smoke condensate from commercial cigarettes, commercial cigars, and experimental cigarettes from the National Cancer Institute. Further, from the data obtained on the smoke condensate it should be possible to calcualte the N-nitrosamine content of the smoke. A secondary objective therefore is to evaluate the method of smoke condensate collection especially as it relates to N-nitrosamine collection from the smoke.

Major Findings: The N-dimethylnitrosamine in the smoke of many filter cigarettes is less than 7 ng/cigarette whereas the non-filter cigarettes tested were over 30 ng/cigarette. The N-dimethylnitrosmaine content of cigar smoke was found to be as high as 340 ng/gram of tobacco or ten (10) times as high as an equal weight of non-filter cigarettes. Cigar smoke in general is much higher in N-dimethylnitrososamine than cigarette smoke.

Significance to Biomedical Research and the Program of the Institute: By identifying and quantitating the presence of known potentially harmful chemicals it is possible to conduct biological tests at more realistic conditions and to take proper steps to decrease the amounts of the harmful substances.

Proposed Course: Further analytical work on the volatile N-nitrosamines in tobacco smoke is to be terminated pending the determination of the significance of the reported results.

Date Contract Initiated: December 22, 1971

Current Annual Level: $16,285

STANFORD RESEARCH INSTITUTE (NIH-NCI-71-2045)

Title: Information System for the Production, Distribution, and Exposure to Man of Substances in the Environment (Formerly, Retrieval and Ranking of Potential Carcinogenic Hazards)

Contractor's Project Director: Mr. Arthur A. McGee

Project Officer (NCI): Dr. John A. Cooper
(Formerly, Dr. Gio B. Gori)

Objective: The initial objective of this project was to define and demonstrate the feasibility of a systematic methodology for information retrieval on chemicals important to man and ranking of estimated risk for carcinogenic potential. As a result of discussions between the contractor and NCI staff members in January 1973, the objective has been modified and broadened. The revised objective is to collect, analyze, and systematize information on the chemical description, production, distribution, and human exposure of chemicals which come in contact with the human population in significant amounts.

Major Findings: Early in the 1972 contract year, in response to a request from NCI, additional documentation to support the system description and feasibility assessment was prepared. It consisted primarily of detailed descriptions of how, and in what order the data are collected and processed and the estimates made. The overall information flow was also presented in the form of flow charts. Included also were descriptions of refinements in methodology that had been made after the previous description was submitted. This elaboration of the methodology included consideration of how to achieve economy of effort by focusing attention on those areas where the payoffs are likely to be the greatest, within the framework of the overall objective.

For the remainder of the 1972 contract year, the project team dedicated its efforts to the implementation of the system. Exposure estimates were developed for approximately 5,000 chemicals from the following eight exposure categories (which were subdivided into 900 product types representing 18,000 chemical-product combinations):

Intentional food additives	Cosmetics
Pesticide residues in food	Air pollutants
Proprietary drugs	Water Pollutants
Prescription drugs	Soaps and detergents

Available data concerning these items are in computer-readable form and contain the following information for each combination of chemical product and route of exposure:

Identification number for the product

Product name

Quantity of the product available for exposure

Routes of applicable exposure--oral, dermal, respiratory, and parenteral

Exposure factors pertinent to each applicable route

Identification number for each ingredient chemical (CAS registry number or an assigned identification number)

Names of ingredient chemicals

Strength (percent) of each ingredient chemical in the product

Uncertainty factor associated with each quantity estimated

Reference to data source

Concurrent with the production of exposure estimates, a computerized chemical classification scheme was completed that contains 223 nodes or end points. This development has allowed a mode assignment to be made for each of the 5,000 chemicals for which exposure estimates have been made. As additional biological data become available it may eventually be possible to make informed guesses as to a molecule's carcinogenic potential based on such a chemical classification scheme.

In addition to the work on the 5,000 chemicals in the above eight exposure categories, a data bank of approximately 25,000 chemicals has been developed which includes many of the substances to which the human population is most likely to be exposed. These chemicals were drawn from eighteen recognized sources of information on such products as cosmetics, food additives, medicinals, etc. For each of the 25,000 chemicals in this data bank, the following information is stored in computer readable form:

Chemical identifiers (CAS registry number and structural representation, chemical name, and synonymns)

Activity parameter estimates for each of the four routes of exposure

Confidence limits of the estimates (i.e., uncertainty factors)

The node of correspondence on the activity tree

Significance to Biomedical Research and the Program of the Institute: The system being developed through this study will supply pertinent information on those chemicals which are of interest to the Carcinogenesis Program and possibly to other activities within NCI and to other Government programs concerned with the impact of chemicals on man.

Proposed Course: The project will continue to provide data useful for the selection of compounds to be introduced into the Carcinogenesis Bioassay Program. As part of the broadened objective of the project, the information needs of the following activities are to be determined and incorporated into the system where feasible: (1) activities in the overall Carcinogenesis Program

and in other organizational elements within NCI, and (2) activities within other Government programs concerned with health hazards arising from exposure to chemicals.

Date Contract Initiated: October 30, 1970

Current Contract Level: $450,903

STARKS ASSOCIATES, INC. (NIH-NCI-72-3203) (Supplement to Chemotherapy for Animal Production)

Title: Procurement, Purification, Characterization, and Distribution of Standard Reference Compounds for Carcinogenesis Bioassay

Contractor's Project Director: Dr. Fred Starks

Project Officer (NCI): Dr. Elizabeth Weisburger

Objectives: The contractor is to acquire stated quantities of specific compounds in a high state of purity and repackage in smaller quantities for storage and distribution to contractors and others working in cancer research who do not have facilities for preparation or purification of chemical carcinogens required to meet program needs.

Major Findings: Forty-two standard reference compounds have been transmitted to the National Cancer Institute. These standards were subdivided into multiple units ranging in size from 5 mg. to 100 g. Packaging containers and shipping methods were devised to retain the chemical integrity of the compounds. All materials were packaged under argon and shipped in appropriate containers sealed with teflon lined covers.

Forty of the compounds supplied had a minimum purity of 99%. Two of the materials were acceptable standards at a purity of 97-98%. Chemical constitution and purity of each product were defined by a combination of elemental analyses, assay methods, trace element analyses, thin-layer chromatography, gas liquid chromatography, and spectrophotometric characteristics. Information was supplied for each material describing the analyses, synthetic approach, and purification methods.

Approximately 25% of the standards were synthesized. Commercially available compounds were refined to meet the 99% minimum purity standard.

Safety measures to prevent worker exposure to carcinogens were devised. Further refinements both in personnel protection and safety equipment design are under continuous development. Environmental protection has been accomplished by disposal of all carcinogenic wastes through incineration or decontamination.

Significance to Biomedical Research and the Program of the Institute: In order to establish a quantitative basis for comparison of carcinogenic effects observed in various laboratories, it is important that standard

reference materials be used. The availability of such materials will facilitate several phases of research on chemical carcinogenesis. Programs especially effected would be those aimed at identifying carcinogenic hazards for man, biochemical studies on the effects of chemical carcinogens, and prevention or modification of the effects of carcinogens. High purified and well-characterized chemical compounds to be acquired include known carcinogens, non-carcinogenic structural analogs, and selected metabolic products.

Proposed Course: The chemicals provided by the contractor will be used by the Bioassay Segment and other programs wherever needed.

Date Contract Initiated: June 21, 1972

Current Annual Level: $145,000

THOMPSON, JOHN I. COMPANY (NIH-NCI-71-2266)

Title: Literature Search, Retrieval, and Compilation of Data Relating to Chronic Tests in Experimental Animals

Contractor's Project Director: Mr. Anthony Lee

Project Officer (NCI): Dr. Sidney Siegel

Objectives: To compile and publish data from the 1970-1971 scientific literature on the carcinogenic activity of chemicals in experimental animals. The resultant publication will be the 1970-1971 Volume of the PHS Publications Series No. 149, entitled Survey of Compounds Which Have Been Tested for Carcinogenic Activity.

Major Findings: This activity represents a continuation of the work carried out under NIH Contract 69-2086, in which volumes covering the 1961-1967, 1968-1969 scientific literature prepared. The 1968-1969 Supplement has been published while the 1961-1967 and 1970-1971 volumes are in press.

The Contractor has searched the scientific literature, selected appropriate documents, extracted specific data from these documents, and indexed selected items from the extracted data. Indexes are based on the following : (1) literature reference, (2) route of administration, (3) site of application, (4) animal species/strain, (5) tumor site, (6) vehicle in treatment, (7) chemical names of compound administered, (8) CAS registry number of compound administered, (9) molecular formula of compound administered, and (10) Wiswesser Line Notation of the compound administered.

The chemical names including CAS names; CAS registry number, molecular formula, Wiswesser Line Notation, and a bibliography by compound accession will be cumulative with respect to the entire PHS Publication No. 149 Series.

Significance to Biomedical Research and Program of the Institute: The 1970-1971 Volume of PHS Publication No. 149, will bring the series of volumes up-to-date and provide a tool useful in searching the chemical carcinogenesis literature. The cumulative bibliography, chemical name, Wiswesser Line Notation, CAS Registry Number, and molecular formula indexes were enhanced and included utility of the entire series.

Date Contract Initiated: June 9, 1971

Current Annual Level: $79,230

SUMMARY REPORT

LUNG CANCER SEGMENT

July 1, 1972 through June 30, 1973

The Lung Cancer Segment is devoted to studies of the pathogenesis of lung cancer and its prevention. The ultimate goal of the program is the prevention of development of lung cancer in man. A spectrum of experimental techniques is used in many investigations involving long-term animal studies, as well as short-term morphological and biochemical studies, all of which are coordinated to establish a correlation between animal models and human lung cancer and to develop a targeted approach to the inhibition or prevention of this most prevalent form of fatal cancer in man. The need for such a program to lessen the incidence of this widespread, lethal disease scarcely requires further comment. Significant accomplishments of this program during the past year are described below.

At the Oak Ridge National Laboratory, the pathogenesis of lung cancer is being investigated in a number of long-term animal studies that are of direct relevance to the human disease. The potential co-carcinogenicity of several irritant gases, that are common combusion products (such as nitrogen dioxide) or are prevalent in the gas phase of tobacco smoke (such as formaldehyde), is being tested in different animal models. The toxic response of the respiratory tract epithelium to these irritants is also under investigation. Studies are under way to investigate the effects of both high and low vitamin A intake on the induction and growth of lung cancer. It was shown that administration of high doses of vitamin A to rats partially protects the respiratory tract epithelium from the tumorigenic effects of a polynuclear hydrocarbon such as methylcholanthrene. Exfoliative cytology studies during the development of bronchogenic carcinoma in hamsters suggest that pre-invasive carcinoma can be detected cytologically and that accurate diagnosis of early invasive carcinoma is feasible. This finding is of great importance with respect to the problem of early diagnosis of human lung cancer. Studies with a cyclic nitrosamine indicate that tumor type and incidence is influenced by the route of carcinogen administration: squamous cell carcinomas of the lung were found to be induced in high frequency in rats receiving N-nitroso-heptamethyleneimine in the drinking water, but were very rare if the same carcinogen was administered subcutaneously.

In the program at New York University School of Medicine, Department of Environmental Medicine, a major emphasis is on the identification of hazardous materials which may pose a serious threat to man as respiratory carcinogens. Great effort has been spent in the design and establishment of an inhalation chamber facility which can accomodate over 1,000 animals. Systems for the generation of aerosols, vapors, and industrial dusts have been developed for testing the carcinogenicity of many substances by the inhalation route. Among the important substances which have been found to be carcinogenic or co-carcinogenic for the respiratory tract are calcium chromate dusts, sulfur dioxide, dusts formed from polyurethane foam (workers in the building trades industry may be exposed to this type of dust), and bis-chloromethyl ether.

The last compound has been found to be an extremely potent carcinogen by the inhalation route. These findings have been reported to the National Institute of Occupational Safety and Health, and human exposure in industrial plants to this dangerous chemical has been drastically curtailed.

In a study of "Factors Influencing Experimental Respiratory Carcinogenesis by Alpha Radiation and Chemical Carcinogens", Harvard University School of Public Health, the potential synergism between radiation and chemical carcinogens in causing lung cancer is being investigated. Such synergism has been implicated in the high incidence of lung cancer in uranium miners. The animal studies in the present contract will take another three years to complete, but it has already been found that the alpha-emitter, polonium-210, is more carcinogenic to the hamster lung when it is uniformly distributed throughout the lung, particularly to the distal airways. The long-term studies in which the potential synergism between polonium-210 and benzpyrene will be evaluated are currently in progress.

In "Susceptibility States and Modulating Factors in Respiratory Carcinogenesis", IIT Research Institute, genetic, as well as environmental, factors, are being assessed for their influence on development of lung cancer in experimental animals. The carcinogenicity of benzpyrene for the respiratory tract of different strains of inbred hamsters is being measured; it is hoped to identify strains that are highly susceptible or highly resistant to development of lung cancer. This is an important study in terms of human relevance; if biochemical and cellular differences can be identified in these animal experiments, significant leads may be found for eventual assessment of the risk of individual human beings to exposure to respiratory carcinogens. Another important factor which may modify host response to respiratory carcinogens is viral or bacterial infection of the respiratory epithelium; this is being evaluated in studies in which APR/8 influenza virus is administered together with benzpyrene. The ability of 13-cis-retinoic acid, a synthetic analog of vitamin A, to prevent the development of lung cancer in animals that have been previously treated with the carcinogen, benzpyrene, is being assessed in a major long-term study. Since the practical usefulness of natural vitamin A in preventing the development of lung cancer is limited by its intrinsic toxicity, it is hoped to develop and test new synthetic analogs of vitamin A for their ability to prevent lung cancer. The immediate goal is to develop a new, synthetic form of vitamin A that will be more potent and less toxic than natural vitamin A. This contract is also beginning to use a new tool, the scanning electron microscope, to follow changes that occur in the respiratory epithelium during carcinogenesis; this instrument has definite ultimate potential for improvement of diagnostic studies in man.

The results of many of the above animal studies are being utilized in several human studies that have already been started, or will be started shortly, by the Lung Cancer Segment. In the contract, "Morphogenesis of Human Lung Cancer", St. Mary's Hospital, an intensive effort is being made to improve the technique of sputum cytology and to use it effectively for earlier diagnosis of lung cancer in man. A large group of uranium miners is being followed with sputum cytology, in order to achieve a detailed documentation of the stages and time of development of lung cancer and its morphologic precursors in this population of workers who are at such high risk for development of

lung cancer and for which quantitative data are collected on the exposure level to radiation and to smoking. An atlas of human sputum cytology is being compiled that will also be of help in diagnostic centers throughout the country. In a related study at the Oak Ridge National Laboratory, methods were developed to obtain cytology samples from the tracheobronchial tract of laboratory animals during the development of their carcinogenic responses. A good correlation has been found between animals and man, in terms of the cytologic stages that occur during the development of bronchogenic squamous cell cancer. These studies are important because they indicate that findings made in the animal model should be of direct relevance to study of the human disease.

Since a significantly large number of deaths from lung cancer in man are caused by oat cell carcinoma (which is a particularly invasive and metastatic type of tumor), a new contract, "Studies on Oat Cell Cancer of the Lung", Stanford University School of Medicine, to investigate the morphological and biochemical properties of this type of cancer has been started. It is believed that oat cell carcinomas arise from a specific type of cell (K-type cells) in the respiratory tract. Studies in this contract are aimed at identifying the unique morphological and biochemical properties of the K-type cells, as well as the factors or mechanisms involved in the neoplastic transformation of these cells. Normal human bronchial tissues, as well as specimens of oat cell carcinomas, are being grown in organ culture in order to pursue these studies.

In order to effectively pursue many of the above studies on lung tissue from both man and animals, improved cytological methods such as radioautography, are required. In the contract, "Autoradiographic Study of the Cellular Response of the Respiratory Tract during Chemical Carcinogenesis", VA Hospital-Tampa, the technique of light microscopic radioautography of the respiratory tract has been improved, so that the actual amount of radioactivity in cellular and subcellular compartments of respiratory epithelium can be quantitatively measured. The distribution and binding of radioactive carcinogens has been measured. In other experiments, the administration of the respiratory carcinogens, benzpyrene or N-methyl-N-nitrosourea, has been found to cause a marked increase in the rate of cellular proliferation in respiratory epithelium. Moreover, states of vitamin A deficiency have also been found to be associated with a high rate of cellular proliferation in this tissue. This is an important finding, because it suggests that there may be a synergistic effect between vitamin A deficiency and carcinogen action in respiratory epithelium.

Since vitamin A has such a profound effect on cellular differentiation in respiratory epithelium, and since it has been shown to be capable of inhibiting carcinogenesis in this epithelium, the biochemical bases of these phenomena are being further investigated, with the long-term goal of achieving more effective use of vitamin A or vitamin A analogs in cancer prevention. In the contract, "Role of Vitamin A in the Control of Differentiation and Carcinogenesis in the Respiratory Tract", Massachusetts Institute of Technology, the role of vitamin A in controlling the synthesis of specific glycoproteins in respiratory epithelial cells is being studied. Vitamin A has been found to be required for the synthesis of specific glycopeptides in these cells. These glycopeptides may be important determinants of membrane structures within the

cell, and thus determine overall cellular morphology and physiology. The various parameters that affect the ability of vitamin A to block the development of lung cancer in the experimental animal are also being evaluated in this contract.

Since the intrinsic toxicity of natural vitamin A is a limiting factor in its usefulness in cancer prophylaxis, studies are underway to identify new synthetic analogs of vitamin A that will be more potent and less toxic than the parent substance. In the contract, "Organ Culture Assay of Vitamin A Analogs", Southern Research Institute, such potential analogs are being evaluated, by both morphological and biochemical techniques. The morphological effects are being evaluated in organ cultures of chick skin and mouse prostate, while the biochemical studies have employed tissue from mouse liver, hamster trachea, calf trachea, and calf bronchi. It is planned to use human bronchial material in the biochemical assays in the very near future. The biochemical studies performed thus far have already made the important new observation that vitamin A and analogs can prevent the metabolic activation of benzpyrene to a presumed "proximate carcinogen". This is an important observation, because it indicates that vitamin A and analogs can modify the metabolism of the class of hydrocarbon carcinogens which are believed to be important agents in development of lung cancer in man.

In summary, the Lung Cancer Segment is pursuing a coordinated program of studies at the human, animal, cellular, and molecular level, to investigate the factors which are involved in the cause and prevention of lung cancer. It is hoped that within this coordinated framework there will be the most rapid dissemination and utilization of new information obtained at the basic levels, and that we thus may hopefully be able to shorten the time before we achieve the desired goal of prevention of lung cancer in man.

AEC-NCI INTERAGENCY AGREEMENT, OAK RIDGE NATIONAL LABORATORY (NCI-FS-64-13)

Title: NCI-AEC Carcinogenesis Program

Contractor's Project Director: Dr. Francis T. Kenney

Project Officer (NCI): Dr. Allen Heim

Contract narrative is reported under the Office of the Associate Scientific Director for Carcinogenesis.

HARVARD UNIVERSITY SCHOOL OF PUBLIC HEALTH (NIH-NCI-71-2136)

Title: Factors Influencing Experimental Respiratory Carcinogenesis by Alpha Radiation and Chemical Carcinogens

Contractor's Project Director: Dr. John Little

Project Officer (NCI): Dr. Norbert Page

Objectives: To examine some of the factors which influence the carcinogenic response to respiratory carcinogens, and to determine in particular whether a synergistic or potentiating effect exists between alpha radiation and chemical carcinogens in the production of lung cancer. Polonium-210 will be used as a local source of alpha radiation, benzo[a]pyrene (BP) as a local chemical carcinogen and diethylnitrosamine (DEN) as a systemic carcinogen.

Major Findings: In an earlier study by Dr. Little, lung cancer was induced in hamsters by the intratracheal instillation of either polonium-210 or BP adsorbed onto hematite particles. No tumors were found in the untreated or hematite-treated controls. Distribution studies with polonium either in saline or in various concentrations of hematite particles indicate that the uniform distribution of alpha-emitting radionuclides as occurs in saline instillation is more carcinogenic than non-uniform distribution or "hot-spots" as seen with the particulate instillation. The major radiation dose from intratracheally administered polonium-210 is to the epithelium of the distal airways, the region in which the malignant tumors arise.

Significance to Biomedical Research and the Program of the Institute: The proposed studies are addressed to a very critical problem in the evaluation of human lung cancer hazards which may result from the combined effects of radiation and chemical carcinogens. Synergistic relationships between

alpha radiation and chemical carcinogens have been implicated in the high incidence of occupational lung cancer in man (uranium, hematite, and flurospar miners). Previous studies have shown that polonium-210, a component of tobacco smoke, accumulates in the bronchial wall, especially the segmental bifurcations of smokers. This is a region where a high percentage of the bronchial carcinomas are found. The possible role of polonium-210, acting as a cofactor along with other chemicals in tobacco smoke, needs clarification. Results from this project could have a direct bearing on the evaluation of lung cancer hazards in man, and on future developments in the whole Lung Cancer Program.

Proposed Course: It is expected that these studies will require another three years to complete. Specific experiments now underway are to (1) determine the microscopic deposition and localization patterns of intratracheally administered BP and polonium-210 by use of a freeze-dried frozen section technique, (2) identify doses of BP and polonium-210 which given individually will result in a small but measurable tumor incidence, and (3) administer both BP and polonium-210 simultaneously and sequentially to determine synergistic action. Future studies will continue to examine synergistic effects between local carcinogens, and also involve administration of DEN systemically following treatment with either BP or polonium-210 to determine possible synergism of local and systemic carcinogens.

Date Contract Initiated: May 30, 1971

Current Annual Level: $79,614

IIT RESEARCH INSTITUTE (NIH-NCI-69-2148)

Title: Role of Vehicles and Particulates in Respiratory Carcinogenesis Bioassays

Contractor's Project Director: Dr. Mary Henry

Project Officer (NCI): Dr. David Kaufman

Objectives: To determine the effect of intratracheal instillation of particulates of varied chemical and physical properties and of various types of solvents on the induction of epidermoid carcinoma of the lung by chemical carcinogens in the hamster.

Major Findings: Chemically and physically defined particulates have been produced by another contract at this institution (NIH-NCI-70-2245). Long-term carcinogenesis studies were conducted to determine the effect of these particles of different size and composition on the induction of lung tumors in hamsters treated systemically with diethylnitrosamine (DEN). The experimental results indicate that the particle size or type of particle does not have a marked influence on tumor incidence or location of tumors in the respiratory tract. The numbers of hamsters carrying peripheral lung tumors were similar in particle-treated and vehicle groups. Experimental results

available to date indicate that intratracheal instillation of dimethylnitrosamine along or suspended with hematite particles produces marked pathologic effects on the liver and induction of a few malignant tracheal and bronchial tumors. The physical characteristics of the carcinogen-dust combination may have a marked influence on tumor induction. Incomplete results of a study where three suspensions of benzo(a)pyrene (BP) and hematite were prepared by different methods, indicate that the carcinogen must be in close association with the dust particle in order to produce a high tumor incidence. The type of association is also important in determining the time to onset of tumor formation. Preliminary results of a study to determine the influence of mode of anesthesia or anesthetic agent on the carcinogenicity of BP-hematite show that fewer tracheal and bronchial tumors were found in hamsters anesthetized with methoxyflurane than in animals treated with Brevital or ether. A long-term carcinogenesis bioassay was done to investigate the effect of small particles in a large particle preparation coated with BP on the tumor-inducing properties of the latter preparation.

Studies utilizing scanning electron microscopy have been directed toward determining the effects of BP-hematite preparations on the morphology of respiratory epithelial surfaces in the trachea. When particles coated with the carcinogen are present in the respiratory tract, loss of cilia, focal areas of squamous metaplasia and small foci of protuberant cells with abnormal surface structure were observed. The effects of vitamin A deficiency on tracheobronchial epithelial surface morphology and the reversal of these changes upon vitamin A administration are being examined.

Significance to Biomedical Research and the Program of the Institute: This project is one of several designed to develop techniques for studying the most common type of lung cancer found in man. It is based on previous observations that bronchogenic epidermoid carcinomas could be induced in hamsters by the intratracheal administration of benzo(a)pyrene with iron oxide dust. These studies have shown the importance of the physical characteristics of inhaled carcinogens in development of epidermoid carcinomas.

Proposed Course: The long-term carcinogenesis studies now under study with histopathologic examination of the respiratory tract and other selected tissues will be completed. The effects of the various treatments on tumor induction and evidence will be determined.

Date Contract Initiated: June 27, 1969

Current Annual Level: $191,752

IIT RESEARCH INSTITUTE (NIH-NCI-72-3292)

Title: Susceptibility States and Modulating Factors in Respiratory Carcinogenesis

Contractor's Project Director: Dr. Curtis Port

Project Officer (NCI): Dr. David Kaufman

Objectives: The objectives of this contract are to develop a single-dose or contracted multiple-dose carcinogenesis model using a nonmetabolizable carcinogen and to determine the following: (1) do there exist states of increased or reduced susceptibility to the development of respiratory tract tumors in hamsters?, and (2) do conditions exist which, after the administration of a carcinogenic regimen, can modulate (i.e. increase or decrease) the ultimate tumor response in the respiratory tract of hamsters?

Major Findings: Two separate studies have been initiated with nitrosomethylurea (NMU) as the nonmetabolizable carcinogen. The dose response data from the first study indicate that protracting the same total dose results in lowered toxicity, with an LD_{50} of 11.0 mg administered as equal weekly increments for a 4 week period. The second study was established to determine what effect a second dose of carcinogen, at varying time intervals from an initial dose, would have on the carcinogenesis process. Not enough time has elapsed to adequately assess tumor incidence in either study.

A virus-carcinogen study was established to determine if the introduction of a carcinogen onto a hyperplastic epithelium results in a greater tumor incidence than would occur with the carcinogen alone. Previous work at IIT Research Institute had demonstrated that APR/8 hamster-adapted influenza virus administered intranasally and followed, at selected intervals, by an additional insult produced a hyperplastic bronchial and bronchiolar epithelium. Accordingly, APR/8 influenza virus was administered intranasally to hamsters, followed by gelatin-saline as an additional insult to produce epithelial hyperplasia. Then BP-hematite, BP, or hematite was administered intratracheally. An assessment of tumor response will be made.

A study to identify carcinogen-susceptible or resistant hamster inbred strains has been initiated. Two potentially susceptible strains and one potentially resistant strain have been selected and will be compared to currently used outbred hamsters, using a BP-hematite carcinogen regimen. Tumor response will be assessed as the animals expire.

A vitamin-A analog study has been designed to determine the effectiveness of 13-cis-retinoic acid in reducing the number of carcinogen-induced tumors of the respiratory tract. The animals will receive two dose levels of BP, as a BP-hematite preparation, and two dose levels of vitamin A analog. Corresponding control groups have been included.

Significance to Biomedical Research and the Program of the Institute: This contract is one of an integrated program of contracts examining facets of the induction of respiratory tract tumors in animal models. Studies in this contract are intended to identify situations which place experimental animals at reduced or increased risk to the development of respiratory tumors. Identification of situations which increase carcinogenic risk may be exploitable by preventive measures. Situations of reduced risk may be exploitable as prophylaxis. By analogy with these animal model studies exploitable situations in human respiratory cancer may be suggested.

Proposed Course: The NMU experiments, virus-carcinogen study, inbred hamster study, and vitamin A analog study will continue and a histopathologic evaluation of the type and location of the respiratory tumors produced will be made.

Date Contract Initiated: June 26, 1972

Current Annual Level: $240,000

MASSACHUSETTS INSTITUTE OF TECHNOLOGY (NIH-NCI-69-2083)

Title: Role of Vitamin A in the Control of Differentiation and Carcinogenesis in the Respiratory Tract

Contractor's Project Directors: Dr. George Wolf
Dr. Paul Newberne

Project Officer (NCI): Dr. Michael B. Sporn

Objectives: The specific aims of this contract are the following: (1) To investigate the biochemical response, with respect to glycoprotein synthesis and secretion, of tracheal epithelium to vitamin A deficiency, vitamin A excess and to benzpyrene treatment, and their interactions; (2) To study the effect of vitamin A and benzpyrene and their interactions on the glycoproteins secreted by tracheas in organ culture; (3) To investigate the histological response of respiratory epithelium (in terms of tumor incidence, tumor type, etc.) to benzpyrene carcinogenesis with retinol treatment, at different dose levels and times.

Major Findings: (1) Biochemical - Work continued on the purification and characterization of the hamster tracheal glycopeptide, isolated previously and defined by its elution pattern from DEAE-sephadex. The level of this glycopeptide is severely depressed in vitamin A-deficient animals. Sephadex exclusion chromatography showed that the glycopeptide consists of several components, only one of which contains fucose. In vitamin A deficiency, fucose and glucosamine uptake into this component are greatly reduced. This component was further purified and shown to be homogeneous by polyacrylamide gel electrophoresis, and its carbohydrate and sialic acid composition determined. Moreover, fucose levels returned to normal within 1-2 days after feeding vitamin A to deficient hamsters. These studies were further extended this year to benzo(a)pyrene-treated hamsters. It was found that uptake of both glucosamine and fucose were greatly reduced in the same glycopeptide fraction as was previously found in vitamin A-deficiency (where the fraction was again defined by its elution pattern from DEAE-sephadex).

(2) Animal Studies - The effect of vitamin A on BP-induced carcinogenesis of the lung. Male Syrian hamsters were given a series of 12 intratracheal BP-Fe_2O_3 treatments and assigned to 1 of 3 groups receiving either 100, 1600 or 3300 µg of retinyl acetate/week. One-hundred-thirty of the 240 hamsters completing the treatment and fed a chow diet and 168 of the 250 hamsters completing the treatment and fed semisynthetic diet are still being given retinyl acetate.

Significance to Biomedical Research and the Program of the Institute: A complete understanding of the phenomenon whereby vitamin A inhibits respiratory carcinogenesis is one of the most important goals of the entire Lung

Cancer Program. This is of great significance for the problem of lung cancer in man, as well as for the problem of inhibition of carcinogenesis in the experimental animal. In other aspects of the program, plans are being made for pilot studies to see if vitamin A or vitamin A analogs can lessen cancer incidence in human populations at high risk for the development of lung cancer. The optimal outcome of these studies will depend on how fully we can develop good animal models for the intelligent use of vitamin A or vitamin A analogs as anti-carcinogenic agents. These studies in turn will depend on a better understanding of the cellular and biochemical processes controlled by vitamin A. The present contract is immediately concerned with obtaining further information in the above areas.

Proposed Course: To continue the long-term animal studies already begun, so that complete evaluation of the gross and microscopic pathology can be made. To continue with biochemical studies on the role of vitamin A in controlling normal differentiation of respiratory epithelium.

Date Contract Initiated: June 18, 1969

Current Annual Level: $203,947

NEBRASKA, UNIVERSITY OF (EPPLEY INSTITUTE FOR RESEARCH IN CANCER (NIH-NCI-68-959)

Title: A Resource for Carcinogenesis Bioassays and Related Research

Contractor's Project Director: Dr. Philippe Shubik

Project Officer (NCI): Dr. Gio B. Gori

This contract narrative is reported under the Office of the Associate Scientific Director for Carcinogenesis

NEW YORK UNIVERSITY (NIH-NCI-66-962)

Title: Studies in Pulmonary Carcinogenesis

Contractor's Project Directors: Dr. Marvin Kuschner
Dr. Sidney Laskin

Project Officers (NCI): Dr. Carl Smith
Dr. David Kaufman

Objectives: The objectives of this contract have been the investigation of the mechanisms of pulmonary carcinogenesis and the examination of the carcinogenic potential of suspected materials. Its aims are to illuminate the process of malignant change as exemplified in the lung while, at the same time, providing immediate means for the prevention of lung cancer by the identification of hazardous materials. In particular, since it has become more and more evident that the induction of human lung cancer is multi-factorial in nature and dependent upon long-term, low level exposures to combined

agents, one objective of this contract is to evaluate this combined action in a wide variety of animal models using a variety of experimental techniques.

Major Findings: In order to define the progression of changes which result in cancer, an extensive dose-response study using hamsters and intratracheal intubations of methylcholanthrene was performed. Results show an excellent dose-response relationship, a shortening of time needed to develop cancer as dose increases, and cancer incidence approaching 100% at 24 intubations of 1 mg methylcholanthrene.

An extensive series of studies are being performed in this laboratory to study the effects of several components of air pollution. This laboratory was the first to produce lung cancer in rats with combined inhalation exposures to sulfur dioxide and benzpyrene. Studies with combined carcinogen-irritant exposures are proceeding now in an effort to reinforce the original results and define dose-response relationships. Findings to date are beginning to confirm the original results.

The hamster intubation technique has also provided a valuable model for carcinogen-irritant studies. Animals intubated with carcinogen are currently being exposed by inhalation to sulfur dioxide atmospheres. Early results have shown the findings of adenocarcinoma in an animal given 10 intubations of methylcholanthrene and lifetime exposures to sulfur dioxide. Benzpyrene, which has shown negative results by intubation only, in combination with exposures to sulfur dioxide has shown 2 squamous cell carcinomas and 1 adenocarcinoma.

Experiments with tobacco tar exposure by means of intratracheal pellet implants with large numbers of animals have been performed. Test materials in cholesterol carrier included benzpyrene alone, tobacco tar alone, and mixtures of the two. Preliminary results, to date, suggest specific effects of the acid washed tobacco tar.

The chrome industry has long been suspect in the human cancer problem. In previous studies, this laboratory was the first to produce bronchogenic carcinomas in rats by use of the intrabronchial pellet technique. Of a special interest in the pellet study is the production of both squamous cell and adenocarcinoma. The identification of calcium chromate as one of the major agents in the chromate problem has resulted in inhalation and clearance studies with this compound. In a current study at 2 mg/m^3, three cancers have already been seen in rats and one in hamsters. Early clearance studies suggest retention of high levels of chromium in the lung.

Two industrial plastic dusts were studied to determine their possible effects on workers in the industry. Reinforced polyester-fiberglass plant dust was studied utilizing the intubation technique and short-term inhalation exposures. Centrilobular emphysema and other suggestive changes of interest were noted. With polyurethane foam, animals were given 30 inhalation exposures to freshly generated foam dust at two levels. Centrilobular emphysema and squamous cell carcinoma were seen in rats at both levels. Ultrastructural studies of hamster lungs after intratracheal instillation of polyurethane foam dust suspensions were made by electron microscopy. Findings included particle retention, and marked changes in capillaries, alveolar epithelial cells and macrophages.

Inhalation studies were completed this year with two chlorinated ethers used widely as industrial reaction intermediates. In lifetime exposures to chloromethyl methyl ether at 1 ppm, carcinomas were seen in three rats and one hamster. With bis(chloromethyl)ether, a high incidence of tumors of the olfactory epithelium and squamous cell carcinomas of the lung have been seen in rats. In a series of limited exposures at 0.1 ppm of bis(chloromethyl)-ether, 40 cancers have been seen in a group of 200 animals examined. Seventeen of these were esthesioneuroepitheliomas and 13 were squamous cell carcinomas of bronchogenic origin. Evaluation of the data demonstrates a dose-response relationship with a 50% cancer incidence at 88 exposures. The extremely low levels used and the magnitude of the carcinoma response with bis(chloromethyl)ether suggests a very serious new industrial hazard. These findings have attracted the attention of the National Institute of Occupational Safety and Health who have recently reported the occurrence of human cases. As a result, the bis compound is currently included in the TLV carcinogen list and will be included in the NIOSH Criteria package for carcinogens.

Significance to Biomedical Research and the Program of the Institute: This contract represents a component of a broad effort to develop bioassay techniques for studies on lung cancer. It is also important for its study of potential hazards of materials which are in use industrially and may be found in the environment.

Proposed Course: To extend previous studies and develop inhalation studies with other types of suspected materials.

Date Contract Initiated: June 29, 1966

Current Annual Level: $362,500

OHIO STATE RESEARCH FOUNDATION (NIH-NCI-69-2144)

Title: Study of the Role of Vehicles and Particulates in Respiratory Carcinogenesis Bioassay

Contractor's Project Director: Dr. Robert Farrell

Project Officer (NCI): Dr. David G. Kaufman

Objectives: To determine effect of intratracheal instillation of fine particulates of varied chemical and physical properties and of various types of solvents on the induction of carcinoma of the lung by chemical carcinogens.

Major Findings: A long-term carcinogenesis experiment to examine the effects of Tween 80 given together with benzo(a)pyrene (BP) via intratracheal instillation in a gelatin-saline vehicle, has been completed. The hamsters treated with Tween 80 in addition to BP developed fewer tumors than did the animals treated with BP alone. For another group of experiments, chemically and physically defined fine particles, both coated with BP, and uncoated, have been produced by another contract (IIT Research Institute, NIH-NCI-70-2245). Long-term carcinogenesis studies to determine and compare the carcinogenicity

of these preparations are nearing completion. Preliminary data show that animals instilled intratracheally with carbon particles coated with BP developed more pulmonary tumors than did animals instilled with ferric oxide particles coated with BP or with aluminum oxide particles coated with BP. Ferric oxide + BP induced more tumors than did aluminum oxide + BP. Also nearing completion are long-term carcinogenesis studies initiated to determine the effect of uncoated particles of different size and composition, administered via intratracheal instillation, on the induction of lung tumors by diethylnitrosamine (DEN).

Significance to Biomedical Research and the Program of the Institute: This project is one of several designed to develop techniques for studying the most common type of lung cancer found in man. It is based on previous observations that epidermoid carcinomas of the lung could be induced in hamsters by the intratracheal administration of iron oxide dusts along with the intratracheal administration of benzo(a)pyrene. Further elaboration of these observations is needed to determine the role of atmospheric dusts or cigarette smoke particulates on the development of bronchogenic carcinoma in man.

Proposed Course: Studies will be completed on the essential characteristics of particles coated with BP, and uncoated, as they affect the induction of epidermoid carcinoma of the lung. Most of the work will be completed on the studies on the enhancement by chemically and physically defined particles, of respiratory carcinogenesis by DEN.

Date Contract Initiated: June 27, 1969

Current Annual Level: $55,209

ST. MARY'S HOSPITAL (NIH-NCI-72-3233)

Title: Morphogenesis of Lung Cancer

Contractor's Project Director: Dr. Geno Saccomanno

Project Officers (NCI): Dr. Norbert Page
Dr. John Berg

Objectives: The objective of this contract is to learn more about the morphogenesis of human lung cancer by sputum cytologic analysis.

Major Findings: 1400 sputum samples from uranium miners have been collected and slides prepared, read, filed and reported. Through this process, five cases of marked atypical squamous cell mataplasia have been identified which are on the borderline of becoming carcinoma in situ. In addition, ten new cases of carcinoma in situ have been identified for follow-up. Collaborative hamster cytology studies with Drs. Nettesheim and Schreiber at Oak Ridge National Laboratory continue. Carcino-embryonic antigen testing of uranium miners was initiated under this contract and in cooperation with Hoffman-LaRoche, Inc.

Significance to Biomedical Research and the Program of the Institute: Identification of early stages in the morphogenesis of human lung cancer and the determination of the characteristics of this high risk population are of vital importance. Experimental study applicable to these goals in the hamster model is thus relevent.

Proposed Course: This project will continue to collect, process and analyze sputa from uranium miner and control populations, and to continue the collaborative studies with ORNL.

Date Contract Initiated: May 1, 1972

Current Annual Level: $137,000

SOUTHERN RESEARCH INSTITUTE (NIH-NCI-72-2064)

Title: Organ Culture Assay of Vitamin A Analogs

Contractor's Project Directors: Dr. Lee Wilkoff
Dr. Donald Hill

Project Officer (NCI): Dr. Michael B. Sporn

Objectives: The objectives of this contract are: (1) to develop adequate bioassays to evaluate the biological activity of new vitamin A analogs, as measured by control of epithelial differentiation; (2) to perform bioassays on selected analogs; and (3) to develop new quantitative assays for vitamin A activity in organ culture or tissue culture.

Major Findings: Organ culture (in a chemically defined medium) of metatarsal skin from 13-day-old chick embryos is being developed to monitor for specific biological properties of vitamin A: the inhibition of keratinization and the production of mucus. All trans-retinol inhibits keratinization with production of histologically detectable amounts of mucus. However, optimum culture conditions have not yet been achieved, since only 50-60% of cultured explants remain viable and differentiate. Organ culture of mouse prostate (2-to-3 month old C3H or BDF_1 mice) in a chemically defined medium is also under development to test analogs for the vitamin A property of maintaining glandular epithelium and mucus production. Preliminary experiments indicate that explants remain viable for up to four days in culture.

A gas-liquid chromatographic assay for mucus production, based on the sugar content of explants, has been developed. Normal chick embryo skin has no detectable mannose or fucose, but mouse prostate contains both.

Preliminary cytotoxicity data using a KB cell culture system indicate that vitamin A aldehydes (i.e., all-trans-retinal, 9-cis-retinal and 13-cis-retinal) are more toxic than other vitamin A compounds and analogs.

Release of acid phosphatose and deoxyribonuclease from mouse liver lysosomes by vitamin A compounds has been measured. Most effective are retinyl acetate,

retinol, and 13-cis-retinoate. Least active are methyl and ethyl retinoate.

Vitamin A compounds inhibit the cytochrome P450-linked oxidase of mouse liver microsomes. With either cyclophosphamide, nicotine, benzo(a)pyrene, 7,12-dimethyl-benzanthracene, or 3-methylcholanthrene as substrates, the degree of inhibition correlates with the labilization of lysosomes.

Significance to Biomedical Research and the Program of the Institute: Vitamin A is definitely known to be required for the normal differentiation and growth of the epithelium in the following organs, all of which are major sites of primary cancer in man: bronchi and trachea, colon, stomach, uterus, kidney and bladder, testis, and skin. The role of vitamin A in controlling normal differentiation and growth in tracheobronchial and intestinal epithelium is currently under intensive investigation in the Lung Cancer Program of the NCI.

In addition to controlling the differentiation of many types of normal epithelium, vitamin A is also able to alter the process of chemical carcinogenesis in several different epithelia, and to block the effects of several different carcinogens. Thus, it has been found that vitamin A can block the carcinogenic effects of polycyclic hydrocarbons on tracheo-bronchial, gastric, and uterine (cervical) epithelium. In some of these studies it has been shown that the effect of vitamin A may not be due to alterations of the immediate metabolism of the carcinogen, since vitamin A is able to inhibit carcinogenesis even when administered several weeks after the carcinogen. Studies are currently in progress in the Lung Cancer Program of the NCI which are attempting to elucidate the role of vitamin A in blocking epithelial carcinogenesis. Presumably, this effect is directly related to the biochemical and cellular role of vitamin A in determining normal differentiation in these tissues, but the proof of this is not yet at hand.

The ultimate implications of these studies on the inhibition of epithelial carcinogenesis are very important. If vitamin A is indeed functioning as a differentiation hormone, then it should be possible, with the skill and cooperation of a group of organic chemists, to design and synthesize new analogs of vitamin A which will have even greater potency than vitamin A itself on differentiation of epithelium. The precedents in steroid chemistry for this approach to vitamin A are very impressive; presently there are many synthetic steroid analogs which are much more potent (and often more specific and less toxic in action) than the naturally occurring estrogens, progestational agents, androgens, or adrenocortical steroids. If this type of approach could be applied to the hormonal action of vitamin A, then the potential for inhibiting carcinogenesis in many types of epithelia would be greatly increased.

Proposed Course: Continue the development of bioassay methods using organ culture systems to evaluate the biological activity of vitamin A analogs. Devise new methods, using morphological, histochemical, and biochemical end points for quantitative bioassay of vitamin A and vitamin A analogs. Determine the toxicities of vitamin A analogs to both KB (epithelial-like) and fibroblastic cell lines.

Measure the inhibition by vitamin A compounds and analogs of microsomal oxidases of liver and epithelial tissues which are responsible for metabolism of polycyclic hydrocarbons. Investigate the possibility that vitamin A must be converted to a biologically active form before it influences differentiation of epithelial tissue.

Date Contract Initiated: December 10, 1972

Current Annual Level: $185,000

STANFORD UNIVERSITY SCHOOL OF MEDICINE (NIH-NCI-73-3207)

Title: Studies on Oat Cell Cancer of the Lung

Contractor's Project Director: Dr. Klaus G. Bensch

Project Officer (NCI): Dr. Michael B. Sporn

Objectives: Development of methods for the identification of cells in the human lung that are the counterpart of the intestinal argentaffine cells (K-type cells), determination of the distribution of these cells in human bronchopulmonary tissues and their adaptation to growth in vitro, and study of factors in their neoplastic transformation.

Major Findings: During the past six months, the contractor has begun the organ culture of human bronchi in order to study the survival and cell kinetics of K-type cells. These studies are still in their preliminary stages during this first contract year.

Significance to Biomedical Research and the Program of the Institute: Oat cell carcinomas of the lung arise from a specific type of cell of the human respiratory tract. Although these cells appear to be present as a relatively small proportion of the population of the bronchial and bronchiolar lining, the incidence of this type of tumor is very high in males. Work in progress should shed light on the distribution of these cells in human adult bronchi, and studies on in vitro cultures of the K-type cells and of carcinoid and oat cell tumors will attempt identification of factors or of mechanisms active in the neoplastic transformation of these cells.

Proposed Course: After development of a method of rapidly identifying the K-type cells, their distribution in human bronchi will be determined. The survival and turnover of these cells in organ and tissue cultures will be studied, and the uptake of compounds which are supposed to play a role in the genesis of oat cell tumors will be attempted. Particular attention will be paid to the effect of these compounds on the replication and growth pattern of these cells.

Date Contract Initiated: September 4, 1972

Current Annual Level: $47,115

VETERANS ADMINISTRATION HOSPITAL (TAMPA, FLORIDA) (FS-73-206)

Title: Autoradiographic Study of the Cellular Response of the Respiratory Tract in Chemical Carcinogenesis

Contractor's Project Director: Dr. Hollis Boren

Project Officer (NCI): Dr. Curtis C. Harris

Objectives: The objective of this project is to determine the proliferative cellular response of various segments of the respiratory tract following exposure to respiratory carcinogens and particulates, using primarily the hamster intratracheal instillation model which is studied in other respects in several other contracts. These measurements are correlated with the histopathological response to carcinogens and particulates. In addition, radioactive carcinogens and related compounds will be localized in the respiratory epithelium by quantitative light microscopic autoradiography.

Major Findings: Significant refinements in light microscopic autoradiography have made it a quantitative tool. Internal standards of known specific activity allow one to determine the efficiency of each autoradiogram. Experiments have determined saturation, geometric error and latent image fading. The use of 1μ sections of plastic embedded tissue allows the exact identification of cell types and cell compartment.

The cell population in the tracheal epithelium is dependent upon the vitamin A state of the animal. With low levels of vitamin A the number of basal cells increases and the number of ciliated cells diminishes. As the vitamin A intake is increased, the number of basal cells diminishes and the number of ciliated cells increases. Both retinyl acetate and retinoic acid were active in this effect on tracheal cell populations. Vitamin A deficiency was associated with a marked increase of labeling of mucous cells and basal cells with ^{3}H-thymidine. The rapidity of the changes in cell populations was observed by administering vitamin A compounds to vitamin A deficient hamsters. Significant changes occurred in 2-7 days.

Quantitative autoradiography has been used to determine intracellular binding of ^{3}H-benzo(a)pyrene. *In vivo* exposure to benzo(a)pyrene followed by *in vitro* exposure to ^{3}H-benzo(a)pyrene demonstrated increased binding of ^{3}H-benzo(a)-pyrene in basal, mucous, and ciliated cells at 48 hours and diminished binding at 7, 10, and 14 days after *in vivo* exposure. A second series of collaborative studies examined the effects of benzo(a)pyrene pretreatment, sham treatment, temperature, and 7,8 benzoflavone on ^{3}H-benzo(a)pyrene binding. Pretreatment with benzo(a)pyrene gave increased autoradiographic counts. The counts were less after either the addition of 7,8 benzoflavone to the incubation mixture or incubation at 4°C. There is no difficulty in transplanting normal hamster tracheas into hamster cheek pouch. Tracheas containing benzo(a)pyrene and ferric oxide showed multiple changes when transplanted. Rat tracheas were transplanted into male weanling hamsters. These xenografts can be maintained for at least 4 weeks in hamsters, which were immunosuppressed by rabbit anti-hamster lymphocyte serum and cortisone acetate.

Significance to Biomedical Research and the Program of the Institute: This contract is an integral part of an interlocking group of contracts designed to define the role of carcinogens and the physico-chemical factors (e.g., particulates) required for respiratory carcinogenesis. The other studies, including long-term tests, have been designed so that they are closely related to the present one with maximum interaction but no overlap of activity, in an effort to define the key role of vehicles and particulates in the process of respiratory carcinogenesis.

Proposed Course: Quantitative methods of studying turnover of the various cell types in the respiratory epithelium now have the added feasibility of using colchicine blockade of mitosis in the hamster, double labeling using ^{3}H-thymidine plus ^{14}C-thymidine, and continuous labeling with ^{3}H-thymidine. Tracheal cell kinetics can be accurately determined following carcinogen exposure or exposure to possible inhibiting or promoting agents.

Studies of benzo(a)pyrene binding will be extended beyond those on the effects of the vitamin A state of the animal. The effect of genetic differences in three different inbred hamsters (LSH, ITG, Warren) on binding of benzo(a)pyrene will be determined.

The major in vivo study will be that of the histogenesis of lung cancer induced after ten weekly intratracheal instillations of benzo(a)pyrene and ferric oxide. All hamsters will be fed the modified vitamin A free gelatin diet. The effect of three levels of vitamin A, (15 µgm/week, 150 µgm/week, 1500 µg/week by intragastric feeding) will be observed in three groups of 150 hamsters each over a period of 90 weeks. The serial-sacrifice study will attempt to identify preneoplastic lesions by high-resolution histology and by the incorporation of ^{3}H-thymidine by autoradiography.

Date Contract Initiated: June 2, 1972 (Formerly: Medical College of Wisconsin, NIH-NCI-69-2082).

Current Annual Level: $85,588

SUMMARY REPORT

TOBACCO RESEARCH SEGMENT

July 1, 1972 through June 30, 1973

The program of studies on tobacco and other smoking products is continuing with the expert advice of the Tobacco Working Group, under the chairmanship of the Associate Scientific Director for Program, DCCP. The task of this group is to advise NCI on the program aimed at the development of a less hazardous cigarette. The activity within the Tobacco Research Segment is articulated in the following phases: selection, preparation, and characterization of different types of tobacco and tobacco smoke condensates for subsequent bioassay and analysis; bioassay of tobacco smoke and its fractions in a battery of animal systems; chemical analysis of the tobacco smoke and its fractions; the evaluation of the data obtained in relation to the human situation.

The bioassay studies are designed to utilize different types of tobacco smoke and condensates in bioassay with existing animal systems, with particular emphasis on topical carcinogenicity by mouse skin application of condensates, and for a cytotoxic and ciliary inhibitory effect on in vitro and in vivo systems.

Mouse skin painting bioassays (Hazleton Laboratories) of 21 different types of experimental cigarette condensates have been completed and preliminary correlation of data from these studies indicates that cigarettes made from reconstituted tobacco sheet show major promise for being less tumorigenic than control cigarettes representing products on the American market today. Also, there is a strong indication that paper porosity can be used effectively for altering the tumorigenic effect of the cigarette.

A second series of bioassays is underway utilizing 25 different types of experimental cigarettes. These studies are in the 52nd week of an 80 week mouse skin painting schedule. Emphasis has been given to low nicotine and high and low nitrogen fertilization as well as expanded types of tobacco in these studies. The protocol used in the first series of cigarettes for mouse skin painting will be followed exactly so that correlation of results can be made.

The first series of feeding tests and intratracheal instillation tests (Hazleton Laboratories) are nearing completion and results from these studies will be correlated with those obtained by other tests. The smoke condensate used in these tests were selected from the first series of experimental cigarette "tars" used in mouse skin painting experiments.

Improved inhalation devices are being developed and tested with collaboration of NIH staff and the AEC-Oak Ridge National Laboratories. Studies to date indicate that definite improvement can be made in the existing apparatus for smoke inhalation studies on small animals. Along with the inhalation chamber

development, a concerted effort is being applied toward development of a cannulation device for bypass of the nose and pharynx in the small experimental animal.

Inhalation studies on high and low nicotine cigarettes have been initiated in beagle dogs. (Veterans Administration, supported in collaboration with the National Heart and Lung Institute). This project will determine the effect of high and low nicotine levels on the lung as well as the cardiovascular system.

Projects have been planned and protocols written for studies on anti-smoking drugs. The first of these studies will be initiated within this fiscal year with screening of specific chemical compounds which show promise as being effective. Based on screening results, specific compounds will be selected for study involving the human.

Support has been given to the National Clearinghouse for Smoking and Diseases for pilot studies in smoke cessation clinics. Preliminary results from these studies should be available within three months for review and evaluation.

Studies are continuing on ciliotoxicity in vivo and in vitro with condensate from the latest series of experimental cigarettes (Arthur D. Little, Inc.). The effect of whole tobacco smoke on the trachea of the chicken is continuing.

The major accomplishments during fiscal year 1973 in the Tobacco Research Segment are summarized as follows: Tobacco smoke condensates from 21 different types of experimental cigarettes were collected in mechanical smoking machines and utilized in mouse skin painting bioassay experiments. The initial correlations from these studies with chemical analysis of the tobacco condensate indicate that reconstituted tobacco sheet show major promise for use in a cigarette which could be less tumorigenic. The manipulation of paper porosity in conjunction with reconstituted tobacco sheet offers a second area of major promise for reduction of tumorgenicity.

CONTRACT NARRATIVES

TOBACCO RESEARCH SEGMENT

July 1, 1972 through June 30, 1973

AEC-NCI INTERAGENCY AGREEMENT (OAK RIDGE NATIONAL LABORATORY) (NCI-FS-40-117-67)

Title: Collection, Separation, and Elucidation of the Components of Cigarette Smoke. Part I - Chemical Characterization of Cigarette Smoke. Part II - Inhalation Bioassay Methodology Project.

Contractor's Project Directors: Part I - Dr. Michael R. Guerin
Dr. Wilbur D. Shults
Part II - Dr. Paul Nettesheim
Dr. Michael R. Guerin

Project Officer (NCI): Dr. Gio B. Gori

Objectives: Part I - To provide maximally characterized chemical data defining the smoke composition for experimental cigarettes, to develop methodologies allowing a more complete characterization of smokes and to provide chemical and hardware support as needed by the Institute tobacco projects.

Part II - To develop new methods of assessing the relative carcinogenicity of tobacco smoke.

Major Findings: Part I - Chromatographic component profiling has been demonstrated effective for vastly extending the number of smoke components which can be routinely surveyed for possible correlation with bioassay results. Element selective detector gas chromatography has been found effective for conveniently and accurately elucidating the sulfur and nitrogen containing components of smokes. Generally held views as to the sulfur component of smokes appear in error. New methods for determining hydrogen sulfide, carbonyl sulfide, carbon disulfide and sulfur dioxide have been developed while considerable promise is indicated for new methods for NO, HCN, amine, and nitrogen heterocyclics. Profiling techniques are now in hand for TPM, gas phase carbonyls, sulfur and nitrogen fractions of smokes. TPM profiling has resulted in a convenient assay for palmitic, oleic-linoleic-linolenic, and stearic acids in smokes.

Special studies have included surveys of experimental filter cigarettes, a high and low nicotine pair, and ammonium sulfamate treated cigarettes. Analytical procedures including those for hydrogen cyanide and titrimetric acids have been modified for specific applications in short term studies. Data generated by collaborators and/or other Institute contractors have been treated and/or tabulated for final data summaries. Chemical and hardware capabilities have been applied in a collaborative effort to better define dog inhalation methodologies.

The NCI protocol chemical assays of 29 smoke parameters on each of 23 experimental cigarettes have been completed. The first analysis of the 25 condensates from the second primary experimental series is nearing completion. First and second generation computer data handling and formating methodologies have been developed and applied in preparing data reports. New procedures have been developed and applied to the first series cigarettes to provide pilot data on isoprene, free fatty acids, metals (6), and whole smoke hydrogen cyanide for preliminary correlation with bioassay results. Interlaboratory collaborative studies have identified changes in chemical protocols for sample handling and analytical measurements which promise to significantly improve the reliability of correlations between chemical and bioassay results. Substantially improved methods have been developed and are now being applied for condensate sampling and determinations of carbon monoxide, carbon dioxide, polynuclear aromatic hydrocarbons, hydrogen cyanide, and percent dry condensate. Final data have been submitted for the 23 cigarette first primary experimental series, a 12 cigarette series of experimental filter products, a high/low nicotine pair, an ammonium sulfamate treated series (3 types), and marijuana cigarettes.

Part II - Nose bypass devices were developed to increase smoke deposition in tobacco smoke exposed hamsters. The device most thoroughly tested to date is a laryngeal cannula. Studies with radioactive smoke showed a two-threefold increase in smoke particle deposition over that seen in nose breathing hamsters. Respiratory physiology studies showed that this effect was in large part due to altered tidal volume and respiratory frequency. A permanent tracheotomy is being developed as an alternative to the laryngeal cannula.

Chronic smoke exposure schemes for nose breathing hamsters were designed and tested with the intermittent ORNL smoking machine. As many as twelve cigarettes per day are tolerated by the animals if enough time is allowed between cigarettes for detoxification. Animals exposed for six-eight months using one of these smoke exposure schemes showed significant changes of the laryngeal and bronchial epithelium. Similar tests are now being conducted with the Hamburg II smoking generator.

Six different hamster strains were tested for their relative susceptibility to tobacco smoke toxicity; one of the strains showed considerably greater resistance than the other five and will be tested further for susceptibility to the carcinogenic effects of smoke. Studies are currently underway to develop smoke exposure schemes for rats which were shown to be highly susceptible to the carcinogenic effects of polycyclic hydrocarbons and smoke condensate.

A new animal containment tube which maintains restraint while providing greater comfort was developed. The ORNL exposure device was modified to accept the new containment tubes and to allow exposing twenty rather than ten animals per puff mechanism. Improved dosimetry methods and techniques for estimating the chemical effect of nasal filtration have been developed. Studies of the chemical and physical characteristics of the smoke aerosol under actual bioassay conditions are in progress.

Significance to Biomedical Research and the Program of the Institute: The National Cancer Institute has assumed much of the responsibility for defining the carcinogenic potential of cigarette smoking and steps which might be taken to reduce that potential. This facility provides the chemical characterization of cigarette smokes undergoing extensive biological study by other Institute contractors. The data are critical to the proper interpretation of the biological results and necessary for the design and control of future experiments. Steps taken to more completely characterize smokes and to carefully define routine analytical procedures improve the reliability, uniformity, and extent of experimental progress toward the definition of characteristics of a less hazardous cigarette.

Proposed Course: Part I - To continue as previously stated. To adopt protocol changes suggested by the results of the collaborative studies and expedite a final evaluation of component profiling as a means of extending the number of components measured.

Part II - To define the characteristics of a viable inhalation bioassay for tobacco smokes and initiate chronic inhalation experiments to evaluate the methodology.

Date Contract Initiated: Part I - November, 1969
Part II - July, 1971

Current Annual Level: Part I - $340,000
Part II - $500,000

AMERICAN HEALTH FOUNDATION (NIH-NCI-70-2087)

Title: Evaluation of Carcinogenic Agents in Cigarette Smoke;
Biological and Chemical Assays and Epidemiological Studies

Contractor's Project Directors: Dr. Ernest L. Wynder
Dr. Dietrich Hoffmann

Project Officer (NCI): Dr. Gio B. Gori

Objectives: Investigation of the relationships of smoking and health. Laboratory investigations focus on the definition of hazards in smoke and on the development of a less hazardous cigarette. Epidemiological studies are aimed at identifying means of disease prevention by the definition of less hazardous smoking products and habits and by identification of susceptible subjects and unfavorable environmental conditions. A supplemental program is in progress to establish basic information on the carcinogenicity of marijuana by combined chemical and biological techniques.

Major Findings: Up to November 1972 a total of 8,784 hospitalized individuals have been interviewed in New York, Los Angeles and Houston. The epidemiological studies have now been expanded into hospitals in Miami, Birmingham, and New Orleans.

The trend for a reduced lung cancer risk among long-term filter smoking males continues to be substantiated by this study. The same trend is now suggested for females, although the data is still inconclusive on this point. The investigation is also suggesting a reduced risk of vocal cord, mouth and bladder cancers among male long-term filter smokers but larger samples are necessary to permit conclusive statistical evaluation.

Chemical studies continue to identify and isolate tumor initiators, promoters, and accelerators in cigarette smoke. These studies aim at the reduction of carcinogenicity and tumor-promoting activity from the particulate matter as well as whole smoke.

The relationship of tobacco terpenes and tumorigenicity of the tar is being studied as well as another natural plant constituent, N-nitrosamine. Quantitative data is being collected on maleic hydrazide, a chemical in widespread use as a tobacco sucker growth inhibitor in the United States. The residual and transfer rate of this chemical in tobacco and tobacco smoke is being measured with a specially-developed analytical procedure.

Biochemical and biological studies are testing the effect of nicotine in tumorigenic response, the influence of tar application frequency on tumor yield, and the decreased carcinogenicity of reconstituted tobacco leaf and tobacco stems.

Detailed chemical analyses have determined the physical parameters of marijuana cigarettes. Additional data is necessary for final evaluation of our bioassay data with marijuana smoke condensate. After 16 months' biological testing on laboratory animals, a weak but significant activity as a complete carcinogen and a weak activity as a tumor promoter has been exhibited for marijuana.

Significance to Biomedical Research and the Program of the Institute: The NCI has major responsibility in the definition of smoking hazards. Scientific expertise in this field is of vital importance in the current programs of the Lung Cancer Task Force at the NCI. Resources capable and willing to perform biochemical and epidemiological surveys in this field are extemely limited. Therefore, the outstanding position of the American Health Foundation fulfills an essential need of the NCI. Additional opportunities for expansion of scientific efforts by this contractor have been identified, should the funding situation improve.

Proposed Course: To continue as outlined above.

Date Contract Initiated: February 24, 1970

Current Annual Level: $982,833

ARTHUR D. LITTLE, INC. (NO1-CP-33284) FORMERLY: (PH43-69-2147)

Title: Bioassay of the Cytotoxicity of Cigarette Smoke and of Its Effects on Ciliary Function

Contractor's Project Directors: Dr. Philip S. Thayer
Dr. Sam P. Battista

Project Officer (NCI): Dr. Gio B. Gori

Objectives: (1) To evaluate experimental cigarettes provided by the program for development of a less hazardous cigarette in the following two groups of test systems: (a) Effects of smoke on growth and phagocytic activity of *in vitro* cultivated cells, (b) Effects of smoke on ciliary kinetics and dynamics of mucus transport in the respiratory epithelium of experimental animals *in vitro* and *in vivo*. (2) To develop and evaluate a model system to determine whether cigarette smoke has effects on carcinogenesis in the chicken trachea as influenced by experimental removal of the tracheal epithelium.

Major Findings: All tracheal denudations and diethylnitrosamine (DEN) treatments have been completed for all groups, including spare animals. Specimens of tracheal epithelial smears removed from all ten denudations for the Smoked and Smoked-plus-DEN groups have been examined cytologically. An inordinate amount of mucus was found on the smears which prevented differentiation of nuclear detail. After several attempts to remove the mucus, treatment with 0.5% aqueous solution of α amylase for 4 hours at 37°C was found to reduce the mucus content of the slide. The amylase treated smears have now been evaluated through the seventh abrasion.

Three cytological changes attributed to the treatment and abrasions were observed: cells exhibiting (1) active and reactive nuclear chromatin patterns, (2) nuclear atypia and (3) morphological features characteristic of metaplasia. The greatest percentage of chickens having cells showing active and reactive chromatin patterns occurred in the Smoked, DEN-treated, and Vehicle Control groups, respectively. The trend is identical in groups of chickens having cells showing nuclear atypia. The percentage of chickens having cells exhibiting metaplastic changes was greater in the Smoked group, followed in order by those in the Smoked plus DEN-treated, DEN-treated and Control groups, respectively. Malignant cells have not been found in specimens from any group.

The groups receiving Smoke and Smoke-plus-DEN will continue to receive smoke. Construction of four additional smoking machines plus a second set of restraining stalls was completed in order for one operator to expose simultaneously eight animals to smoke. During the last week of March 1973, a number of the DEN-treated, Vehicle-treated Controls and Untreated Controls will be sacrificed to determine changes produced after one year. Some of the animals in the Smoked and Smoked-plus-DEN groups will be sacrificed the first week of May 1973. All animals not sacrificed will be maintained until such time that neoplasms are produced or the experiment terminated.

Fourteen Vehicle-treated Controls (7 Abraded, 7 Non-abraded), 3 Untreated Controls, 14 DEN-treated (7 Abraded, 7 Non-abraded), 6 Smoked (3 Abraded, 3 Non-abraded), and 4 Smoked-plus-DEN (2 Abraded, 2 Non-abraded) are to be sacrificed.

Significance to Biomedical Research and the Program of the Institute: The overall program for the formulation of a less hazardous cigarette needs to identify physiological responses of the respiratory tract surface tissues to cigarette smoke of different characteristics.

This information will be correlated with carcinogenesis data in testing and directing modification of cigarettes towards less hazardous formulations.

Proposed Course: The four bioassay procedures are available for evaluation of subsequent sets of experimental cigarettes. The development and evaluation of the neoplastic model system is proceeding.

Date Contract Initiated: June 19, 1969

Current Annual Level: $108,886

BATTELLE NORTHWEST LABORATORIES, INC. (PH43-68-1372)

Title: Inhalation Cocarcinogenicity of Industrial Pollutants and Cigarette Smoke

Contractor's Project Director: Dr. Alfred P. Wehner

Project Officers (NCI): Dr. Norbert P. Page
Dr. Thomas B. Owen

Objectives: The objective of this lifespan inhalation bioassay is to investigate the effect of combined exposures to cigarette smoke and aerosols of chrysotile asbestos, CoO or NiO, and of nitrosodiethylamine (DEN) injections, under controlled laboratory conditions. Male Syrian golden hamsters have been exposed to cigarette smoke in Hamburg 2 type smoking machines 3 times/day, 5 days/week. Exposure to aerosols of chrysotile (30 μg/l) NiO (60 μg/l) and CoO (10 μg/l) is for 7 hours/day, 5 days/week. Two groups received 12 injections of 0.25 mg DEN and DEN + smoke, respectively. Detailed pathological studies are being conducted on all animals. Aerosol concentrations have been monitored by continuous sampling.

Major Findings: An aerosol exposure system for long-term exposure of rodents has been developed as well as an improved asbestos aerosol generator. The smoke exposed groups lived significantly longer and weighed significantly less than the comparable groups not exposed to cigarette smoke. Asbestosis caused appreciably increased mortality in the asbestos exposed groups. While the computerized histopathological results are awaiting statistical analysis and evaluation, it appears that the number of tumors in the treatment groups expected to show a high tumor incidence (asbestos, smoke and DEN exposed groups) is much less than anticipated.

Significance to Biomedical Research and the Program of the Institute: This project is designed to test the combined effects of exposures to cigarette smoke and environmental dusts. The three dusts were selected on the basis of previous studies where direct administration of the single materials in the respiratory tract was employed.

Proposed Course: The experiment is now about 28 months into its chronic exposure phase and about to be concluded. After evaluation of all data, a final report will be issued.

Date Contract Initiated: June 28, 1968

Current Annual Level: $179,238

CENTER FOR DISEASE CONTROL (NCI-72-205)

Title: Study of Smoking Intervention Techniques

Contractor's Project Directors: Dr. Ernest L. Wynder
Mr. Alvin R. Jaffin

Project Officer (NCI): Dr. Gio B. Gori

Objectives: Investigation of the efficacy of various smoking intervention techniques and the relationships between success and failure, techniques used, and personality and smoking characteristics of subjects. Work is being conducted in two major areas: minimal interventions that physicians and allied health personnel can employ in their routine procedures and more maximal clinic-type interventions both group and individual. Study is intended to clarify variables indicative of success with minimal intervention as opposed to more intensive approaches.

Major Findings: To date, the minimal intervention program and the clinical program have been developed separately, largely because the populations involved in each case are very different. Minimal intervention subjects are smokers showing no particular interest in stopping smoking, while clinic subjects are relatively highly motivated.

Work with clinic patients is proceeding actively and is conceived of as a training ground for development and improvement of techniques. Extensive individual feedback and expression, as well as follow-up data and statistical analyses of characteristics of successful patients all are leading to refinement of techniques. This work has been built upon the development of an intensive questionnaire, as well as various group and individual techniques. Knowledge gained from this "in-house" service program will be useful in later stages of the carefully designed minimal-maximal study as coordinated through the National Clearinghouse for Smoking and Health.

In the minimal intervention program, work has focused on preparation for the subject contract, with some administrative aspects causing some delays. Final approval of the questionnaire and the specific interventions to be used will lead shortly to the actual interventions. To date, relevant smoker types have been defined, based on Clearinghouse formulations, and preparatory arrangements have been secured with the physicians who will give the minimal interventions during their exit interviews with patients in a multiphasic health screening examination.

Significance to Biomedical Research and the Program of the Institute: The results from these studies should provide a basis for aiding those individuals at high risk to reduce or eliminate smoke intake from cigarettes.

Proposed Course: To continue program of testing efficacy of various smoking intervention techniques.

Date Contract Initiated: June 21, 1972

Current Annual Level: $152,817

HAZLETON LABORATORIES (NIH-NCI-69-2145)

Title: Carcinogenicity Bioassays by Intragastric Intubation of Cigarette Smoke Condensates in Experimental Animals

Contractor's Project Director: Mr. James Gargus

Project Officer (NCI): Dr. John Cooper

Objectives: The objective of this contract is the evaluation of the carcinogenic potential of cigarette smoke condensates following repeated oral administration in rodents.

Experimental variables in the smoke condensates include nicotine content and total dose. If a satisfactory bioassay can be standardized using the oral route of administration, it will be utilized in the comparison of tobacco condensates of experimentally modified cigarettes. These bioassays are a part of the research program directed toward the development of a less hazardous cigarette.

The cigarette smoke condensates have been furnished by Meloy Laboratories (NIH-NCI-69-2084) under contract to NCI.

Major Findings: During the past support period, compound administration has continued to be administered in the rats and mice. Two tobacco condensates, Variables No. 2 and No. 5, are being administered at 10 mg/kg and 20 mg/kg. The mice will complete 18 months of dosing in March 1973 and the rats in June 1973.

Significance to Biomedical Research and the Program of the Institute: This project is an integral part of the program for the formulation of a less hazardous cigarette. This program requires that new bioassay techniques be devised which will be both sensitive and specific. The selection of this route of administration for emphasis is justified by the large quantity of tobacco tars and condensate which are ingested into the stomach in the course of smoking and subsequent ciliary clearance of the respiratory tract.

Proposed Course: The study will be terminated during this support period and necropsy information will be recorded at the time of death or sacrifice. Histopathological examinations will be performed on specified organs and tissues.

Date Contract Initiated: June 24, 1969

Current Annual Level: $49,200

HAZLETON LABORATORIES (NIH-NCI-69-2149)

Title: Skin Carcinogenesis Bioassay of Cigarette Smoke Condensates in Mice

Contractor's Project Directors: Mr. James L. Gargus
Dr. Marcelina B. Powers

Project Officer (NCI): Dr. Gio B. Gori

Objectives: The objective of this research program is to provide a standardized bioassay for the determination of the carcinogenicity of cigarette smoke condensates by repeated dermal applications in mice.

Whole smoke condensates are supplied by Meloy Laboratories under NCI Contract (NIH-NCI-69-2084). The experimental variables included in these coded samples are tobacco of different origin; methods of cultivation, including fertilization, suckering agents, and soils; curing and aging methods, blends of tobacco plant parts such as leaves, stalks, and stems; reconstituted tobacco, cutting of tobacco, cigarette paper, and filters.

Female ICR mice are treated six times weekly with dermal applications of the whole smoke condensates dissolved in acetone. Skin tumors are recorded and measured. All animals are necropsied and tissues are fixed and preserved for histopathological examinations. Data on each animal are entered in a computer memory once monthly. Computations and printed reports from NCI provide monitoring data for continuous evaluation of the experimental results.

Major Findings: The mouse skin painting bioassay on the first series of experimental cigarettes has been completed. Histological findings have been reported and are being correlated with the chemical analysis from the smoke condensates. A second series of dermal bioassays of 25 different smoke condensates were initiated in February, March, and April 1972. The experimental design employed in this new series is the same as that previously employed in the first series. Monthly animal data are submitted for computer storage and computations. There are 6,500 experimental animals in this second series.

The histopathological examination of the skin tissues from all tumor-bearing mice in the first series of bioassays is in progress.

Significance to Biomedical Research and the Program of the Institute: The comparative bioassay results along with other monitoring bioassays and chemical determinations will permit evaluation of the biological effects of the variables in the different smoke condensates. These comparative evaluations will provide data for testing and developing less hazardous cigarette formulations.

Proposed Course: Continue treatment and observations of mice through 18-month experimental period.

Date Contract Initiated: June 24, 1969.

Current Annual Level: $298,573.

HAZLETON LABORATORIES (NIH-NCI-72-3275)

Title: Chronic Carcinogenesis Bioassays by Intratracheal Instillation of Cigarette Smoke Condensate in Syrian Hamsters

Contractor's Project Director: Dr. Marcelina B. Powers

Project Officers (NCI): Dr. Michael B. Sporn
Dr. John A. Cooper

Objectives: The objective of this contract is to evaluate the carcinogenic effects of various cigarette smoke condensates in hamsters resulting from repeated intratracheal administration.

The cigarette smoke condensates have been furnished by Meloy Laboratories (NIH-NCI-69-2084) under contract to NCI.

Major Findings: Fifty weeks of repeated intratracheal instillation of three tobacco condensate variables have been completed. Animals received an estimated weekly dose of 1 mg of condensate per hamster. The animals are currently on post-treatment observation for a period of 30 weeks.

Significance to Biomedical Research and the Program of the Institute: Work performed by Saffiotti and co-workers has established that benzpyrene and other purified hydrocarbons (which are constituents of tobacco smoke) will cause tracheal and bronchial epidermoid squamous cell carcinoma when administered intratracheally in hamsters. The histology of these tumors closely resembles the most common form of lung cancer in man. Thus an excellent animal model is available to investigate the human disease. The present contract is an attempt to evaluate the potential of various types of tobacco tars to induce lung cancer by repeated intratracheal instillation in hamsters. These studies are an important parallel to similar work being done with purified hydrocarbons and could ultimately represent an important bioassay for a less hazardous cigarette.

Proposed Course: The study will come to an end during this support period. Necropsy observations will be recorded at time of death or sacrifice. Histopathologic evaluation of specified tissues will be undertaken and special attention will be given to sections of the respiratory tract.

Date Contract Initiated: May 20, 1971

Current Annual Level: $59,269

MELOY LABORATORIES (NIH-NCI-69-2084)

Title: Preparation and Analysis of Cigarette Smoke Condensate Samples

Contractor's Project Director: Dr. A. R. Patel

Project Officer (NCI): Dr. Thomas B. Owen

Objectives: Meloy Laboratories is responsible for mechanically smoking cigarettes and collection of the smoke condensates having, as nearly as possible, uniform composition for use in a number of bioassay programs to be carried out under contracts in the Chemical Carcinogenesis/Lung Cancer Task Force Program.

The following quality control analyses are performed on each of the 23 types of cigarettes: a) nicotine in tobacco; b) nitrate in tobacco; c) reducing sugars in tobacco; d) ash; e) hexane; f) potassium and sodium in tobacco; g) calcium and magnesium in tobacco; h) phosphorus in tobacco; i) static burning rate of cigarettes; j) average temperature of firezone at different butt lengths.

Major Findings: This is a complex service contract, the scope of which is production of cigarette smoke condensate on schedule and involves quality control on each batch of smoke condensate.

Significance to Biomedical Research and the Program of the Institute: This contract is designed to produce the cigarette smoke condensate required for the execution of four other projects which are funded under the Lung Cancer Task Force: (1) Skin Carcinogenesis Bioassay of Cigarette Smoke Condensate, (2) Intratracheal Bioassay of Cigarette Smoke Condensate for Carcinogenesis, (3) Bioassay of Cigarette Smoke Condensate by Oral and Parenteral Routes, (4) Skin Carcinogenesis Bioassay of Marijuana Cigarette Smoke Condensate.

Proposed Course: Activities of this contract started in February 1970, as experimental cigarettes became available. Analytical work on the unsmoked tobacco from these experimental cigarettes is being carried out. Production activities on the second series of NCI cigarettes are proceeding on schedule.

Date Contract Initiated: June 30, 1969

Current Annual Level: $393,734

NEW YORK UNIVERSITY (NO1-CP-33241) FORMERLY: (NIH-NCI-64-938)

Title: Studies on Carcinogenesis Principles of Processed Tobacco and Tobacco Smoke

Contractor's Project Director: Dr. Benjamin L. Van Duuren

Project Officer (NCI): Dr. Thomas B. Owen

Objectives: To determine the nature of deleterious agents that are known or suspected components of cigarette smoke condensates. These include promoters, initiators, cocarcinogens and other factors. To develop cell culture systems for the rapid bioassay of tumor-promoting agents.

Major Findings: Using the mouse skin carcinogenesis assay system, it was found that the presence of aromatic hydrocarbon carcinogens in these tars is inadequate to explain their biological activity. Furthermore, tobacco tar possesses marked tumor promoting activity in two-stage carcinogenesis tests on mouse skin. In order to determine which chemical components of tobacco tar are responsible for its activity, a series of substances known to be present in tobacco leaf or cigarette smoke condensate was tested in the two-stage mouse skin system for studying tumor promotion. Nine leaf or smoke components or related compounds have been shown promoting activity, e.g., decyl acetate, linalool, dodecane, phenol, and anthralin. Since exposure to tobacco smoke condensate or smoke, respectively, in mouse skin or human lung, involves simultaneous exposure to a variety of initiating agents, carcinogens, promoters and cocarcinogens, it was important to employ a concomitant exposure in mouse skin experiments with the compounds of interest. (Benzo(a)pyrene was selected as the carcinogen since it occurs in tobacco tar.) The compounds tested as carcinogens included both active tumor-promoters and compounds which showed no tumor-promoting activity. Some of the compounds tested showed cocarcinogenic activity, e.g., linalool esters whereas others were tumor-inhibitors, e.g., phenol, rutin and morin. The inhibitory compounds either delayed the appearance of tumors or decreased the tumor yield. In recent work it was shown that catechol, benzo(e)pyrene and pyrene are potent cocarcinogenic agents.

In lists of carcinogenic aromatic hydrocarbons, weak or borden line carcinogens are frequently omitted yet these compounds probably play some role in tobacco carcinogenesis as initiating agents, e.g., chrysene and dibenz(a,c)-anthracene.

A cell culture system was developed for the rapid assessment of the tumor-promoting activity. This system depends upon the measurement of the enhancement of transformed cell outgrowth in mixed culture (3T3 mouse fibroblasts and SV-transformed 3T3 cells). A series of compounds which are either known or suspected components of tobacco leaf and/or tar were tested in this system. A major possible benefit of this bioassay is that it may be of use in shortening the duration of a test from one year or more to a few weeks. Good agreement has been found between the *in vivo* and *in vitro* test systems, the exceptions are those in which the material on test has high toxicity.

Significance to Biomedical Research and the Program of the Institute: Lung cancer is one of the leading causes of cancer death and the importance of cigarette smoking in the etiology of this disease is well established. Therefore, the identification of the agents responsible for the deleterious health effects of cigarette smoke is of critical importance for the ultimate reduction of cancer risk due to cigarette smoking. The identification of these agents, an understanding of their biological effects, and the mode of action can lead to their removal from tobacco or cigarette smoke or possibly the neutralization of their biological effectiveness as tumor inducing substances.

Proposed Course: The examination of the tumor-promoting and cocarcinogenic activity of a variety of phenolic compounds will be continued. The cocarcinogenic activity of a series of alkanes, alkenes, terpenes, fatty acids and alcohols of tobacco leaf or cigarette smoke condensate will also be evaluated. The relationship of aryl hydroxylase enzyme systems to the tumor-inhibiting activity of various agents tested in cocarcinogenesis studies will be further examined. The mixed culture system will be reexamined with the goal of establishing a test system using human cells of pulmonary tissue origin. Previous efforts to grow untransformed mouse epidermal tissue in vitro for long periods of time have not been successful. As a part of the present work methods of growing cells derived from mouse skin papillomas or carcinomas will be explored. These tumor cells will be used in a mixed culture system along with various untransformed cell populations.

Date Contract Initiated: June 29, 1964

Current Annual Level: $80,620

VETERANS ADMINISTRATION HOSPITAL (EAST ORANGE, N.J.) (FS-72-66)

Title: Test Effects of High and Low Nicotine Cigarettes on Male Beagle Dogs

Contractor's Project Directors: Dr. Oscar Auerbach
Dr. Edwin Howard

Project Officer (NCI): Dr. Gio B. Gori

Objectives: The purpose of the proposed experiment is to determine if, and to what degree, nicotine plays a significant role in the harmful effects resulting from long term cigarette smoking.

Major Findings: Pilot studies were initiated to evaluate two different smoking systems from the standpoint of efficiency and number of personnel required to conduct a major dog smoking study, and to evaluate the biological effectiveness of the two methods of introducing cigarette smoke into beagle dogs.

These studies will be completed by May of 1973, and based on histological findings, a smoke delivery system will be selected for use in the chronic studies. Preliminary results indicate that a mechanical smoking device will be used.

Significance to Biomedical Research and the Program of the Institute: This project will provide needed information that will indicate what role the nicotine present in cigarettes plays in the pulmonary and cardiovascular disease associated with cigarette smoking. It is known from previous studies that cigarette smoking by beagle dogs results in pulmonary emphysema, pulmonary neoplasia, and arteriolosclerosis. By doing this study with high and low nicotine level cigarettes, it will be possible to determine the biological significance of nicotine intake during cigarette smoking. This will contribute significantly to the overall program designed to develop a less harmful cigarette for public use.

The pilot study to compare the two different smoking systems described will provide other researchers with information needed in the selection of a smoking system for other programs designed to study the mechanisms of cigarette induced damage, and in the evaluation of other test cigarettes.

Proposed Course: To continue the program as outlined above.

Date Contract Initiated: April 1, 1972

Current Annual Level: $308,437

IV. PUBLICATIONS OF THE CARCINOGENESIS PROGRAM

A. COLLABORATIVE PROGRAM

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C. AUTHOR INDEX[1]

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[1] Publications with authors from the intramural staff and from contractors' staff are reported under both lists; such cross references are provided in parentheses.

SUMMARY REPORT

OFFICE OF THE ASSOCIATE SCIENTIFIC DIRECTOR FOR VIRAL ONCOLOGY (ASDVO)

July 1, 1972 - June 30, 1973

J. B. Moloney

The Annual Report for Viral Oncology, Division of Cancer Cause and Prevention, NCI, is presented as follows:

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I. VIRAL ONCOLOGY AREA - SPECIAL VIRUS CANCER PROGRAM (SVCP)

A. Introduction:

The Viral Oncology Area is responsible for planning and conducting the Institute's program of coordinated research on viruses as etiological agents of cancer. Scientists within this Area not only provide the broad operational management for intramural and collaborative research but also conduct investigations on oncogenic viruses and their interaction with host cells. Any information obtained from these coordinated studies is rapidly applied (1) to search for viruses or virus genetic information which may be etiologically related to the initiation of human cancer and (2) to develop therapeutic and preventive measures for control of human cancers when such causative agents are found. This program, as it is now structured, contributes to six of the seven goal-oriented and key objectives set forth in the National Cancer Plan, the ultimate goal of which is control of all human cancers.

Contract supported research is conducted within the Viral Oncology Program under the Special Virus Cancer Program (SVCP). The yearly budget from the inception of this Program in 1964 to the present and the number of professional positions are given in a separate section of this report. A history of events leading to the development of the SVCP may be found in previous Annual Reports of the NCI. These documents highlight the extensive research on the role of viruses as etiologic agents of cancers in man. The Program has succeeded in marshaling many of the nation's finest

virologists, biochemists, immunologists, molecular biologists, epidemiologists and physicians for this strongly goal-oriented effort. From the beginning, it was clear that an understanding of the suspected relationship between tumor viruses and human neoplasia would not only require an interaction among these groups of scientists but sound and contructive administrative support as well.

The Research Logic for the SVCP, based on the premise that a virus or viral genetic information persists in the host and is an indispensable element for the induction of certain kinds of human cancer, is continually updated. The plan is reviewed regularly by the Director, NCI; the Scientific Director for Division of Cancer Cause and Prevention, NCI; the National Cancer Advisory Board, NCI; the Executive Committee, NCI; and the DCCP Management Group, NCI. This year at the request of the Viral Oncology Area, the National Cancer Advisory Board appointed an ad hoc committee to review the research efforts of the SVCP and suggest any relevant changes.

B. Organization:

1. Viral Oncology Area. In keeping with the new format imposed by the Divisional status given to the Etiology Area, now Division of Cancer Cause and Prevention (DCCP), the Office of the Associate Scientific Director for Viral Oncology will in the future be known as Office of the Associate Director for Viral Oncology. Within this office are: The Office of Biohazards and Environmental Control with two sections, the Biohazards Research Section and the Environmental Control Section; The Office of the Coordinator for Ultrastructural Studies with a Viral Studies Section; The Office of Program Analysis and Communications with one Information Unit; and The Office of Program Resources and Logistics.

The Viral Biology Branch is composed of six research sections: Cell Biology Section, Electron Microscopy Section, Microbiology Section, Experimental Pathology Section, Human Tumor Studies Section, and Virus and Disease Modification Section. The Molecular Biology Section has been transferred to the Viral Carcinogenesis Branch.

The Viral Leukemia and Lymphoma Branch is composed of seven sections: the Primate Studies Section, Tumor Virus Section, Clinical Studies Section, Immunology Section, Viral Pathology Section, Genetics Section, and Viral Biochemistry Section. Three of these sections--Primate Studies, Viral Biochemistry and Clinical Studies -- were created this year.

The Viral Carcinogenesis Branch is composed of four research sections: the Molecular Biology Section; the Ecology and Epizoology Section, with the Field Studies Unit; the Solid Tumor-Virus Section; and the Viral Genetics Section, with the Serology Unit, and the Trailer Unit.

2. Special Virus Cancer Program

By the end of the fiscal year the Special Virus Cancer Program will be renamed the Virus Cancer Program. Under the Chairman, the Associate

Director for Viral Oncology, the following 6 working segments will be maintained: Developmental Research Segment, Immuno-epidemiology Segment, Biohazards Control and Containment Segment, Solid Tumor Virus Segment, Breast Cancer Virus Segment, and Tumor Virus Detection Segment.

The Tumor Virus Detection Segment was established on September 1, 1972. This working group provides advice to the Associate Director for Viral Oncology for determining the role of viruses in human cancer by studying the interactions between viruses and cells involved in the process of oncogenesis and applies this knowledge to devising methods for modifying or blocking these interactions. The current membership (listed elsewhere in this report) consists of 14 scientists, predominantly members of the scientific community outside the government. Each has been selected on the basis of his outstanding individual qualifications and recognized expertise in the fields of molecular biology, immunology, or virology as related to cancer research.

It is anticipated that in the next year, the name of the Immuno-epidemiology Segment will be changed to the Viral Immuno-epidemiology Segment. In conformance with the directive from Office of the Director, NCI, all collaborative studies in which the major thrust is tumor immunology will henceforth be transferred to the Tumor Immunology Program, Division of Cancer Biology and Diagnosis, NCI. The research orientation of the present working group is and always has been directed to tumor virus immunology and epidemiology, an area exempted from the scope of the Tumor Immunology Program; therefore, no significant research changes are expected.

The SVCP management requested an independent survey group to determine the extent to which various types of resource and logistics management systems may apply in the implementation of the Special Virus Cancer Program within the National Cancer Program. The findings are summarized as follows: This pilot study was conducted to determine the acceptability of alternative resource management and logistics system to the SVCP community. Twenty-six Principal Investigators and 19 Contract Administrators from 19 organizations were interviewed in the course of the survey. Tabulation and analysis of interview data indicate an overwhelming preference for an NCI-SVCP operated centralized system. Those interviewed felt that only NCI was qualified to operate the SVCP resource management and logistics system. While some participants believed that a qualified Prime Contractor could be found within the SVCP program, they doubted whether the contractor could be truly objective in placing program interests ahead of company research interests. Very few believed that a qualified Prime Contractor could be found outside the SVCP program. No centralized system at all was judged least acceptable on the premise that the SVCP program would become unmanageable and result in chaos.

By invitation, a delegation of U.S. virologists, all working within the SVCP, visited the Soviet Union during the period November 10-21, 1972. The members were taken to research centers in Moscow, Sukhumi, and Leningrad, and met with various Soviet representatives to discuss problems in viral oncology within the framework of the U.S.-U.S.S.R. Agreement on Health Cooperation. During the many meetings both delegations interacted extremely well and

following a series of very frank discussions developed a Memorandum of Understanding for further cooperation in the study of the microbiology, the immunology, and the molecular biology of cancer viruses. This Memorandum, signed on November 18, 1972, establishes clearcut procedures for joint studies through the exchange of (1) information--reprints, preprints, and summary reports; (2) materials--tumor virus isolates and viral diagnostic reagents; and (3) scientists--lecturers, guest workers, and participants in relevant cancer-virus meetings. (For a detailed account of scientific progress see Part C of this report.)

Administrative Structure of SVCP

Segment Chairmen and Vice Chairmen. The segment chairmen are responsible for planning the projects in each working group. As senior scientists, they must review, analyze and integrate studies which fulfill the objectives of their working group and the total Program.

Executive Secretaries. Executive secretaries assist the chairmen in managerial duties of contract operation. They are responsible for optimal review and complete documentation of each project within the working group.

Project Officers. Project Officers are the direct extension of program segment chairmen. To assure progress in accomplishing the goals set forth in the workscope of a project, they are called upon to advise principal investigators on scientific matters and coordinate segment and program decisions.

Working Groups. The program segment working groups are the peer review units for the Program. Composed of both intramural and extramural scientists and chaired by senior NCI scientists, the groups review individual contracts or proposals for scientific excellence and technical competence within a given funding level. Their recommendations provide the Segment Chairmen, Associate Director for Viral Oncology, and the Director, DCCP, with a basis for program decisions.

Contract Specialists. Contract specialists are responsible for negotiating research contracts. They provide valuable advice on fiscal and legal matters to the project officers, executive secretaries, segment chairman and Associate Director. Some specialists are well conversant with the scientific aspects of the Program. They are assigned to Program Areas by the Research Contracts Branch, NCI.

Contract Review. The projects within the total Program are reviewed at many levels:

(1) Each contract is reviewed for relevance, priority, and need to total Program at the Program Segment Chairmen's meeting.

(2) Each contract is reviewed for scientific excellence and technical competence at the Program Segment Working Group meeting.

(3) Each contract is continually monitored for performance by the Project Officer.

(4) Each contract above the annual funding level of $1 million and with multifaceted workscope undergoes a third review by an ad hoc committee appointed by the Associate Director for Viral Oncology.

(5) Each contract is reviewed by the Associate Director for Viral Oncology; the Director, Division of Cancer Cause and Prevention; the Chief, Research Contracts Branch, NCI; and the Director, NCI.

As an aid to the review processes, key staff members receive progress reports on all contracts on a biannual basis. Collection and distribution of these reports is the responsibility of the Office of Program Analysis and Communications. A single comprehensive report is prepared annually by the Associate Director.

This year, the NCI has obtained permission to begin incremental funding. Those research projects for which long term commitments are clearly indicated will be funded in this manner. This should add a new dimension of administrative flexibility to the Program.

Administrative Highlights. Implementation of the National Cancer Plan and elevation of NCI to the level of Bureau has meant the delegation of increased responsibilities to Viral Oncology. In order to support the program in meeting these responsibilities, the Viral Oncology Administrative Office now provides total financial management support of both the contract and the in-house research programs. A monthly report of the status of contract funds is provided by the Administrative Office. The office has become the control point for the obligation of all contract funds, providing more extensive support in the financial management of the program.

The FY 1973 budget provided a substantial increase in funds for NCI. However, in spite of this, Viral Oncology received an increase of only $894,000 for contracts and $545,000 for the in-house program.

In the area of personnel there were three administrative policies which had drastic effects on Viral Oncology Area and consequently the members of the staff of the SVCP: (1) the grade de-escalation program designed to reduce the average GS grade; (2) the manpower reduction effort to reduce federal employment; and (3) the freeze on hiring and promotions from December to March. The result of these programs has been a cutback in personnel positions in FY 73 from 226 to 217, the promotion of very few employees this fiscal year, and a general lowering of employee morale. In spite of these restrictions, however, we were able to convert 10 staff fellows to permanent civil service positions.

Some additional space was provided in this fiscal year when NIH acquired the Landow Building in Bethesda. However, this did not supply immediate relief to Viral Oncology since there was not sufficient space to move entire program elements from Building 37. Even with the acquisition of the new space we approached the end of the fiscal year still pressed for both

office and laboratory space. With no new plans on the part of NIH for the acquisition of additional space, Viral Oncology is making plans for the conversion of the Building 41 Open Bay Area to laboratories.

The funding history of the Special Virus Cancer Program and the earlier Special Virus Leukemia Program from its inception in 1964 is given below. The table indicates that in recent years the number of professional staff vital for program management has not kept pace with increases in funding levels.

Funding History - SVCP Contracts
(in thousands)

Fiscal Year	Positions	VO	SVLP	SVCP	Totals
64	30	4,926	-	-	4,926
65	90	5,433	8,723	-	14,156
66	95	3,064	13,556	-	16,620
67	138	3,137	13,505	-	16,642
68	140	-	-	17,241	17,241
69	157	-	-	17,985	17,985
70	167 (59)*	-	-	17,340	17,340
71	187 (68)	-	-	34,091	34,091
72	226 (79)	-	-	41,889	41,889
73	217 (83)	-	-	42,511	42,511

*Figures in parentheses represent professional staff, GS-13 and above.

Program Management Personnel

Science Management Team

Dr. J. B. Moloney, Associate Scientific Director for Viral Oncology
Dr. L. R. Sibal, Special Assistant for Program Coordination, VO
Dr. R. J. Goldberg, Assistant for Collaborative Research, VO
Dr. H. J. Hearn, Scientific Coordinator for Viral Oncology, Frederick Cancer Research Center

Administrative Staff

Mr. N. A. Olimpio, Acting Administrative Officer, Division of Cancer Cause and Prevention, NCI
Mr. Robert Velthuis, Administrative Officer, Viral Oncology
Mr. Thomas Jones, Administrative Assistant, Viral Oncology

Contract Specialists*

Mr. John Gibbons	Mr. Thomas Louden
Mr. Wm. Caufield	Ms. Amy Simpson
Mr. J. Thomas Lewin	Mr. Jack Leibowitz
Mr. Thomas Porter	Mr. Charles Fafard

Program Resources and Logistics Advisory Group

Dr. Jack Gruber, Chairman
Dr. David Howell, Executive Secretary

Dr. Robert Bassin	Dr. Wade Parks
Dr. James Duff	Dr. Gary Pearson
Dr. Robert Holdenried	Dr. Deward Waggoner
Dr. Maurice Guss	Dr. Garrett Keefer

Frederick Cancer Research Center Advisory Group

Dr. Henry J. Hearn, Chairman

Dr. James Duff	Dr. Louis Sibal
Dr. Maurice Guss	Dr. Bernard Talbot
Dr. Jack Gruber	Mr. Robert Velthuis

Program Management Segment

Dr. J. B. Moloney, Chairman
Dr. L. R. Sibal, Executive Secretary

Dr. A. J. Dalton	Dr. Robert Manaker
Dr. James Duff	Dr. Robert Holdenried
Dr. Michael Chirigos	Dr. George Todaro
Dr. Robert Huebner	Dr. Jack Gruber

Dr. Alfred Hellman

* Members of Research Contracts Branch, NCI

PROGRAM SEGMENTS

Developmental Research Segment
Dr. Robert Manaker, Chairman
Dr. Michael Chirigos, Vice Chairman (Acting)
Dr. Maurice Guss, Executive Secretary
Dr. Sam Dales, Public Health Res. Inst, N.Y.C.
Dr. Anthony Girardi, Wistar Institute
Dr. Arthur Brown, University of Tennessee
Dr. Mathilde Krim, Sloan-Kettering Inst.
Dr. Malcolm Pike, University of Oxford
Dr. Paul Gerber, DBS, NIH
Dr. Bernice Eddy, DBS, NIH
Dr. Alan Rabson, NCI
Dr. Robert Gallo, NCI

Solid Tumor Virus Segment
Dr. Robert Huebner, Chairman
Dr. James Duff, Vice-Chairman
Ms. Harriet Streicher, Executive Secretary
Dr. Stuart Aaronson, NCI
Dr. Janet Hartley, NIAID, NIH
Dr. Jerard Hurwitz, Albert Einstein College of Medicine
Dr. Howard Igel, Children's Hospital, Akron, Ohio
Dr. Henry Kaplan, Stanford University
Dr. Edmond Klein, Roswell Park Memorial Institute
Dr. Hans Meier, Jackson Laboratories
Dr. Guy Newell, Tulane University
Dr. Wade Parks, NCI
Dr. Fred Rapp, Hershey Medical Center, Pa. State U.

Tumor Virus Detection Segment
Dr. George Todaro, Chairman
Dr. Bernard Talbot, Vice-Chairman
Dr. Roy Kinard, Executive Secretary
Dr. J. Thomas August, Albert Einstein College of Medicine
Dr. Paul Black, Massachusetts General Hospital
Dr. Dani Bolognesi, Duke University
Dr. Friedrich Deinhardt, Presbyterian St. Luke's Hosp., Chicago
Dr. Peter Fischinger, NCI
Dr. Charlotte Friend, Mt. Sinai Hospital, N.Y.C.
Dr. Clarence Gibbs, NINDB, NIH
Dr. Adeline Hackett, Naval Biological Laboratories
Dr. Edward Scolnick, NCI
Dr. Howard Temin, McArdle Laboratory, U. of Wisconsin

Immunology-Epidemiology Segment
Dr. Paul H. Levine, Chairman
Dr. Gary Pearson, Vice-Chairman
Dr. Clarice Gaylord, Executive Secretary
Dr. Tadao Aoki, NCI
Dr. Dharam Ablashi, NCI
Dr. Barry Bloom, Albert Einstein College of Medicine
Mr. Roger Connelly, NCI
Dr. M. Lieberman, Stanford University
Dr. Richard Morrow, Harvard School of Public Health
Dr. Herbert Oettgen, Sloan Kettering Institute
Dr. Ken Takemoto, NIAID, NIH
Dr. David Yohn, Ohio State University
Dr. Michael Blaese, NCI

Biohazards Control and Containment Segment
Dr. Alfred Hellman, Chairman
Dr. Emmett Barkley, Vice-Chairman
Dr. Garrett Keefer, Executive Secretary
Mr. Mark Chatigny, Naval Biological Laboratories
Dr. Michael Chirigos, NCI
Dr. Peter Gerone, Tulane University
Dr. Richard Griesemer, U. of California, Davis
Dr. Seymour Kalter, Southwest Foundation for Res. and Ed.
Dr. George Michaelsen, U. of Minnesota
Dr. Maurice Mufson, Westside VA Hospital, Chicago
Dr. William Payne, Div. of Environmental Health Sciences, NIH
Dr. J. A. Schneider, U. of California, LaJolla
Dr. Arnold Wedum, Fort Detrick

Breast Cancer Virus Segment
Dr. Robert Depue, Acting Chairman
Vice Chairman - To be appointed
Dr. Ernest J. Plata, Executive Secretary (Acting)
Dr. Richard Bates, NCI
Dr. Maurice Black, N.Y. Medical College
Dr. Harish Chopra, NCI
Dr. Joseph Fraumeni, NCI
Dr. Raymond Gilden, Flow Laboratories
Dr. M. Meyers, NCI
Dr. E. Vollmer, NCI

(Four additional extra-governmental members to be appointed)

C. Scientific Activities and Progress Highlights

The scientific activities of the Office of the Associate Director for Viral Oncology and of the Offices and Branches within this Office are presented in the following sections.

Summary of Scientific Activities

Introduction. A considerable number of viruses are known which either directly or indirectly cause tumors in different vertebrate species. Many of these replicate in and cause transformation of cultured human cells. The RNA viruses are responsible for many naturally occurring tumors in animals, and this group is most likely to be the cause of some human neoplasms. Certain DNA viruses may also be involved in oncogenesis either as causative agents or as necessary co-factors in cell transformation. Thus far, the only DNA viruses associated with natural cancers of animals and man are herpesviruses. Although candidate viruses have been isolated more frequently in recent years, none as yet has met the rigorous criteria to be considered a human tumor virus. Nevertheless, there is cause to be optimistic, for scientists have acquired the knowledge, techniques, experimental systems, and insight to study this problem. Even if virus particles may not be the cause of human cancer, virologists are pursuing the exciting possibility that cellular genes--genes containing information similar to that of RNA or DNA tumor viruses--are expressed in human cancer.

Current knowledge suggests several methods for analysis of human tumors for virus-specific genetic information: (1) Molecular hybridization between cancer cell RNA and natural and synthetic DNA prepared by viral RNA → DNA polymerase; (2) Search for RNA → DNA polymerase activity in human cancers; (3) Search for human virus-specific antigens related to known tumor virus antigens in human cancers; (4) Application of new techniques (e.g. chemical treatment) for the induction of virus or virus genetic information in human tumor cells. All of these methods are being vigorously applied to the etiology and regulation of human cancers and currently more than 50% of the manpower and funding of the Program are allotted to investigating this problem. The success of this program is dependent upon extensive participation of in-house and collaborating scientists in long-term, multidisciplinary studies, as recommended in the new National Cancer Plan.

A history of the major advancements in studies on RNA and DNA tumor viruses was presented in the Annual Report for Fiscal Year 1972. Only newer findings and their relationship to human disease will be emphasized in this report.

RNA VIRUSES

Characteristics. RNA tumor viruses: (1) possess a number of similar biological and biochemical characteristics, (2) are widely distributed in vertebrate species, and (3) are transmitted vertically under natural circumstances. Infections are often covert, resulting in only partial expression of the viral genome. Each Type C virus, regardless of origin, possesses a major internal antigen which permits species delineation (gs-1), and shares a determinant which is interpsecies specific (gs-3). Antigens with

biochemical characteristics similar to those of Type C viruses have been found in Type B viruses, but the two groups of RNA viruses are antigenically totally dissimilar. There is now good evidence that internal antigens can be detected in embryonic, adult, and tumor tissues of various animal species. For example, natural expression of mouse gs-1 antigen was found in all mouse tissues examined, suggesting that a continuous synthesis of this polypeptide is an integral part of the murine cell macromolecular synthesis. This finding is highly significant because the antigen can be found in situations where no virus can be demonstrated. Thus, it provides a highly useful marker for virus expression because viruses which can replicate in widely divergent species maintain their species-specific reactivities. Since the antigen is a structural component of the virus, its detection presents a strong argument for the presence of virus activity.

Recently the gs-1 antigen from each of the following Type C viruses--mouse, cat, woolly monkey, gibbon ape, and RD-114--was purified to homogeneity and used in species-specific, highly-sensitive radioimmunoassays. The close relationship of gibbon and woolly monkey viruses and their difference from RD-114 was demonstrated. Of greater importance in terms of understanding the determinants of cancer is the reported linkage between RNA tumor virus and early viral gs antigen expression with the development of cancer late in life. In certain mouse strains a high percentage of the cancers observed in old age occurred in animals whose spleens were gs-positive at weaning; only a small percentage occurred in mice gs-negative at weaning. The results indicate that exogenous structural and regulatory genes are critical determinants of RNA tumor virus expression.

All of these viruses contain 60-70S RNA and an enzyme known as RNA-dependent DNA polymerase (RDDP) which permits transcription of viral RNA into DNA. As an antigen, RDDP, like the gs antigen, has both species and interspecies characteristics. The murine polymerase has been partially purified and used to produce antisera in other species. The antibody to the mouse enzyme inhibits the activity of hamster, cat, and rat polymerases indicating antigenic cross relationships. Crosses with other mammalian Type C viruses are only partial thus allowing precise identification of the species of origin of an unknown Type C virus. For example, RDDP from M-PMV is unrelated to known Type C or primate syncytium (foamy) virus RDDP. Among the Type C viruses, polymerases from woolly monkey and gibbon viruses are very closely related immunologically; both differ widely from the enzyme found in RD-114 virus.

To provide a basis for further understanding of this process, information was sought to elucidate the mechanisms involved in: (1) the integration of RNA tumor virus information into host cells, and (2) the relationship of this reaction to cell transformation. Recent results indicate the initial product of the RDDP is covalently linked RNA-DNA that is primed for DNA synthesis by a 4S RNA associated with 60-70S RNA. The yield of DNA is also increased by a stimulatory viral protein. A hybridase (RNAse-H), also associated with RDDP, and apparently active only on the RNA of free ends of DNA-RNA, provides single-stranded DNA which may be the form integrated into nuclear DNA. In the presence of nuclear enzymes causing DNA repair, a DNA-DNA structure is effected.

The RNA of all known RNA tumor viruses contains long stretches of poly A sequences, up to fifty times more than that found in non-oncogenic RNA viruses. A technique involving hybridization with labelled poly A was developed which permits quantitative estimates of the number of virus particles in virus preparations. The presence of poly A in tumor virus RNA also suggests that the origin of the virus is in the nucleus of a cell.

The protein products of virus gene expression in relation to cell transformation are also being studied. Representative viruses having sarcomagenic and leukemogenic properties contain up to 5 polypeptides. The purified preparations are antigenically active. End group analyses and amino acid composition of individual polypeptides and glycoproteins are under intensive study. The capability to detect these polypeptides within cells should provide a powerful tool for following virus gene expression in the transformation process.

It is evident that within the past year tremendous progress has been made toward the understanding of the biochemical (molecular) events which occur in the process of cell transformation. These studies are important because they can be applied to human normal and tumor cells to identify virus-specific sequences that have characteristics similar to those of animal RNA tumor viruses. Such studies also help to provide information on mechanisms of virus expression and regulation which could lead to new methods for controlling human cancer at the cellular level.

RELATIONSHIP TO HUMAN CANCER

Analysis of cancers for genetic information of RNA tumor viruses. The powerful tools of molecular hybridization have permitted the analysis of human tumors for viral genetic information. Evidence from animal model systems indicate thus far that every cell line infected with or transformed by RNA tumor viruses possesses virus-specific RNA sequences readily detectable by molecular hybridization. Corresponding neoplasias of murine and human origin were found to exhibit remarkable similarities. For example, human breast carcinomas contained RNA possessing sequence homology to that of murine mammary tumor virus (MMTV). This type of RNA was not detectable in normal breast tissues. Breast cancer RNA did not hybridize to DNA complementary to the RNA of MuLV (Rauscher). Later, it was shown that human leukemic cells and human sarcomas both contain RNA showing homology to that of MuLV (Rauscher) and not to MMTV. Of further interest is the recent demonstration that Hodgkin's lymphomas and Burkitt's tumors possess RNA uniquely homologous to that of the MuLV (Rauscher). Since a causal relationship between EBV and Burkitt's tumor has not been conclusively established, it was pertinent to examine this lymphoma for MuLV-related RNA. The data show that at least a portion of the RNA exists in the form of a 70S RNA associated with an RDDP in a particle having a density between 1.16 and 1.19 g/ml. Furthermore, Hodgkin's and Burkitt's lymphomas showed no significant reactions with either AMV-DNA or MMTV DNA. The presence in human lymphomas of RNA related MuLV (Rauscher) strongly suggests the involvement of a viral agent in these diseases.

Analysis of cancers for viral reverse transcriptase (RDDP). Following reports of RDDP in the virions of RNA tumor viruses, it was important to determine whether the enzyme is restricted to tumor viruses and whether it is restricted to tumor cells. All of the oncogenic RNA viruses tested so far have RDDP as indicated by both the endogenous reaction using viral RNA and by synthetic polymer-stimulated reactions; with few exceptions, non-oncogenic viruses show no evidence of this activity. Attempts are now being made to determine whether the polymerase is present in human cells. RDDP obtained in the particulate fraction (1.16 g/m) of human leukemic cells was purified and characterized. The enzyme could be distinguished from major DNA polymerases of normal cells by its properties, such as acceptance of an RNA heteropolymer template. Four RDDP preparations were purified from human leukemic cells. An antiserum prepared against the gibbon leukemia virus RDDP showed complete or partial cross reactivity in 3 of 4 cases with human RDDP preparations. The enzyme was not found in phytohemagglutinin stimulated lymphoid cells from non-leukemic donors.

Analysis of cancers for oncogenic RNA viruses. Considerable effort is being directed to search for RNA viruses which might be oncogenic for man. For the past several years sufficient numbers of observations reporting the finding of particles similar to Type C and Type B viruses in human malignancies have been made to conclude that viruses of these types may also infect man. Of the four candidate viruses isolated in the last year, two, ESP-1 and RD-114, have been well enough characterized to be considered animal in origin. ESP-1 was shown to be murine; RD-114 has been classified as an endogenous feline virus.

Intensive study of the ESP-1 virus produced in cells cultured from a human lymphoma demonstrated the presence of gs-2 and gs-3 interspecies antigens in the virions and of mouse gs-1 in the virus-producing cells. The RD-114 virus, isolated from the culture of a tumor from a fetal cat inoculated with human rhabdomyosarcoma cells also failed to meet the criteria for a human virus. The rapid resolution of the species origin of this virus is a tribute to the efforts of scientists and the availability of highly specific reagents provided by this collaborative program. This virus, nevertheless, is novel and merits further consideration. It is completely distinct from three FeLV viruses which are themselves identical except for envelope antigens. The major internal antigen is distinct from all other Type C viruses; its RDDP is more closely related to two primate viruses than viruses from lower species. Hybridization experiments, however, have shown that RD-114 is a feline tumor virus different from other known FeLV. This endogenous virus is still of particular interest because it readily infects human cells and not cat cells.

In the last year, cells growing in tissue culture from several animal species have been shown to undergo spontaneous malignant transformation and to release endogenous Type C RNA tumor viruses. As a general rule, these viruses are unable to reinfect cells of the species from which they were derived. The recent findings of other endogenous viruses released by cat cells is of particular interest because this agent like RD-114 infects human, but not cat cells. This endogenous virus is very different in its properties from other Type C viruses of the cat which appear to be transmitted

horizontally. In contrast, it remains latent in cells and seldom appears as a whole particle. These studies are important because they suggest that isolation of a tumor virus from a particular species may require cells from a different species for virus propagation. Indeed, human cells may be unsuitable for continuous replication of a human RNA tumor virus. The possibility is now being actively investigated by in-house and collaborative scientists.

Following published reports that endogenous animal RNA tumor viruses could be activated by treatment of cultured cells with halogenated uridine, the technique was successfully applied to a human tumor cell culture, and the agent, released in small amounts, is presently undergoing preliminary characterization, both intramurally and within the research contract program. The human cell derived virus was shown to have a Type C morphology, a density of 1.16 g per ml in a sucrose gradient, and RDDP activity. Upon infection of whole human embryo or human bone marrow cultures, proliferating foci of morphologically altered cells were observed, but no significant amounts of virus were shed into culture fluids.

Recently, budding and extracellular Type C virus has been observed in rhesus monkey placentas and associated embryos. The presence of Type C particles in normal baboon placentas was also observed at various stages of gestation. These findings have stimulated the search for similar particles in placentas derived from normal humans. Type C particles were detected in 4 of 6 human placentas at the junction of the syncytiotrophoblast and the basement membrane. Budding and mature particles were more readily observed in non-term placentas. Confirmation of certain biological characteristics for these particles, namely the presence of RDDP and group specific antigens, as found in RNA tumor viruses is obviously essential. It is interesting to speculate that the presence of these particles may have resulted from alterations in the physiological endocrine balance thus supporting the concept that genetic information for tumor virus synthesis and possible tumor formation is transmitted vertically.

Attempts to confirm earlier findings showing a correlation between the presence of virus-like particulates in human milk, the RDDP activity associated with fractions separated from the milk, and the breast cancer history of the milk donor have not been successful. RDDP activity was found to be no higher in preparations from milk of donors with a family history of breast cancer, or any other cancer, than in those from donors without a history of cancer. Further, no correlation was determined between the presence or absence of particulates in milk and RDDP activity. While the simultaneous test for 70S RNA and RDDP is highly dependable as an assay for RNA tumor viruses, it is not reproducible for studies with human milk. A specific inhibitor in milk which interferes with the assay, a ribonuclease, may be responsible for the inconsistent results.

Human S+L- cells were established by infection of a continuous cell line from human amnion cells with a virus stock containing MSV (FeLV) in excess of helper FeLV. A terminal focus was cloned and established into a transformed cell line demonstrating no release of infectious focus-forming virus

until superinfected with either FeLV or RD-114 virus. These superinfecting helper viruses determined the envelope-associated properties of host range and interference for the rescued MSV pseudotypes. Murine gs-1 antigen was conclusively identified in these human S+L- cells indicating that the sarcoma genome present is from MSV and not a derepressed endogenous human sarcoma genome. No Type C virus production from these human S+L- cells was demonstrable, either by electron microscopy or by release of polymerase into the culture supernatant fluid. The development of the human S+L- cell system may lead to the isolation or induction of a human Type C virus(es).

Finally, under the terms of the US-USSR Agreement on Health Cooperation, USSR scientists presented US scientists with 6 human cell lines producing candidate Type C or Type B particles. Laboratories in the Program have already confirmed that the origin of the lines is indeed human and have found particles in all 6 of the cultures studied. The cell lines are currently being propagated in larger quantities to permit the identification and characterization of the particles; their possible human origin awaits further study.

DNA VIRUSES

Characteristics. As recently as 1964, herpesviruses were generally considered to lack oncogenic activity. Prior to that time, the only evidence linking these viruses to animal neoplasias was the finding of herpes-like particles in cells of renal adenocarcinomas of the frog. The discovery of the Epstein-Barr virus (EBV) in cultured lymphoblastoid cells from patients with Burkitt's lymphoma in 1964 stimulated extensive studies to examine herpesviruses as possible etiological agents of cancer in several animal species, including man. Herpes-induced lymphoproliferative neoplasias in wild rabbits, chickens, and non-human primates have now been described. These animal models have provided valuable experimental systems to study viral oncogenesis.

Herpesviruses interact with their host cells in a productive or non-productive manner. During the productive growth cycle, the synthesis of infectious progeny is invariably accompanied by destruction of specific target cells. The non-productive cycle is very similar to that observed with other oncogenic DNA viruses. Stimulation of cellular DNA synthesis, acquisition of virus-induced antigens, incorporation of viral nucleic acid and transformation of normal cells into established lines capable of indefinite proliferation have all been described. Activation of virus synthesis in non-productively infected cells by exposure to mutagens (BUDR, IUDR) or irradiation is usually paralleled by cell death. Herpesviruses, like the RNA tumor viruses, also establish persistent covert infections. Whether latent herpes infections are the result of low-level productive or non-productive interactions has not been determined. Although transmission of the genome in non-productive cultured cells to progeny has been recorded, there is no definitive evidence that herpesviruses are vertically transmitted *in vivo* as is the case with the RNA tumor viruses. However, horizontal transmission of various herpesviruses in several animals, including man, seems to occur.

The mechanisms underlying herpes-induced oncogenesis remain obscure. The

close association of these viruses with several animal malignancies suggests, but does not prove, their involvement as etiological agents. The possibility remains that these DNA viruses are simply co-factors, passenger viruses, or derepressed latent agents whose expression is enhanced by the oncogenic process. These challenging questions are now being intensively studied.

Relationship to human cancer. The most outstanding evidence for the existence of human oncogenic herpesviruses is derived from seroepidemiological, biochemical, and biological studies, suggesting an association between various herpesviruses and specific malignant diseases of man. The Epstein-Barr virus is clearly suspect as playing some role in the genesis of Burkitt's lymphoma, nasopharyngeal carcinoma and, to a lesser extent, the sarcomatous form of Hodgkin's disease and chronic lymphocytic leukemia. In addition, EBV has been identified as a causative or contributory agent of non-malignant infectious mononucleosis in young adults.

The Epstein-Barr virus is closely associated with Burkitt's lymphoma (BL) but the absence of an experimental animal susceptible to infection with this agent has made it difficult to pinpoint its role in the induction of this disease. Therefore, although the evidence which suggests that EBV is the etiological agent of Burkitt's lymphoma is circumstantial at present, it is so strong that a causal rather than a casual relationship is suggested. The tumor is most prevalent in regions of Africa where conditions are extremely favorable for insect-vectored disease. Cases in certain areas have been shown to cluster in time and space. Most African BL patients were found to have relatively high titers of antibodies to EB viral capsid antigens (VCA) and cell membrane antigens (MA) when compared to control populations of similar age and sex distribution. Loss of anti-MA during chemotherapeutically induced remission has preceded recognition of a recurrent tumor by several months. In addition, BL patients frequently possess antibodies to EBV-induced early antigens (EA). Two antigenic components were differentiated in the EA complex by indirect immunofluorescence tests: the D or diffuse nuclear and cytoplasmic staining complex and the R or restricted cytoplasmic staining component. In the majority of BL patients, anti-R is the dominant or sole antibody to the EA complex. Changes in the titers of antibodies to EBV-related early antigens are prognostically significant. The absence or decline of anti-R antibodies in BL patients is indicative of a favorable prognosis while patients with high or rising titers to this antigen, with or without anti-D formation, are prone to recurrent, ultimately fatal relapses of this disease.

The results of studies on BL tumor biopsies have been very consistent. The majority of BL biopsy cells resemble the cultured lymphoblasts routinely derived from this tumor. No EBV particles have been detected in tumor sections examined by electron microscopy, nor, with rare exceptions, were cells containing viral capsid or early antigens observed. However, EBV-associated cell membrane antigens have been routinely detected in BL biopsy cells. Furthermore, an EBV-determined complement-fixing antigen, unrelated to VCA, MA or EA, has been measured in BL biopsy extracts. Since multiple copies of EBV DNA have been demonstrated in virtually every African BL tumor examined by molecular hybridization, these data indicate that the viral genome is largely repressed in vivo.

The establishment of continuous lymphoblastoid cell lines following exposure of lymphocytes from normal donors to EBV again suggests an oncogenic potential for this virus. Adult peripheral white cells may convert into such lines without the addition of extraneous virus, but the derived cell lines usually carry EBV. Several lymphoblastoid cell lines have been established by direct culturing of the Burkitt tumor itself. Although the antigenic profile and virus productivity patterns vary among these cell lines, EBV-homologous nucleic acid sequences are always detectable. The frequency of cells which make virus or viral products can be increased by arginine deficiency, x-irradiation, or small doses of certain synthetic inhibitors, such as IudR or BudR. Evidently, the virus genome persists in these cells, is expressed in varying degrees, and, at least in vitro, is transmitted to progeny during cell division. Thus, in some respects this virus resembles the RNA tumor viruses.

A serological association has also been described between EBV and nasopharyngeal carcinoma (NPC) that is as pronounced and consistent as that observed in Burkitt's lymphoma. Virtually all NPC patients have detectable antibody to EBV VCA and MA in high titer. Anti-VCA titers have been reported to increase with progression of the disease. In contrast with BL, membrane antigens are not detectable in NPC tumor biopsies. Nasopharyngeal patients have also been shown to possess antibody to EBV-induced early antigens. In Chinese patients, the early antigens are predominantly of the anti-D type and antibody titers to this antigen increase with progression of the disease and decrease with tumor regression. A notable exception to this pattern occurred among Caucasian NPC patients with active disease. Although the VCA titer was elevated to levels similar to those observed in non-Caucasians, the early antigen titer was much lower and there was a higher titer to the R than the D component of the early antigen complex. The differential response of Oriental and Caucasian patients to EBV may be a reflection of the extent of lymphatic involvement in these populations, since reactivity to the D component seems to be dependent on this parameter. The serological picture and the demonstration of EBV-DNA in biopsies and lymphoid cell lines derived from this tumor secure the position of this carcinoma as an EBV-associated tumor.

The relationship between EBV and Hodgkin's disease (HD) is not as clear as that observed between EBV and BL or NPC. Clustering studies strongly suggest a horizontally transmissible agent as one factor in the pathogenesis of the disease. Although increased antibody levels to EBV have been recorded in persons with HD, it is clear that a significant percentage of patients have no detectable titers. In fact, antibody titers in patients with one histological type of the disease (lymphocyte predominance) are not elevated above control values. The finding of elevated antibody levels to Varicella virus, and the occurrence of chickenpox as a complication of HD suggests that these patients may have an altered ability to control the proliferation of other herpesviruses. It is likely, also, that the high anti-EBV titers, observed in a proportion of patients, may reflect the reactivation of latent persistent EBV infections due to depression of cell-mediated immunity observed in this disease.

Although these arguments are highly suggestive, they are inclusive as far

as the etiology of BL and NPC are concerned. While there is good reason to believe that EBV is involved in the oncogenic process, its role could be that of an accessory factor or passenger virus in the tumor. High anti-EBV antibody titers are not always detected in BL patients and tumors of the Burkitt type do occur in temperate regions. No EBV DNA has yet been found in American BL tissues by the RNA-DNA hybridization technique, even though this tumor is histopathologically similar to African BL. RNA sequences homologous to that of the Rauscher murine leukemia virus RNA have recently been described in BL biopsies suggesting the possible interaction of Type C viruses in the genesis of this disease. If EBV acts to prime the cell for neoplastic change, it is possible that one or more combinations of environmental and host factors interact to promote oncogenesis. Thus, the association of EBV infection with more than one disease becomes more plausible. Should this virus prove to be a necessary co-factor in any or all diseases with which it appears to be associated, control of infection would be of paramount importance.

An expanding body of evidence has strengthened the causal relationship between herpes simplex virus type 2 (HSV-2) and carcinoma of the human uterine cervix, the second most common malignancy in women in the United States. Genital HSV-2 infection has been found to be a venereally transmitted disease probably second in frequency only to gonorrhea. HSV-2 infection may be more common in males than females, since this virus can be isolated from urogenital specimens taken from a high percentage of asymptomatic males. These observations indicate that there is a sufficient incidence of genital herpes infections to account for every case of cervical anaplasia, although the epidemiological data is too limited to determine the risk of developing cancer in women infected with this virus.

The frequency of antibody to HSV-2 is generally greater in women with cervical neoplasia than in normal controls matched for promiscuity and other risk factors. In general, the differences between cervical carcinoma and control groups are more impressive in Negro than in Caucasian women, and in the U.S. than in several foreign countries, including Israel and Columbia. Similarly, exfoliative cytology studies have shown more evidence of herpetic cytopathology in patients with cervical anaplasia than in matched controls.

Preliminary molecular hybridization studies performed on one tumor indicate that cervical carcinomas contain less than one HSV-2 genome per tumor cell. Neither virus-specific antigens nor virions and herpes-induced cytoplasmic changes have been detected in biopsied neoplastic cells. However, tumor cells on the surface of neoplastic lesions, as well as exfoliated tumor cells, possess HSV-2 antigens detected by immunofluorescent staining. Complete or incomplete virus particles were absent in these anaplastic cells examined by electron microscopy. However, cytological changes associated with the synthesis of herpesvirus antigens, and absent from biopsied cells, were observed. Cultured cells, established from a biopsy of carcinoma *in situ*, failed to show evidence of viral antigens and complete or incomplete virions unless the cells were exposed to media of high pH. This *in vitro* data suggests that cervical cancer cells harbor the complete HSV-2 genome in a latent or repressed state, and virus expression occurs following exposure of the tumor cells to conditions of stress.

The oncogenic potential of herpes simplex virus has been demonstrated in vitro. Ultraviolet irradiated HSV-2 transformed cultured hamster embryo fibroblasts and these transformed cells were oncogenic in newborn hamsters. HSV-2 antigens were detected in the cytoplasm of 5-to-20% of the cells transformed in vitro. Concurrently, virus-neutralizing and membrane-reactive antibodies appeared in the sera of the tumor-bearing animals, suggesting the presence of genetic information derived from HSV in the tumor. More recently, two additional human herpesviruses, HSV-1 and cytomegalovirus, have been shown to transform cultured hamster embryo fibroblasts, suggesting that the in vitro oncogenicity of these viruses is more widespread than originally suspected.

These findings strengthen the association of HSV-2 with cervical carcinoma but still do not prove a causal relationship between the virus and this malignant disease. Studies to determine if this virus is the sole etiological agent, or an essential co-carcinogen in the induction of this disease will be continued. If genital herpes infection can be shown to be a factor in the development of cervical neoplasia, appropriate control measures may be developed to reduce the incidence of this human cancer.

TREATMENT AND CONTROL

New approaches to the treatment and control of virus-induced cancers are provided by the study of (1) the biochemical pathways of viral replication and/or cell transformation, and (2) the immune responses to tumor viruses and/or tumor virus expressions. A rational approach to tumor therapy may not depend on the isolation of a bonafide human tumor virus. Indeed, existing model systems have provided sufficient information which can be applied to the control of human cancers. For example, chemical inhibitors (anti-enzymes, gene repressors) which act on specific stages of viral replication or stimulation or host immune mechanisms (vaccines) to virus or virus-mediated, tumor-specific antigens may prevent or control oncogenesis.

The systematic screening of rifamycin derivatives which block the RDDP activity of RNA tumor viruses may help in the development of inhibitors that possess properties for the control of specific gene expression. These drugs interact with the enzyme, not with the template, non-competitively and inhibit cell transformation. Some fluoranthrene di-substituted cationic derivatives and analogs are also strong inhibitors of RDDP and of cell transformation. Here the mechanism of action involves specific binding to the template.

Many compounds have been tested for their capacity to inhibit the reproduction of murine leukemia and sarcoma viruses in vitro. A few of the most active ones also showed inhibitory activity against these viruses in vivo. Among the chemicals tested for non-specific stimulation of the RES, imidazolethiazole, pyran copolymer, and Tilaron proved effective in increasing survival of mice in induced remission. Presumably their activity is associated with the capability to stimulate non-specific cellular immune responses.

A DNA binding protein in non-virus producing AKR mouse cells has been

isolated and found to have many of the properties of known repressor proteins described in bacterial virus systems. This finding could lead to understanding the mechanisms by which mammalian cells control gene expressions and how genetic mechanisms regulate RNA tumor virus expression. Further research will be aimed at delineation of the effects of proteins with repressor-like properties on the oncogenic activity of viruses.

Formalin killed vaccines prepared from murine leukemia viruses have produced significant reductions in tumor incidences in mice given potent chemical carcinogens. Because of the reported immunological and biochemical relatedness between MuLV (Rauscher) and human leukemic cells, terminal cancer patients were immunized with inactivated RLV. Evidence for humoral and/or cell-mediated immunity against this virus was found in more than one-half of the immunized patients. Whether this treatment produces beneficial effects for the patients remains to be determined.

The immunogenicity of virus-induced tumor cells has been shown to be increased by infection with influenza virus. Tumor transplantation antigens on these cells can be isolated in cell-free form. This material protected mice against challenge with tumor transplants causing either suppression or complete regression. These studies have obvious implications for immunotherapy of human tumors.

PROGRESS HIGHLIGHTS
(Not considered in Scientific Activities narrative).

Forty-one of 45 human milk specimens tested exhibited RNAse activity; this activity migrates with particles in a centrifugal field. The ability to detect viral RDDP by the simultaneous test is inversely proportional to the RNAse present in the specimens.

A virus resembling M-PMV in ultrastructural morphology has been isolated from the breast tissue of three normal lactating rhesus females. The new isolates were found to be antigenically identical to the prototype M-PMV and exhibited many other properties generally associated with RNA tumor viruses.

Primary human fetal cultures inoculated with material from human milk and from human breast cancers showed evidence of viral proliferation based on the 70S RNA-RDDP assay.

Effective immunotherapeutic methods for skin cancer have been successfully applied to patients with breast cancer and hemangiosarcoma. The type of treatment employs application of skin-sensitizers in sub-threshold doses to neoplastic skin lesions of patients previously sensitized to these substances.

A human breast tumor cell line, HBT-3, has been isolated and characterized biologically. Although virus particles were not seen even after treatment with inducing agents such as IdU, column chromatography using dT-cellulose demonstrated RDDP activity.

MuLV 36S RNA subunits are composed of two chromatographically distinct fractions consisting of poly A subunits representing approximately 60% of the viral RNA and non-poly A subunits comprising 40% of the total RNA. These findings are important because they provide characterization of the genome of RNA tumor viruses.

Mouse cells which are permissive for mouse leukemia virus (3T3) were fused with nonpermissive human cells (W1-38). Essentially all of the heterokaryons examined were nonpermissive for virus expression, indicating that the nonpermissive state is dominant and implying that the nonpermissive cell is able to repress virus synthesis specifically.

RNA of RSV harvested at 3 minute intervals contains mostly 30-40S RNA. Upon incubation at 40°C the 30-40S RNA is converted to 60-70S RNA in the virus. This suggests that 30-40S is a precursor, and not a breakdown product, of 60-70S RNA. Transforming avian sarcoma viruses contain mostly a subunits while nontransforming clones of these viruses contain only b subunits.

To demonstrate that unique genetic information was contained in the human leukemic cell, DNA products of the endogenous reaction between the leukemic cell RNA and the RDDP were exhaustively hybridized with normal human lymphocyte DNA to remove any nucleotide sequences found in normal cells. The residual probe retained sequences that hybridized with leukemic cell DNA but not with normal cell DNA. This finding indicates that nucleotide sequences in leukemic cells differ from those in normal cells.

Normal embryo rat cells treated with BrdU followed shortly thereafter by 3-methylcholanthrene were readily transformed into tumor cells, whereas untreated cells were not. The transformed cells were shown to contain rat RNA tumor virus gs antigen when transplanted into newborn rats. The precise time relationships between virus infection and 3MC induction of transformation is also important. Transformation of cells only occurred when RNA viruses were replicating at significantly detectable levels prior to treatment with 3MC. Virus added at the same time or shortly after 3MC treatment failed to result in neoplastic cell transformation.

Seven thousand wild mice, trapped at several Los Angeles sites, were observed throughout their natural life. Virtually all of the mice were negative for infectious Type C RNA tumor viruses and during their lifetime they developed only 45 tumors. A high percentage of animals developing cancer had RNA tumor virus gs antigen in their tumors and spleens. These studies were significant because they showed that wild mice having little or no virus expression also had very little natural cancer.

Human tumor cells but not normal human cells readily grow in brains of mice given anti-thymocytic serum. Such tumors and the cells derived from them often contain Type C RNA viruses which appear to have properties conferred by both the mouse and human cells. This technique for isolating presumably recombinant human and animal viruses provides an important new method for isolating viruses from human tumor material.

Growth of MSV-induced tumors was inhibited by serum from mice immunized with allogeneic tumor cells and from mice whose tumors were in remission. However, serum from mice with progressively growing tumors enhanced the growth of tumor cell challenge. The degree of effectiveness in preventing tumor outgrowth was related to cell membrane and virus neutralizing antibody levels in donor sera.

Cell strains derived from leukemic patients are generally more susceptible to SV40 transformation than those derived from normal individuals. This extends the previous observation that cells from patients with Fanconi's anemia or Down's syndrome, have increased SV40 transformation susceptibility.

Herpesvirus saimiri (HVS) infections in marmosets and owl monkeys terminate in fatal lymphoma or leukemia. Early antigens were found to be produced in HVS infected cells, mimicking the observations made in human cells infected with EBV. The squirrel monkey shows only a transient antibody response to early antigens and no pathology following primary infection by HVS. The owl monkey and marmoset, however, develop antibodies to this antigen only when there is evidence of abnormal lymphoproliferation. This is a striking parallel to the relationship between antibodies to early EBV-induced antigen and the course of Burkitt's lymphoma in man.

Highly labile non-virion early antigens from HSV-infected cultured cells and complement fixing antibody directed against these antigens have been prepared. Sera from patients with confirmed HSV-2 infections had no detectable levels of HSV-2 non-virion antibodies. However, sera from patients with cervical cancer contained antibodies to this early antigen, while none was detected in the sera of matched controls. The presence of complement fixing antibody for HSV non-virion antigens has also been shown in sera of patients with tumors of the prostate, bladder and kidney. Finally, antisera to HSV non-virion antigens reacted with separated soluble membrane antigens extracted from lip and cervical carcinomas, but not from similar extracts from normal vaginal tissue or intestinal carcinoma. While this information is useful for evaluating the relationship of herpes simplex viruses to various types of human tumors, it may also provide a sensitive diagnostic tool for the early detection of cancer in man.

The HSV-2 cervical cancer hypothesis would be strengthened if it could be demonstrated that genital infection with this virus resulted in cervical anaplasia in a non-human primate model. For this purpose, cebus monkeys have been inoculated intra-vaginally with HSV-2 or virus-free control material. Virus inoculated animals showed clinical herpetic lesions and evidence of infection as determined by virological, cytological, or serological determination, although not all animals responded equally to primary HSV-2 infection. Cross-infection by venereal transmission from infected females to males and occasional viral transmission between female cage mates, possibly by way of a male, has been observed. Cytological data obtained showed no evidence of cervical anaplasia to date. Test animals will be held for several years and closely monitored for evidence of cervical cancer.

Studies in avian systems have demonstrated an apparent necessity for the interaction between Marek's disease herpesvirus (MDHV) and an RNA leukosis virus (RAV-2) to produce classical Marek's disease. Cell-free MDHV did not produce classical Marek's disease in leukosis-free chickens after exposure to infection unless the birds were coinfected with RAV-2. The production of RAV-2 antigens and RAV-2 viral RNA was found to be considerably enhanced by MDHV infection when compared to infection with RAV-2 alone. Cells of human lymphomas, including Burkitt's tumor, contain 70S RNA associated with an RNA-dependent DNA polymerase in a particle having a density between 1.16 and 1.19 g/ml. The particles thus identified in cells from both of these malignancies have at least three of the biochemical and physical features of the RNA tumor viruses. Tritiated DNA, synthesized from the 70S RNA template isolated from Burkitt tumors, hybridized to Rauscher murine leukemia virus RNA but not to RNA extracted from avian myeloblastosis virus. These data suggest that oncogenic expression in human Burkitt's lymphoma and avian Marek's disease may require the interaction of two unrelated viruses acting together with specific genetic or environmental conditions.

D. Projections:

Last year, the Viral Oncology Area set forth a long range research plan for the identification and control of virus-induced cancers of man. Many studies within the following broad categories have already been implemented and will be expanded in the coming year:

1. Virus (or virus-expression) - tumor relationships

   a. Model Studies. Studies on animal, RNA and DNA, tumor viruses, known to cause malignancies in several mammalian species, will be continued. The results of these studies have already provided important information about tumor viruses that is applicable to the isolation and identification of human agents. Special emphasis will be given to determine the characteristics of several newly-isolated primate viruses, since these agents may provide the best probes for detection of Type C virus information in human cells. This work will remain an integral part of the Program.

   b. Human Studies. Efforts to identify viruses or detect virus expression in human tumors have been underway for some time. The Program will continue to increase its activities in the search for viruses which induce malignancies of man.

      (1) To identify and isolate candidate viruses or subviral products in leukemias, lymphomas, sarcomas, and carcinomas.

      (2) To identify and isolate candidate viruses or subviral products in lung, colon, and other carcinomas.

      (3) To develop methods for the detection of high cancer risk groups, i.e. individual susceptibility or predisposition

to transformation by human viruses.

(4) To extend existing and develop new methods to induce tumor virus or virus expression in "normal" cells.

(5) To develop suitable reagents and to improve existing immunological and biochemical methods for mass diagnostic screening for candidate viruses.

(6) To characterize, biologically and biochemically, presumptive human virus isolates.

(7) To increase emphasis on understanding the relationship of environmental agents (e.g. chemical carcinogens) as co-factors in viral carcinogenesis.

2. Molecular Studies

Major progress in the understanding of the molecular pathways of tumor virus replication has been made within the past year. Such advances have already provided the basis for new, extremely sensitive methods for the detection of oncogenic viruses or virus expression. It is now possible to characterize agents detected in specimens of human cancer patients in terms of their content of high molecular weight RNA and of RDDP. Specific hybridization procedures already provide a method for further investigation of host-cell-virus relationships which have been extended into the study of human cancers. Preliminary results offer strong supportive evidence that certain human tumor cells contain genetic information related to that found in known oncogenic viruses.

a. Basic Studies

The Program will continue to broaden its activities for detecting, identifying, and characterizing the spectrum of enzymes and their products required by tumor viruses for replication and/or transformation.

b. Applied Studies

As knowledge of the fundamental molecular events in virus-cell interaction increases, the Program will continue to apply this information to the study of human cancer as follows:

(1) To identify and characterize similar enzymes or enzymatic activities within normal and malignant human cells.

(2) To develop highly sensitive methods for the detection of virus or virus activity in human cells.

(3) To develop a rational basis for therapy or prevention by exploring various approaches to blocking of viral

replication and/or tumorigenesis at the cellular and subcellular levels. The therapy could be directed at any or all of the stages of cell transformation beginning with cell infection by a tumor virus.

Ultimately these approaches will require an intensified program to develop drugs, anti-enzymes, gene repressors, or inhibitors effective at the molecular level.

3. Immunological Studies

Immunologic research has provided extremely sensitive techniques for detection and characterization of tumor viruses, viral antigens, and changes in surface membranes of tumor cells. These studies have also contributed to an understanding of the role of immunological mechanisms in host-tumor and host-virus interactions which provide a rational approach to the prevention and treatment of cancer.

a. Basic Studies. Investigations of selected model systems, representing tumors induced by Type C, Type B, and Herpestype viruses, will be extended to further identify, characterize, and determine the viruses, viral antigens, and membrane antigens of tumor cells. The studies include development and application of improved techniques to detect cellular alterations induced by tumor viruses alone or as the result of interaction with other environmental agents. Research on spontaneous or naturally occurring tumors in model systems relevant to human cancer will be continued as a basis for a rational approach to prevention and treatment.

(1) To study cellular and humoral immune mechanisms and to determine their relative significance in host recognition of and response to virus-induced tumors and/or tumor viruses.

(2) To develop methods to enhance host response to virus-induced tumors or tumor virus antigens.

b. Applied Studies. As basic research provides the framework for identification and characterization of viruses, viral antigens, and virus-induced cell membrane alterations in human cancers, immunological methods will be applied:

(1) To relate candidate human viruses to known oncogenic agents.

(2) To identify and characterize intra- and inter-species viral antigens which are present in known mammalian tumors, as probes for detecting human tumor viruses or viral antigens.

(3) To determine the presence of cross-reacting antigens implying viral causation in various human tumors.

(4) To launch large-scale seroepidemiological surveys which will define populations at high risk to virus-induced cancers.

Clinical studies will be directed toward understanding and manipulation of immune mechanisms in human cancer as a basis for:

(1) Development of vaccines from identified and fully characterized human tumor viruses.

(2) Determination of the role of host immune responses in virus-induced tumor recognition and rejection.

(3) Application of (1) and (2) in the prevention and control of human cancer.

4. Test Systems and Resources

a. Test Systems. In vitro and in vivo (animal) test systems will be carefully selected to evaluate the work outlined in the previous research areas:

(1) To determine the oncogenic potential of candidate human viruses;

(2) To develop bioassay systems for testing viral, and viral/chemical carcinogens;

(3) To begin viral vaccine (conventional or other) testing and immunization programs;

(4) To begin viral therapy testing programs;

(5) To explore special animal tumor systems, especially in primate species particularly relevant to human cancer;

(6) To develop and maintain well-characterized cell culture lines and animal stocks (small mammalian and primate species).

Many of these systems are being developed at the National Cancer Institute's Frederick Cancer Research Center.

b. Resources. Since research efforts undergo continual change in emphasis and scope as new leads emerge, a variety of resources will have to be developed, maintained, and coordinated.

(1) Human Resources - collection and storage of serum and tissue specimens; integration of data on clinical records, storage and distribution; computerization of specimen collection.

(2) Animal Models - maintenance of various mammalian animal

colonies for basic research and special studies;

(3) Reagent production - large scale production of animal tumor viruses for basic research; production of standardized lots of purified viruses; and production of high quality diagnostic reagents;

(4) Candidate Human Virus Production - intensive developmental research effort to isolate and produce human viruses;

(5) Biohazards Control and Containment - controlled environment facilities are required for research on known oncogenic viruses and candidate human tumor viruses as well as for maintaining animal colonies which are protected from extraneous infections.

FUNDING LEVELS OF CONTRACTS WITHIN SEGMENTS
(By Type of Institution)
Viral Oncology Area, Division of Cancer Cause and Prevention, NCI

Segment	Profit		Educational		Non-Profit		Other*		Total	
	No.	Amount**	No.	Amount	No.	Amount	No.	Amount	No.	Amount
Program Management	1	1,708	1	25	0	0	1	708	3	$2,441
Developmental Research	5	2,213	13	4,283	5	1,086	0	0	23	$7,582
Solid Tumor Virus	3	6,682	8	5,087	5	1,210	1	206	17	$13,185
Immunology	2	169	9	1,119	4	447	3	1,081	18	$2,816
Tumor Virus Detection	2	2,555	9	1,246	2	113	1	800	14	$4,714
Resources and Logistics	11	8,059	10	1,380	10	464	1	63	32	$9,966
Breast Cancer Virus Studies	2	508	5	538	3	625	0	0	10	$1,671
Biohazards and Environ. Cont.	1	200	3	356	1	57	1	115	6	$ 728
TOTAL	27	22,094	58	14,034	30	4,002	8	2,973	123	$43,103

* Includes interagency agreements.
**Dollars in Thousands.

SPECIAL VIRUS CANCER PROGRAM

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July 1, 1972 - April 1, 1973

Part A: Published Papers

Part B: Papers In Press

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NATIONAL CANCER INSTITUTE - FREDERICK CANCER RESEARCH CENTER

The President's announcement on October 18, 1971 that the scientific laboratories at Fort Detrick would be converted for use in the national fight against cancer set into motion a series of events that resulted in the establishment of the Frederick Cancer Research Center (FCRC) for research on the cause, prevention, and treatment of cancer. The Center is administered as a government-owned/contractor-operated facility with Litton Bionetics, Inc. (LBI) as the prime contractor. Scientific activities at FCRC are supported by four major organizations: Viral Oncology and Chemical Carcinogenesis (DCCP) each of which supports approximately 40 percent of the effort, and DCBD and DCT, which contribute approximately 10 percent each. The current in-house staff for NCI administration of operations consists of a Project Officer, Dr. William W. Payne, who acts as the overall Scientific Coordinator; a Deputy Scientific Coordinator, Mr. Orley Bourland, Jr., who is Assistant Project Officer for Engineering, Renovation, and Maintenance; a Scientific Coordinator for Viral Oncology (SCVO), Dr. Henry J. Hearn; an Administrative Contracting Officer, Mr. Ronald H. Defelice, and four support personnel including three secretaries. FCRC began operations on June 26, 1972 with an LBI personnel complement of 21. The present staff numbers over 315 personnel many of whom are skilled in virology, biochemistry, immunology, veterinary medicine and other related disciplines required for the understanding and enlargement of knowledge in cancer research.

Program direction for Viral Oncology research at FCRC is derived from the Office of the Associate Scientific Director for Viral Oncology with the assistance of the FCRC Viral Oncology Advisory Board which has the SCVO as its chairman. The major function of the Board is to review all Viral Oncology projects at FCRC on a continuing basis for the purpose of attaining and maintaining program excellence and to insure rapid response of the FCRC project operations to needs related to the overall NCI program objectives. The FCRC Viral Oncology program is implemented through the office of the SCVO which is physically located at Fort Detrick. Program objectives are further served through the close cooperation between the Offices of the SCVO and Program Resources and Logistics. This insures the efficient entry of virus and culture materials into working programs and later dispersal of product.

For the first year of operations, Viral Oncology completely supported 4 of the 12 original contracted tasks performed at FCRC and partially supported an additional 3 tasks. In addition, intramural projects were initiated including the research conducted by Dr. Sabin and Dr. Tarro on herpesvirus associated nonviral antigens, studies on neoplastic cell structure by Dr. Victor Zeve, research on cell surface antigens in conjunction with Dr. Boone, research on the use of immunostimulatory and chemotherapeutic compounds against viruses with Dr. Chirigos, work on Herpesvirus saimiri and related viruses by Dr. Ablashi and an additional project on the isolation of a possible human cancer-related virus.

The tasks or projects that are completely funded by Viral Oncology are defined as follows:

Task 1, Virus Production, has as its objective the production of 150 liters of high quality virus per week. Rauscher Leukemia Virus (RLV) was selected as the starting virus primarily for training the staff and for developing techniques necessary for the efficient utilization of materials and equipment for cell culture growth and virus purification and concentration. The goal of 150 liters per week was met in March; plans already call for the distribution of all of the product for use at NCI, FCRC and in other NCI-associated programs. Plans were also implemented to produce a single 50 liter quantity of herpesvirus to fulfill a program request and to introduce a second virus into the program without discontinuing effort with RLV.

Task 2, Developmental Research, has as its objectives the development of techniques for production of those oncogenic or suspected oncogenic viruses for which no established protocols exist or for which existing protocols have failed to consistently provide a suitable product. Thus far, work has been initiated on the growth of (i) mouse mammary tumor virus, selected as a B-type model, (ii) Epstein-Barr virus, and (iii) gibbon ape virus, as a C-type model. Starting virus cultures, a large variety of cell cultures, and animals for sources of tumor material were acquired for study and appropriate assay techniques were perfected. Task 2 also was responsible for developing, perfecting and applying a number of the quality control techniques for Task 1 products including electron microscopy particle counting, in vivo and in vitro infectivity, and tests for contamination.

Task 3, Preparation of Diagnostic and Test Antigens, covers the preparation of special viral diagnostic and test reagents from selected avian, murine, feline and/or other viruses based on procedures and techniques already established but performed by very few laboratories. In preparation for performing these objectives, which may require developmental research to either improve established techniques or improvise new ones, selected professional and technical personnel were assembled and training programs initiated. Using virus material produced in Task 1, work began on the isolation and purification of gs antigens and viral polymerase and on the preparation of antisera to these substances. It is expected that small quantities of product will soon be available for distribution.

Task 3 also has responsibility for a number of quality control tests on product from the virus production area. These tests include assays for reverse transcriptase, 70S RNA, total RNA, total protein, gs antigens, complement fixation and immunofluorescence.

In Task 5, NCI Office of Biohazards and Environmental Control, effort was initiated to provide and maintain laboratories, animal holding and administrative space for use by the NCI Office of Biohazards and Environmental

Control (OB&EC) in Building 550. Materials and supplies, facility renovation, equipment and travel were provided the OB&EC through Task 5. Also, a technical support staff of six scientific and technical employees was provided.

The Task 5 group was organized to evaluate potential hazards associated with research activities in viral oncology and chemical carcinogenesis and to develop adequate protective measures. A number of applied research programs are being carried out. Each of these programs is a cooperative undertaking between Tasks 4 and 5, and one or more workers from Task 4 are identified with each of the listed research programs.

Those tasks that are partially funded by Viral Oncology are as follows:

Task 4, Environmental Control, efforts are directed towards establishing a safe working environment in FCRC operations, with special emphasis directed towards minimizing human exposure to biological agents, hazardous chemicals and radioactive materials. An important secondary consideration in this safety program is the protection of the validity of experimental results through the containment and isolation of work activities. Environmental Control activities have been concerned with all phases of FCRC activities, including engineering, maintenance and the scientific tasks. Safety activities have been directed towards developing good safety attitudes among all FCRC employees. Total current personnel complement in this task is ten. Viral Oncology supports approximately 35 percent of this effort.

Task 6, Advanced Systems Laboratory, covers the establishment, maintenance, and operation of an advanced systems laboratory for research in viral oncology, chemical carcinogenesis, chemotherapy, and other aspects of cancer. This laboratory is to contain the most modern equipment and safety features available. Laboratory modules shall be specifically designed for electron microscopy, tissue culture, virological, biochemical, and immunological studies. Containment-type holding rooms for small laboratory animals shall be provided and maintained by the Contractor as a part of this project. This facility will be utilized by NCI intramural research personnel and scientists from throughout the world who will be invited by NCI to work in areas of major breakthroughs in cancer research. The Contractor will be expected to provide support services and a technical staff for this facility under the direction of the Project Officer. Thus far, work carried out in this task includes that of Dr. Sabin and Dr. Tarro, Dr. Zeve's project in electron microscopy, and effort directed toward the isolation of a suspected human cancer virus, as discussed above. Viral Oncology is responsible for 40 percent of this task effort. Future work will depend upon recommendations of the recently formed Ad Hoc Committee acting in an advisory capacity for the FCRC program.

In Task 12, Animal Breeding, the Contractor is responsible for the operation of the Animal Farm, in accordance to specific directions and subject to amendment by the Project Officer, for the breeding of rodents to meet, first, the needs of the research programs at FCRC and for shipment to other NCI operations as production permits. Additional species, strains, and hybrids shall be added as directed by the Project Officer. Viral Oncology supports approximately 25 percent of this effort.

The NCI FCRC program for next year is expected to undergo some modification. Administratively, the word "task" will be replaced by the word "project" and all technical and research efforts at FCRC will be grouped into various areas rather than remain as autonomous tasks as originally conceived. The Viral Oncology area will include Projects 1, 2, and 3 (formerly Tasks 1, 2, and 3) plus a new Project 13 which was created to provide an efficient administrative mechanism to cover the performance of basic research at FCRC in direct support of existing programs pertaining to the role of virus in the etiology of human neoplasms. The research objectives of Project 13, determined by NCI staff, will reflect those areas of intramural scientific investigation that require special emphasis and/or expanded activities and that can maximally utilize FCRC facilities. Activities in this project are separate from those in Project 6, the Advanced Systems Laboratory, although some collaborative effort between the two projects is planned in the area of viral immunology.

A few additional professional and technical staff personnel may be required for Tasks 1 through 3 in the second year but the objectives will remain essentially as they were during the first year. Task 5, however, may undergo considerable expansion with the formation of a new Project 5b to accommodate the proposed transfer of work by Dr. Hellman and his staff to FCRC. In addition, Dr. Ablashi's efforts will be transferred in toto to FCRC as part of Project 13. The establishment of these two new intramural programs at FCRC will require expansion of Viral Oncology activities into Building 538. Plans are underway to accomplish this by mid-autumn.

The Office of Biohazard and Environmental Control conducts research pertaining to the physiological and environmental factors that alter the hosts susceptibility and response to oncogenic and non-oncogenic viral infections. It is also responsible for the development and implementation of the environmental control and laboratory biological safety program for the Special Virus Cancer Program.

The Biohazard Section conducts research to elucidate the mechanisms involved in host immunocompetence and the consequence of this on oncogenesis. Furthermore, understanding is being sought of the biochemical factors that lead to the induction of malignancy and how best to detect and modify these inductive factors. Physiological imbalances, induced by controlled stress are being examined in in vivo and in vitro systems in order to assess the host response.

We and the Southwest Foundation for Research and Education (collaborative contractor laboratory) have demonstrated the presences of C-type particles in normal primate reproductive tissue, including man. The significance of this observation is being determined at our two laboratories. It is interesting that we had previously demonstrated that the virogenic markers, group specific antigens (g.s.) and RNA directed DNA polymerase, can be activated by alteration of the physiological endocrine balance. This major finding lends strong in vivo support to the concept that genetic information for tumor virus synthesis and possible tumor formation is transmitted vertically and can be activated by definable physiological mechanism. The potential for such activation has also been noted by us to be present in certain pesticides. Other consequences of these observations in relation to perhaps normal physiological requirements of C-type particles is also being evaluated.

The Environmental Control Section develops and recommends equipment, facilities, procedures and standards for the proper handling of potentially biohazardous materials and disseminates this information to the scientific community through publications, training programs, site visits and consultation activities. Procedures, equipment and facilities are evaluated to identify inherent safety difficiencies and to provide basic information for developing corrective measures. The Section operates the NCI virus containment facility and prototype containment laboratories.

The Section conducts applied research to evaluate potential hazards associated with research activities in viral and other areas of oncology and to develop adequate protective measures. This program is performed at the Frederick Cancer Research Center.

Minimum Standards for Biological Safety and Environmental Control have been issued to all contractors within the SVCP. Consultation and training activities have been expanded to assist contractors in implementing these standards.

This office co-sponsored, along with the National Science Foundation and the American Cancer Society, a three day working symposia on "Biohazards in Virus and Cell Research". Proceedings from this symposia, in which over 100 senior research directors participated, is being prepared for publication in the Cold Spring Harbor Press.

Serial No. NCI-4800

1. Office of the Associate Scientific Director, Viral Oncology, Division of Cancer Cause and Prevention
2. Office of Biohazard and Environmental Control, Biohazard Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Development of Facilities and Procedures to Reduce Biohazardous Exposures

Previous Serial Number: None

Principal Investigator: Mr. Manuel S. Barbeito

Other Investigators: Dr. W. Emmett Barkley

Cooperating Units: Litton Bionetics, Inc.

Man Years:

Total:	1.2
Professional:	1.2
Other:	0

Project Description

Objectives:

The objectives of this project are to: (1) develop an animal room containment system to prevent intercage cross contamination and to provide a safe work environment for the investigator to perform routine animal inoculations, examinations and animal care procedures, (2) to identify sources and mechanisms of tissue culture contamination and to develop tissue culture contamination control methods, and (3) to design, construct and evaluate an automated animal waste handling system.

Methods Employed:

The design of the animal room system will incorporate in-ward air flow cubicles for animal containment, high volume airflow recirculation and high efficiency filtration of the recirculated air. The animal room will be designed for housing several different types of research animals including primates. The room will be of modular design to permit installation within existing facilities. Room performance will be determined by studying the

potential of the room system to prevent the cross contamination of test animals with a horizontally transmissible virus.

A model tissue culture system will be established and methods in which the culture can be contaminated by routine manipulations within the laboratory will be evaluated. The total laboratory environment will be contaminated to determine the contribution of this source to contamination. Air and surface sampling techniques will be employed.

An automated conveyer system for safe transfer of contaminated animal bedding will be designed and constructed. The capability of this waste transfer system to contain a tracer biological contaminant will be evaluated.

Major Findings:

A prototype animal containment test room has been designed, fabricated and installed at the Frederick Cancer Research Center. Performance tests are being performed to demonstrate compliance with design criteria and specifications.

The tissue culture incubator has been identified as a major reservoir which can harbour a variety of potential bacterial contaminants.

An invitation for bid has been solicited by Litton Bionetics, Inc. for the design and construction of the automated animal waste conveyer system.

Significance to Biomedical Research and the Program of the Institute:

Results from these studies will improve the quality and safety of laboratories engaged in cancer virus research. The animal containment room project and the work handling project will provide basic data which can be used to establish practical design and operating procedures for the safe handling of research animals and their associated wastes. The elucidation of tissue culture contamination mechanisms will provide the basis for improving quality control procedures in the virus laboratory.

Proposed Course:

A Newcastle Disease Virus challenge tests will be employed to measure the performance of the animal containment system. Airflow parameters will be modified and tested to establish the most practical design criteria.

The waste conveyer system will be constructed and evaluated.

Additional sources and reservoirs of laboratory contamination will be identified and their potential for affecting tissue cultures will be determined.

Honors and Awards:

None

Publications:

None

Serial No. NCI - 4803

1. Office of the Associate Scientific Director, Viral Oncology
2. Division of Cancer Cause and Prevention
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: A. Lung Cancer in Sheep as a Spontaneous, Natural, Model System

B. Tumor Regression and Relapse in MSV(M) Inoculated Mice and Rats

Previous Serial Number: None

Principal Investigator: Dr. Kalman Perk

Other Investigators: None

Cooperating Units: Inside NIH
Dr. Adi Gazdar, VLLB, NCI
Mr. Edward Russell, VLLB, NCI
Mr. Harvie Sims, VLLB, NCI
Dr. John Pearson, VBB, NCI
Dr. Michael Chirigos, VBB, NCI

Outside NIH
Dr. Kirby Smith, St. Jude Children's Hospital, Memphis Tennessee
Dr. Israel Hod, Hebrew University, Rehovot, Isarel
Dr. Jeffrey Schlom, Columbia University, New York, New York

Man Years:

Total	.75
Professional:	.75
Others:	.00

Project Description

Objectives:

A. The objectives of this project are to define and characterize the natural history, etiology, structure-ultrastructure genesis, host physiology in the

development, pathogenesis and biochemistry of this neoplasm - as a possible lung-cancer model in comparative oncology.

B. The objectives of this study are to examine and correlate the rate of regression and tumor relapse to the age and strain of mice and rats and to the dose of virus inoculum - as a possible model for tumor regression studies.

Methods Employed:

A. Histochemical, immunological, biochemical, tissue culture and electron-microscopic methods are employed.

B. Histochemical, tissue culture and electronmicroscopic methods are employed.

Major Findings:

A. Ultrastructural studies of sheep pulmonary carcinoma showed that the neoplastic cells (in the lungs or in different metastatic loci) were derived from B-type alreolar cell, which is one of the two types of lung alveolar epithelial cells.

Electron microscopic examinations of thin sections of the ovine tumor revealed the occurrence of virus particles morphologically similar to the C-type virus. Further, it was demonstrated that the tumors contain RNA directed DNA polyherase and high molecular weight RNA.

It was also found, that the tumor bearing animals had consistent organ and blood changes (other than metastasis) e.g. lymphnode plasma cell hyperplasia, reticulocytosis, and extreme hyperglobulinemia. Ultracentrifugal analysis and immunoelectrophoresis indicated that the hyperglobulinemia occurred mainly in the 7SIgG fraction.

B. The MSV(M) virus preparations induced sarcomas in all animals; definite strain, age or virus dose dependent variations in the pathogenic spectrum were found. Accordingly four tumor 'stages' may develop: (a) progressive lethal, (b) lethal but long persistant, (c) complete tumor regression and (d) tumor reoccurrence after complete regression. Virus recovery was highest from the progressively growing tumors and in the reoccurred neoplasms while in the long persistant tumors or in tissue at the site of tumor regression, little or no virus could be detected.

Significance to Biomedical Research and the Program of the Institute:

A. Pulmonary carcinoma of Awassin sheep is a spontaneous, malignant disease, which appears usually in multiple cases in a given herd. The ultrastructure of this metastasizing lung tumor strongly resembles that of human alveolar carcinoma.

Perhaps most intriguing in this spontaneous sheep neoplasm, is the detection of virus particles in thin sections by electronmicroscopy and the demonstration

of RNA directed DNA polymerage and high molecular weight RNA in this naturally occurring tumor. Although the etiologic and biologic significance of these particles is still undetermined, additional vistas to the cause of lung cancer become open. Thus, this natural occurring tumor system may provide an appropriate model for studies in comparative oncology.

Proposed Course:

A. Efforts will be concentrated on (1) tissue culture system in an attempt to isolate and propagate the causative organism; (2) hybridization of tumor cell RNA with DNA products of ₹ RLV, MMTV, Visna, Maedi, and DNA from human lung tumors; (3) in vivo studies at a later stage.

B. Further characterization of the host response in the found four tumor growth pattern.

Publications:

Perk, K., Hod, I., Nobel, T. A. and Klopher, U.: Some pathogenetic aspects of ovine pulmonary carcinoma. Refuch Vet. 29: 15-19, 1972

Hod, I., Perk, K., Nobel, T. A., Klopher, U.: Lung carcinoma of sheep. III. Lymph node, blood and immunoglobulin. J. Nat. Cancer Inst., 48: 487-494, 1972

Perk, K. and Hod, I.: Nuclear bodies in some human and animal tissues. Refuch Vet. 29: 51-53, 1972

Perk, K., Shachat, O. A. and Moloney, J. B.: Hypogammaglobulinaemia and lipaemia initiated by the MSV(M) in rats. Lab. Animals 6: 315-320, 1972

Perk, K., Russell, E. and Smith, K. L.: Tumor regression and relapse in MSV(M) inoculated mice. Lab. Animals 9in press)

Perk, K. and Hod, I.: Ultrastructure of sheep osteosarcoma: Vet. Research (in press)

Presentey, B. and Perk, K.: Simultaneous staining of phospholipids, basic proteins, and glycogen on the same slide. J. Clin. Path. 25: 608-610, 1972

Serial No. NCI-4805

1. Office of the Associate Scientific Director, Viral Oncology, Division of Cancer Cause and Prevention
2. Office of Biohazard and Environmental Control, Biohazard Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Parameters of Immunosuppression in the Malignant State

Previous Serial Number: Same

Principal Investigator: Dr. Alfred Hellman

Other Investigators: Dr. Arnold K. Fowler
Dr. Phyllis D. Kind
Dr. James E. Strickland

Cooperating Units: Inside NIH:
None

Outside NIH:
Southwest Foundation for Research and Education, San Antonio, Texas

Man Years:

Total	0.6
Professional:	0.2
Others:	0.4

Project Description

Objectives:

The evaluation of host immunocompetence and the physiological and pathological significance of endogenous C-type particles.

Methods Employed:

Cellular immunocompetence of the host during oncogenic and non-oncogenic viral infection is being investigated by means of cellular host immunological responses during phases of host development.

Major Findings:

Genetic as well as physiological influence on endogenous virus marker expression have been identified. The demonstration of the presence of C-type particles in reproductive tissue of primates, permits speculation as to their significance in the hosts physiology.

Significance to Biomedical Research and the Program of the Institute:

The elucidation of the events leading to malignancy with endogenous C-type marker expression in vivo as well as its implication in immunocompetence will provide a more rational means for determining the significance of such particles in oncogenesis. An understanding of inducing or co-inductive factors associated with malignancy will provide for more meaningful means of control and prevention.

Proposed Course:

The development of in vitro assay system for studying early inductive changes during viral produced malignancy and to determine the genetic and immunologic factors associated with such inductions and its modification.

Honors and Awards:

Organizational committee member for the Biohazards meeting, Biohazards in Cancer Research, held at Asilomar, Pacific Grove, California, 1973.

Invited lecturer in Oncology at TCA Institute.

Publications:

Hellman, A., Fowler, A. K., Steinman, H. G. and Buzzerd, P. M.: Studies of the Blastogenic Response of Murine Lymphocyte: III. Specific Viral Transformation. Proc. Soc. Exp. Biol. and Med. 141: 106-109 (1972)

Wedum, A. G., Barkley, W. E. and Hellman, A.: Handling of Infectious Agents. J.A.V.M.A. 161: 1557-1567 (1972)

Fowler, A. K., Reed, C. D., Todaro, G. J. and Hellman, A.: Activation of C-Type RNA Virus Markers in Mouse Uterine Tissue. Proc. Nat. Acad. Sci. 69: 2254-2257 (1972)

Fowler, A. K., McConahey, P. J. and Hellman, A.: Strain Dependency of Hormonally Activated C-Type RNA Tumor Virus Markers. JNCI (in press)

Kalter, S. S., Helmke, R. J., Heberling, R. L., Panigel, M., Fowler, A. K., Strickland, J. E. and Hellman, A.: Presence of C-Type Particles in Normal Human Placentas. JNCI (in press)

Fowler, A. K., Hellman, A. and Dimmick, R. L.: Environmental Pollutants as Activators of C-Type RNA Tumor Virus Information. In Winkle, R. J. (Ed.): Proc. of IVth Inter. Sym. on Aerobiology (1972)

Hellman, A., Oxman, M. and Pollock, R. (Eds.): Biohazards in Virus and Cell Research. Proc. from Biohazards in Cancer Research, Asilomar, Pacific Grove, California. Cold Spring Harbor Press (1973)

Serial No. NCI-4806

1. Office of the Associate Scientific Director, Viral Oncology, Division of Cancer Cause and Prevention
2. Office of Biohazard and Environmental Control, Biohazard Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Evaluation and Development of Biological Safety and Environmental Control Equipment

Previous Serial Number: Same

Principal Investigator: Dr. W. Emmett Barkley

Other Investigators: None

Cooperating Units: The Dow Chemical Company

Man Years:

Total:	0.5
Professional:	0.5
Other:	0

Project Description

Objectives:

The objectives of the biological safety and environmental control equipment project are: (1) to develop primary barrier systems for the containment of oncogenic viruses, (2) to establish a Qualified Products List for safety equipment to assure compliance with safety performance standards, (3) to evaluate equipment performance testing methods and develop standardized testing procedures, (4) to design and evaluate modular prefabricated containment rooms.

Methods Employed:

Standard engineering methods for the measurement of airflow, air velocity and filter penetration are employed. Physical and biological tracer assay systems are used to evaluate equipment performance. Environmental sampling procedures are routinely employed.

Major Findings:

An evaluation of design parameters for laminar flow biological safety cabinets demonstrated that face velocity is the critical design parameter on which personnel safety is dependent. A face velocity of 75 feet per minute was found to be required to achieve optimum safety. This observation indicated that most laminar flow biological safety cabinets are not adequately designed to provide sufficient protection to the equipment user. The results of this study have been used to establish and design criteria for new laminar flow safety equipment. A new design has been completed using these criteria.

A reproducible test method was developed to measure the performance of laminar flow biological safety cabinets. The test has the capability to measure cabinet leakage with sensitivity of 1 part in 10,000. The test consists of a highly concentrated challenge aerosol of Bacillus subtilis var. niger spores which is disseminated near the cabinet opening into an area of controlled air disturbance.

Initial studies on in-place filter testing procedures have shown that the particle size of a cigarette smoke challenge is comparable to the standard DOP smoke challenge. Particle penetration through pinhole filter leaks has been found to be a function of particle size. These findings demonstrate that the development of a simple filter challenge technique to measure filter efficiencies is feasible.

A practical design approach for creating containment space in existing conventional laboratories has been developed. This approach uses "off-the-shelf" modular components which allow assembly within a variety of institutional settings.

Significance to Biomedical Research and the Program of the Institute:

This project will improve the performance capabilities of safety equipment used for the containment of potentially hazardous biological materials. The establishment of a Qualified Products List for safety equipment will insure that safety equipment procured for biohazard containment will meet necessary safety standards. The development of modular containment systems will provide a feasible approach for upgrading individual laboratories for isolation of high hazard activities.

Proposed Course:

The Qualified Products List for safety equipment will be established by testing and certifying equipment provided by interested manufacturers. Containment equipment for centrifuges and for special process application will be designed, fabricated and evaluated. Equipment certification tests will be standardized.

Operational and containment characteristics of a modular safety laboratory will be evaluated.

Honors and Awards:

None

Publications:

Wedum, A. G., Barkley, W. E., Hellman, A.: Handling of Infectious Agents. J. of Amer. Vet. Med. Assoc. 161: 1557-1567, 1972.

Barkley, W. Emmett: Facilities and Equipment Available for Virus Containment. Biohazards in Cell and Virus Research, Cold Spring Harbor Press. (In Press.)

Serial No. NCI-4807

1. Office of the Associate Scientific Director, Viral Oncology, Division of Cancer Cause and Prevention
2. Office of Biohazard and Environmental Control, Biohazard Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Immunocompetence and Susceptibility of Animal Systems to Oncogenesis

Previous Serial Number: Same

Principal Investigator: Dr. Arnold K. Fowler

Other Investigators: Dr. Alfred Hellman
Dr. James Strickland
Dr. Phyllis Kind

Cooperating Units: None

Man Years:

Total	2.0
Professional:	1.0
Others:	1.0

Project Description

Objectives:

The objectives of this project are to define and characterize biological mechanisms contributing to host immunological regulation and to ascertain their role in oncogenic expression. Physiological imbalances, produced by controlled stresses, are to be examined at the biochemical level as initiators of host immunological inadequacy. In vivo and in vitro model systems will be developed to assess and monitor host immunocompetency.

Methods Employed:

The in vivo and in vitro activation of virus or virogene marker expression by a variety of physiological stimuli is being characterized using conventional methods: group specific antigens of the C-type RNA tumor viruses (gs-1, gs-3) by complement fixation, radioimmunoassay and gel precipitation techniques; virus synthesis by electron microscopy and in vitro culture assays; RNA directed DNA polymerase by biochemical, biophysical and serological methods. These criteria of oncogenic

detection are being studied concomitantly with various parameters of host immunocompetence. These include the in vivo delayed hypersensitivity and graft vs host in vitro reactions and the in vitro response of lymphocytes to specific and nonspecific antigenic stimulation.

Major Findings:

The previously described observation of the in vivo activation of group specific antigen of the C-type RNA tumor viruses in mouse uterine tissue by estrogen (Nature New Biol. 233: 142, 1971) has been further characterized and extended by a more recent finding that an RNA-directed DNA polymerase, showing serological cross-reactivity to Rauscher leukemia virus "reverse transcriptase" but having a heavier molecular weight, is also activated by estrogen therapy. Significantly, hormonal induction of these "virogene marker" expressions are genetically controlled, and in the mouse strains examined thus far appear to parallel each other as well as the strain's incidence of tumorigenesis. The observation that the expression of viral information is induced by hormones, at physiological levels, in genetically predisposed mice demonstrates a definable biological mechanism by which viral genetic information may be derepressed and possibly repressed in the animal system. Additional numerous other in vivo stimuli including a variety of synthetic compounds (contraceptive preparations and pesticides) and radiation have been shown to influence virogene marker expression. (Contract #NIH-71-2348) In a collaborative study with the Southwest Research Foundation, San Antonio, Texas, the biochemical and immunological characterizations of C-type viruses found in normal primate tissues are in progress.

The developmental aspects of a fully automated "flow-through-filter" lymphocyte culture harvest device to permit a very rapid and controlled means to selectively assay different parameters of lymphocyte cellular mediated recognition are continuing.

Significance to Biomedical Research and the Program of the Institute:

The essential role of host immunity, as dictated by genetic and environmental control mechanisms, in the etiology of some, if not all cancers, has become increasingly apparent during recent years. The concept that degradation of natural immunity processes under certain environmental and/or physiological stresses precede or are concomitant with infection by oncogenic viral agents is not new, although it remains poorly defined. The frequent implication of host physiology in the development and pathogenesis of cancer, as well as their involvement in host immunocompetence, points to the need of further research directed toward elucidating the biochemical mechanisms and the drgree of its participation in these phenomena as a prerequisite to the development of means to control human cancers.

Proposed Course:

The development of an in vivo system permitting the "at will" control of virogene marker expression by the manipulation of the host's endocrine milieu now provides a model to comprehensively examine the relevance of virus information in tumorogenesis. Efforts will be concentrated on physiological imbalances as they interact with host genetics and immunocompetence in the induction and propagation of the malignant state. Furthermore attempts will be directed toward the characterization of the underlying biochemical mechanisms of these interrelationships.

Honors and Awards:

None

Publications:

Fowler, A. K., Reed, C. D., Todaro, G. J. and Hellman, A.: Activation of C-Type RNA Virus Markers in Mouse Uterine Tissue. Proc. Nat. Acad. Sci. 69: 2254-2257 (1972)

Fowler, A. K. and Reed, C. D.: Estrogen Analysis of Neonatal Bovine Urine Using Gas-liquid Chromatography. J. Animal Sci. 35: 843-847 (1972)

Hellman, A., Fowler, A. K., Steinman, H. G. and Buzzerd, P. M.: Studies of the Blastogenic Response of Murine Lymphocytes: III. Specific Viral Transformation. Proc. Soc. Exp. Biol. and Med. 141: 106-109 (1972)

Fowler, A. K., McConahey, P. J. and Hellman, A.: Strain Dependency of Hormonally Activated C-type RNA Tumor Virus Markers. JNCI (in press)

Fowler, A. K., Hellman, A. and Dimmick, R. L.: Environmental Pollutants as Activators of C-Type RNA Tumor Virus Information. In Winkle, R. J. (Ed.): Proc. of IVth Inter. Sym. on Aerobiology (1972)

Kalter, S. S., Helmke, R. J., Heberling, R. L., Panigel, M., Fowler, A. K., Strickland, J. E. and Hellman, A.: Presence of C-type Particles in Normal Human Placentas. JNCI (in press)

Serial No. NCI-4809

1. Office of the Associate Scientific Director, Viral Oncology, Division of Cancer Cause and Prevention
2. Office of Biohazard and Environmental Control, Biohazard Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: A. Characterization of GS Antigen in Mouse Uterus

B. Immunosuppression by Rauscher Leukemia Virus

Previous Serial Number: Same

Principal Investigator: Dr. Phyllis D. Kind

Other Investigators: Dr. Alfred Hellman
Dr. Arnold K. Fowler
Dr. James E. Strickland

Cooperating Units: None

Man Years:

Total	2.0
Professional:	1.0
Others:	1.0

Project Description

Objectives:

A. To characterize immunologically the estrogen induced antigen in the mouse uterus and determine its relationship to the RNA tumor virus gs antigen.

B. To describe the cellular event that is altered in RLV infected animal and results in a reduced immune response.

Methods Employed:

A. Immunodiffusion, complement fixation and radioimmuno precipitation are being used to detect and characterize the antigen and to investigate its cross-reactivity with the RNA tumor virus antigen.

Mice and rabbits are being immunized to obtain antisera capable of detecting gs antigenic determinants.

Sephadex chromatography, isoelectric focusing and polyacrylamide gel electrophoresis are being used to isolate and characterize the antigen.

B. Humoral immunity was evaluated by measuring the number of hemolytic plaque forming cells in the spleens of mice injected with sheep red blood cells.

Cellular immunity was evaluated by mixed lymphocytes reaction and by PHA stimulation.

Cells bearing immunoglobulin on their surface (B cells) were identified and counted by a direct fluorescent antibody test.

Major Findings:

A. Injection of mice with uterine tissue from estrogen-treated mice resulted in antisera that reacted with tween-ether extracted FeLV and with tween-ether extracted MuLV.

Gs antigenic activity from stimulated AKR mouse uteri eluted from a Sephadex G-100 column in two peaks. Gs antigenic activity from stimulated NIH mouse uteri eluted in a single peak earlier than the peak of viral gs activity. These findings suggest that the antigens activated by hormonal stimuli have a somewhat higher molecular weight than viral gs antigen. All fractions from mouse uteri reacted in immunodiffusion with a line of identity with tween-ether extracted MuLV and partial identity with tween-ether extracted FeLV. These data suggest that the uterine material contains both gs-1 and gs-3.

B. Late in the course of infection with Rauscher leukemia virus, the percentage but not the total number of B cells in the spleen was depressed. Although it is known that B cell function is depressed in Rauscher infection, this finding indicates that the depression in function does not occur by decreasing the number of B cells or by eliminating the immunoglobulin from the surface of B cells.

Significance to Biomedical Research and the Program of the Institute:

A. Characterization of the tissue gs antigenic will permit further studies of the control of their expression as well as studies of the relationship of gs antigens to oncogenesis and perhaps to normal physiological function.

B. Elucidation of the interaction of host factors and RLV should increase the understanding of the pathogenesis of malignant disease and of the cellular events in the immune response.

Proposed Course:

A. Major efforts will be directed toward isolation and characterization of gs antigens from mouse uteri and in the development of greater specificity

in these assays. Special emphasis will be given to characterizing antigen in the mouse uterus that has gs antigenic determinants but an apparent greater molecular weight than viral gs antigen.

B. We will attempt to reverse the immunosuppression caused by RLV with a variety of B cell adjuvants.

The mechanism of immunosuppression will be studied on the level of interaction of T cells and B cells.

Honors and Awards:

None

Publications:

Abramoff, P. and LaVia, M. (Eds.): Biology of the Immune Response. Responsible for chapter entitled Immuno Enhancement. (in press)

Serial No. NCI-4810

1. Office of the Associate Scientific Director, Viral Oncology, Division of Cancer Cause and Prevention
2. Office of Biohazard and Environmental Control, Biohazard Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Studies on RNA-Directed DNA Polymerases of Mammalian Tissues

Previous Serial Number: None

Principal Investigator: Dr. James E. Strickland

Other Investigators: Dr. Arnold K. Fowler
Dr. Alfred Hellman
Dr. Phyllis D. Kind

Cooperating Units: Inside NIH:
None

Outside NIH:
Southwest Foundation for Research and Education, San Antonio, Texas

Man Years:

Total	2.5
Professional:	1.0
Others:	1.5

Project Description

Objectives:

To determine the structure and biological function of RNA-directed DNA polymerases of normal tissues, to elucidate the mechanisms of control of expression of genes for these enzymes and to determine the relationship, if any, of these enzymes to similar enzymes of oncorna viruses.

Methods Employed:

Homogenates of various tissues have been surveyed for DNA polymerases, utilizing several template-primers to assay fractions from sedimentation velocity gradients and gel filtration and ion exchange chromatography. Kinetics of changes in enzyme activity in response to hormonal stimulation have been examined. The reactions of some of these polymerases with antisera to specific viral DNA polymerases has been studied.

Major Findings:

A DNA polymerase which uses poly rA·oligo dT as template-primer appears in the uterus of certain strains of mice in response to estrogen stimulation. Though of larger molecular weight than the polymerase of Rauscher murine leukemia virus, the uterine polymerase is antigenically very similar to the former, whereas all other cellular polymerases tested do not cross react. The enzyme is absent or at very low levels in the uterus of ovarectomized non-stimulated animals of the same strain. In certain other strains, however, this enzyme does not appear, regardless of stimulation. Time course experiments are consistent with the idea of a newly synthesized enzyme in response to hormonal stimulus. Similar polymerases have been found in fetal tissues of several species including primates.

Significance to Biomedical Research and the Program of the Institute:

The presence of an enzyme which appears to be virally related in normal tissues which do not contain viruses seems to indicate that viral genetic information is present. If these same genes exist but are not expressed in other strains in response to similar stimuli, one is hopeful that an understanding of the mechanism would offer a means of suppressing undesirable genetic information.

Proposed Course:

Purification of polymerases which appear to be immunologically related to viral enzymes and structural comparisons will answer questions regarding the nature of this relationship, e. g. could the cellular enzyme be a viral polymerase precursor? Such information would shed light on the mechanism of synthesis and processing of viral components. Furthermore, the mechanism of appearance of polymerase activity in response to hormonal stimulation will be examined. Is this, in fact, gene derepression? Antibodies will be prepared toward purified cellular polymerases so that we can determine whether these antibodies cross react with viral enzymes and so that when we have a polymerase which does not cross react with antibody against virus polymersae, we can test it for reaction with antibody to cellular enzymes.

Honors and Awards:

None

Publications:

Kalter, S. S., Helmke, R. J., Heberling, R. L., Panigel, M., Fowler, A. K., Strickland, J. E. and Hellman, A.: Presence of C-Type Particles in Normal Human Placentas. JNCI (in press)

Serial No. NCI-4819

1. Office of the Associate Scientific Director, Viral Oncology, Division of Cancer Cause and Prevention
2. Office of Biohazard and Environmental Control, Biohazard Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Safety and Environmental Control Survey

Previous Serial Number: None

Principal Investigator: Dr. Garrett V. Keefer

Other Investigators: Dr. Alfred Hellman
Dr. W. Emmett Barkley

Cooperating Units: Litton Bionetics, Inc.
The Dow Chemical Company

Man Years:

Total:	1.5
Professional:	1.5
Other:	0

Project Description

Objectives:

The Office of Biohazard and Environmental Control (OB&EC) has the responsibility for surveying facilities, equipment and laboratory procedures at the various collaborating laboratories within the Special Virus Cancer Program with the view toward recognition and reducing potential biohazards and enhancing the research environment.

Methods Employed:

The OB&EC has assembled a group of staff personnel and consultants with individual expertise in microbiology, biohazard safety, and engineering to perform these surveys. The survey team is comprised of 2 or 3 individuals and is headed by a member of the OB&EC. The project officer usually accompanies the survey team to provide the link to program and is available to answer any questions concerning funding that might arise. The team meets with the principal investigator, his staff, and facility engineer with access

to current building drawings, and a member of the facilities safety group.

The principal investigator describes his project and identifies various laboratory functions as well as their support groups. This presentation also includes the quantities and types of oncogenic viruses under study as well as the flow of materials and personnel. The leader of the survey team discusses the "Minimum Standards of Biological Safety and Environmental Control for Contractors in the SVCP". Suggestions by the principal investigator as to how he feels the Standards could be improved are also noted. After the meeting, the team tours the facility observing normal laboratory practices and operations. At the completion of the survey the visitors again meet with the principal investigator to identify major deficiencies and provide recommendation for their removal. Each member of the survey team submits a trip report including his observations. These are discussed and the salient points are compiled into a letter addressed to the principal investigator. Eleven contractors were surveyed in the past year.

Major Findings:

The observations from the surveys emphasized three major deficiencies that must be resolved: (1) a lack of uniformity was observed among SVCP laboratories as to the degree of containment needed to protect both personnel and product. As a result the OB&EC developed, compiled and promulgated the "Minimum Standards of Biological Safety and Environmental Control for Contractors in the SVCP". The implementation of this document is required of all contractors in the SVCP, (2) funds were provided to improve facilities and procure necessary safety equipment. The OB&EC, in conjunction with the Contracts Construction Office, has established a review and administrative mechanism to provide safe, adequate and efficient research facilities for institutions receiving construction or renovation funding support through the Division of Cancer Cause and Prevention (DCCP) area of the National Cancer Institute, (3) a lack of awareness on the part of principal investigators and technicians to the potential hazards associated with laboratory procedures was quite prevalent.

Significance to Biomedical Research and Program of the Institute:

The significance to biomedical research or to any research effort is that this program provides a safe environment for the researcher and increased protection for the product.

Proposed Course:

Fifteen to twenty contractor laboratories are scheduled for survey in the coming year. The present survey protocol will be expanded to include the presentation of a seminar prior to a tour of the laboratories.

Honors and Awards:

None

Publications:

None

Serial No. NCI-4820

1. Office of the Associate Scientific Director, Viral Oncology, Division of Cancer Cause and Prevention
2. Office of Biohazard and Environmental Control, Biohazard Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Development of a Carcinogenic Chemical Safety Program for the SVCP

Previous Serial Number: None

Principal Investigator: Dr. Harry G. Steinman

Other Investigators: None

Cooperating Units: Carcinogenesis Branch, DCCP

Man Years:

Total:	1
Professional:	1
Other:	0

Project Description

Objectives:

An increasing number of the contract laboratories of the SVCP are using chemical carcinogens in conjunction with their virologic studies. These range from overt carcinogens to virus activators and immunosuppressive agents. Also, some possess mutagenic and teratogenic properties. It is planned to develop a carcinogenic chemical safety program to complement the ongoing biologic safety program and thus provide environmental control of all types of carcinogenic agents for the operations of the SVCP.

Methods Employed:

A search of the literature was made to ascertain the "state of the art". The principal source of information was the NIH Medlines Service, which draws on the NLM Medlars System. Because the subject of HAZARDS was not treated directly, it was necessary to learn how the system was programmed in order to retrieve the desired information.

Several meetings were held with the Carcinogenesis Branch, DCCP, to learn of their experience in this area and to discuss the future plans of both groups. This liason will continue as the chemical safety program develops.

Contact will be made with other governmental agencies, such as the Occupational Health and Safety Administration, who have an interest in this field.

Major Findings:

Although attention has been drawn to the problem of hazards to chemical carcinogens in the laboratory in several recent articles, there have been surprisingly few publications dealing explicitly with procedures for handling such materials. Many individual laboratories have developed safety procedures for specific purposes but, to date, there is no set of standards or safety manual of procedures available for general use.

Significance to Biomedical Research and the Program of the Institute:

Safe handling of hazardous materials is of obvious importance to all research programs. The environmental control program required to accomplish this will not only provide a safe-guard for laboratory workers and the general community but will also help eliminate a source of error in experimental systems (e.g., animal studies) subject to cross-contamination.

The safety procedures to be developed for the SVCP will probably become important elements of the total safety program of the NCI, which in turn should be the model for the whole scientific community.

Proposed Course:

In order to meet the needs of both the Viral Oncology Branch and the Carcinogenesis Branch of DCCP it has been decided to establish a contract operation to develop standards and procedures for the safe handling of various types of carcinogenic chemicals.

This will probably be accomplished as an expanded activity of the Environmental Control portion of the Litton-Bionetics contract at the Frederick Cancer Research Center.

Because the planned course is a long range project requiring much development before full implementation and because the needs in the SVCP are immediate, it is proposed to establish a set of guidelines based on existing information for interim use within the SVCP.

Honors and Awards:

None

Publications:

None

SUMMARY REPORT

The Office of Program Analysis and Communications (PAC) has actively pursued its role in data and information management for the SVCP. Major services rendered during the past year were in the general areas of analytical statistics, systems analysis, computerized data and information storage, retrieval, and distribution. Specific activities were the following:

Automated Inventories of SVCP Resources

1. SVCP Serum Bank: Maintained a computerized inventory of serum specimens held in the Bethesda area; PAC managed the continuous data input, updating, and distribution of specimens for testing to program scientists, from repositories containing around 100,000 serum specimen units.

2. Local Systems: Planned and installed complete institutional automated inventories in three laboratories participating as resource repositories for the SVCP: Naval Biological Research Laboratories, Huntingdon Research Center, and Simpson Memorial Hospital-University of Michigan.

3. Central PAC Inventory: PAC has promoted compatible automated systems and codes for specimen inventories in all institutions participating in the SVCP. This included the capture and automation of complete clinical demographic, and laboratory information on specimen donors. Goal has been a central specimen inventory at NIH making available for all of SVCP the many large specimen repositories throughout the United States. During the last year several SVCP resource repositories were brought into the system: Communicable Disease Center, Atlanta, Memorial Hospital, New York City, African Burkitt collection of NCI.

Statistical and Data Services

All researchers in the SVCP are offered a comprehensive statistical service (consultation on problems of research design, data procurement, data management, and statistical analysis of survey or experimental findings). A completely automated data processing routine directed by experienced technicians maintains direct access to the main NIH computer. Consultation and planning service is also rendered to SVCP contractors in developing local automation systems for managing and processing experimental data.

Progress Reports

A system of managing regular (triannual and annual) progress reports from all SVCP research contracts was maintained. Early in 1973 the system was

Progress Reports (continued)

changed from triannual to semiannual reports. In addition to the logistics of monitoring and report procurement, PAC compiles and distributes to program scientists condensed summaries of the actual progress reports.

Scientific Information Management

The Information Unit continued to focus attention on scientific information retrieval in the area of viral oncology, and its dissemination to program scientists. Sources of information are scientific journals, books, and other notifications and summaries of current research in the field. Major contributions of the Information Unit in FY 1973 were as follows:

1. Bibliographic Service: A semi-automated system is maintained for storage, rapid search, identification, and reference print-outs covering almost any desired topic in the published literature on viral oncology.

2. Viral Tumorigenesis Report: This semi-annual publication contains indexed summaries of current pertinent research projects, thereby presenting a panoramic view of viral oncology research.

3. Cooperative Literature Searching Service: Cooperative arrangements from this office have been established with the information offices of six other federal agencies whereby their literature collections and other user services are made available to Viral Oncology program staff members on request.

4. Viral Oncology Contractor Directory: This quarterly publication contains the names, addresses, and telephone numbers of contractors, principal investigators, project officers, and contract specialists within the Viral Oncology Program. Its purpose is to facilitate and expedite communications between staff members and contractors.

5. Compilation of Journal Instructions to Authors: This displays in one volume the instructions-to-authors from a majority of pertinent scientific journals. It is a reference aid for research investigators in writing papers and also for the secretaries who type them. The compilation is updated and expanded periodically.

Other responsibilities of the Information Unit are: administration of the Viral Oncology library facility containing subscriptions to seventy scientific journals and also a collection of 350 reference books; the collection and lending of recorded tapes on NIH seminars related to viral oncology; continuous compilation of the SVCP bibliography containing citations to all papers published by Viral Oncology staff members and research contractors; and the preparation of special bibliographies both manually and by computer on request.

SUMMARY REPORT

OFFICE OF THE COORDINATOR FOR ULTRASTRUCTURAL STUDIES
July 1, 1972 - June 30, 1973

This office and the Virus Studies Section are involved in a number of projects concerned with viral replication and oncogenic virus-host cell interactions. These projects consist of:

A. The search for viruses in biopsies and tissue culture isolates of human solid tumors.

B. The study of ultrastructural variations in different oncogenic viruses.

C. The study of the interaction between viruses and host cells and the relationship between viral and host cell nucleic acids by biochemical and ultrastructural methods.

D. The study of the early events in viral penetration into cells and of replication in host cells.

E. 1. The study of ethidium bromide in comparison with other inhibitors on Rous sarcoma virus transformation. 2. Morphological and biochemical studies on chick embryo cell transformation by Rous sarcoma virus.

A. Search for Viruses

A total of 10 cell lines have developed as suspension cultures from a series of biopsies of human solid tumors. A series of tests including compliment fixation, karyology, immunoflourescent staining, HLA, immunoglobulin and electron microscopic analysis together indicate that these 10 lines are independent and distinct from one another. Eight of the lines were shown to contain herpes type virus and EBV cell antigens while 2 did not. However, in these 2 cell lines, after long term treatment with BudR, early EBV antigen appeared as well as herpes type virions. Electron microscopic studies of these cell lines indicated that many of them continued to exhibit morphologic evidence of considerable differentiation after relatively long intervals in culture. The results of these studies are further evidence for the importance of the presence of the EBV genome for the continued growth of both normal and malignant cells in suspension cultures.

B. Ultrastructural Variations in Virions

In continuing studies on the fine structures of oncogenic viruses it has been demonstrated that because of differing reactions to different fixatives, type C virus indigenous in certain species can be distinguished from one another, i.e., ESP-1, RLV in JLSV-5 mouse cells or in HEK cells, FeLV and AMV. RD-114 virus appeared to be more like FeLV than any other. This similarity in ultrastructure between the RD-114 virus and FeLV has since been supported by biochemical RNA-DNA hybridization studies. These results suggest that certain morphologic features of some virions are under viral genome, not host cell control. On the other hand, other studies, have shown that the treatment given some viruses prior to fixation has a good deal to do with the appearance of virions when finally viewed with the electron microscope.

C. Viruses vs. Host Cells

Recently it has been shown that heat-treated, non-cytopathogenic herpes virus saimiri is capable of inducing lymphomas in owl monkeys. Enveloped particles showed no morphological changes following heat treatment but naked particles exhibited modified capsids with hardly recognizable capsomeres. It is possible that only the enveloped particles are capable of inducing neoplasia following heat treatment. There is also evidence to suggest that in highly infectious, rapidly replicating herpes simplex virus type I, little but the viral nucleocapsid is included in the enveloped particle, whereas in herpes type viruses of low infectivity and minimal replication, more cytoplasmic matrix is incorporated within the envelope. The inclusion of this cytoplasmic, presumably non-viral, material may be related to the low infectivity levels of these herpes type viruses.

Studies are in the process of being carried out comparing host cell RNA with viral RNA at both the ultrastructural and biochemical levels. Biochemical methods for this comparison are already available but ultrastructural visualization requires further technical developments which are presently being worked on.

D. Viral Penetration and Replication

Studies are continuing which involve attempts to identify and clarify the molecular events which occur during the eclipse phase of infection with Rous sarcoma virus. Because of the failure of NIH to pay its bills in England it was impossible to obtain satisfactory photographic nuclear track emulsion for a good part of the year. This has delayed work in this area. Recent results, however, have not supported the working

hypothesis that differences in sensitivity and binding affinity of nucleoli of RSV infected cells to antinomycin D might be the result of virus induced changes in the distribution of nucleolar DNA. What has been demonstrated is the presence of thymidine label in the cytoplasm near the plasma membrane within 10 minutes after infection. If a difference in nucleolar vs. nuclear labelling does exist, it must be below the 7 to 10% limits of standard errors.

Studies on the penetration of vesicular stomatitis virus (VSV) into cells has demonstrated that under optimum conditions, the virus penetration occurs by viro pexis, that is by the incorporation of virus into specialized vacuoles comparable to pinocytotic vesicles. Previous studies have indicated that virus penetration occurred by means of fusion and direct absorption. Efforts are being made to determine the conditions under which the latter type of penetration occurs.

E. RSV Transformation

Chick embryo cells exposed to ethidium bromide contain 75-90% degenerated mitochondria yet such cells infected with RSV continue to produce virus at an uninhibited rate. On the other hand chick embryo cells exposed to actinomycin D exhibit a greatly reduced production of virus. Thus functional mitochondria are not essential for RSV production.

Characteristic vacuoles appear in chick embryo cells transformed by RSV and serve as a clearly recognizable marker of transformed cells. The detailed structure and composition of these vacuoles are under study with various available techniques including scanning electron microscopy.

The Electron Microscopy Technique Unit

The staff serviced includes, Doctors Dalton, Heine, Bader, Suskind, Dahlberg and Mrs. Michele Fox and visiting scientists, Doctors Perk and Botchorov.

Total number of specimens received 952.
Total number of specimens processed 942.
Total number of specimens cut and stained 674.
Total number of specimens negatively stained 85.
Total number of specimens shadowed, chrome or platinum-carbon 92.

The Tissue Culture Unit

For members of the staff of the Virus Studies Section, approximately 2300 flasks and L and S plate styles dishes were transferred monthly. In addition 10 new biopsy specimens from St. Joseph's Hospital have been isolated in tissue culture, one of which has developed into a suspension culture. Two suspension cultures were maintained for Dr. Barinski, Atlanta, Georgia. Seven human suspension culture lines were supplied to Doctors Ablashi, Houts (St. Joseph's Hospital) Klein (Sweden) Barinski (Atlanta) and zur Hausen (Germany).

Rauscher virus in JLSV-5 and RSV in CE have also been prepared, as well as SR-RSV pools and Spafas embryos free of Marek's disease and influenza.

Primary chick embryo cultures for VSV production and L-929 mouse and L-2 guinea pig embryo cultures have been grown for biochemical studies.

Photographic Unit

Routine printing from electronmicrographic plates from the section's four scopes:

Dr. A. V. Bader	699
Drs. A. J. Dalton & J. Dahlberg	642
Dr. U. I. Heine & Mrs. M. Fox	1201
Dr. R. G. Suskind	1180

Publications:

Two (2) publications worked on with large numbers of special prints for each illustration for Dr. Bader.

Three (3) publications worked on with large numbers of special prints for each illustration for Dr. Dalton.

Four (4) publications worked on with large numbers of special prints for each illustration for Dr. Heine.

Light Micrographs	Approx. 250
Lantern Slides	Approx. 50
2 x 2 Slides	Approx. 400
4 x 5 Copy Negatives	Approx. 400

Special projects for Dr. Moloney, Dr. Chirigos, Dr. Pearson, Dr. Wolf, Dr. Perk, Dr. Boone, Dr. Stewart and Mr. Patterson.

Guest Speakers for Virus Studies Section Seminars

Dr. A. Howatson; Dr. H. zur Hausen; Dr. K. Porter; Dr. G. H. Weber; and Dr. N. C. Yew.

Serial No. NCI - 4811

1. Office of the Associate Scientific Director, Viral Oncology, Division of Cancer Cause and Prevention
2. Office of the Coordinator for Ultrastructural Studies
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Electron microscopic studies on oncogenic viruses and on host cells.

Previous Serial Number: Same

Principal Investigator: Dr. Albert J. Dalton

Other Investigators: Dr. Ursula Heine
Dr. D. V. Ablashi
Dr. G. H. Klein
Dr. Gary Pearson
Dr. U. Fabrizio
Dr. P. I. Terasaki
Dr. K. Nilsson

Cooperating Units (Inside NIH): Viral Pathology Section, Viral Leukemia and Lymphoma Branch
Experimental Pathology Section, Viral Biology Branch

Cooperating Units (Outside NIH): Litton-Bionetics
St. Joseph's Hospital, Tampa, Florida
Karolinska Institute, Stockholm, Sweden
University of Uppsala, Uppsala, Sweden
U.C.L.A.

Man Years:

Total	3.0
Professional:	2.0
Other:	1.0

Project Description

Objectives:

A. To study biopsy and tissue culture isolates from human solid tumors

for the presence of candidate oncogenic viruses.

B. High resolution electron microscopic studies on oncogenic viruses to determine whether or not consistent inter-strain and inter-species differences exist.

Methods Employed:

A. The same methods of fixation and preservation for electron microscopy and of tissue culture isolates have been used here and at St. Joseph's Hospital to allow direct comparison of results.

B. Methods of embedding, sectioning and staining have been held constant but different methods of fixation were used: Glutaraldehyde for 1 to 24 hours followed by chrome-osmium; glutaraldehyde for 1 to 24 hours followed by phosphate buffered osmium; and chrome-osmium 1-2 hours followed by 10% neutral formalin either containing or not containing 0.5% uranyl acetate.

Major Findings:

A. Ten continuous suspension cultures have been developed of which 7 are still growing. Of these 10, 8 were shown to contain a herpes type virus by electron microscopy and to possess EBV antigens. The other 2 originally contained cytomegalovirus antigens but have since, on stimulation with BudR, been demonstrated to possess early EBV antigen and to produce herpes type virions. Studies on these cell lines involving immunoglobulin analysis, chromosome studies, HLA-testing, immunoflourescent staining and electron microscopy, indicate that each of these lines is distinct and separate and not the result of contamination. Electron microscopic analysis of these cell lines showed that many of them continued to demonstrate morphologic evidence of differentiation, such as well organized ergastoplasm, phagocytosis and variations in mitochondrial size and shape. Five of these lines must have been derived from normal stromal cells since the cells had none of the characteristics of the original tumor cells.

B. Differing reactions of virions to different fixatives and to different fixation times has made it possible to distinguish between mouse leukemia-sarcoma virus, ESP-1 virus and FeLV. These differences relate to the thickness and clarity of separation of the capsid layer of the nucleocapsid of the type C virions. RD-114 virions were shown to be similar to FeLV virions and not to those of ESP-1. It has also been shown that budding type B and type C particles can be distinguished from one another providing proper fixation and post fixation treatments are carried out. With

chrome-osmium fixation followed by post fixation with neutral formalin containing 0.5% uranyl acetate, the inner shell of the nucleocapsid of budding type C particles is much more electron dense than the same component in budding type B particles.

Significance to Biomedical Research and the Program of the Institute:

A. In spite of the current popularity of the protovirus and oncogene hypotheses, it remains important to learn more about DNA viruses exhibiting a consistent relationship with human tumors. In addition, while there are many efforts underway to discover candidate human oncogenic viruses, none of these efforts can be considered to have been successful as of the present date. It would appear to be obvious that the discovery of a candidate human oncogenic virus being replicated in sufficient amount in human tissue culture cells would make possible signal advances in the SVCP program.

B. The effort to characterize by ultrastructural features the type C viruses indigenous to different species has as its ultimate aim the ability to determine whether a particular type C virion is of mouse, cat or human origin. This would then become one of the criteria needed to identify a human type C virus.

Proposed Course:

As indicated under the previous heading, both of these approaches are considered of importance to the general program of the SVCP and it is planned to continue then with modifications as deemed necessary during the coming year.

Note: Because of the continual efforts at type C particle characterization, many investigators from this country and abroad have consulted this Office in regard to the reality of type C virions in their material. This has been particularly notable with Dr. R. J. Huebner.

Three scientists from abroad have been associated with this Office and have worked in the Virus Studies Section during the past year.

Honors and Awards:

Chairman of a session on Oncogenic Viruses at the Annual M. D. Anderson Symposium on Fundamental Cancer Research, 1972.

Chairman of a session on Viruses and Cancer at St. Joseph's Hospital, Tampa, Florida, 1972.

Participation in a Symposium on Cytogenetics at Fairleigh Dickinson, 1973.

Consultant in Electron Microscopy, M. D. Anderson Hospital and Tumor Institute, 1972-73.

Member of the Scientific Advisory Committee of the Institute for Medical Research, Camden, N. J., 1972-73.

Publications:

Dalton, A. J.: RNA tumor viruses-terminology and ultrastructural aspects of virion morphology and replication. J. Nat. Cancer Inst. 49: 323-327, 1972.

Dalton, A. J., Heine, U. I., Kondratick, J. M., Ablashi, D. V., and Blackham, E. A.: Ultrastructural and complement-fixation studies on suspension cultures derived from human solid tumors. J. Nat. Cancer Inst. April 1973, (in press).

The Ultrastructure of Animal Viruses and Bacteriophages--An Atlas. (A. J. Dalton and F. Haguenau, co-editors). Academic Press, New York, (in press).

Dalton, A. J., and Hagueanu, F.: "Introduction to the RNA Tumor Viruses". In: "The Ultrastructure of Animal Viruses and Bacteriophages--An Atlas". Academic Press, New York, (in press). Editors, A. J. Dalton and F. Haguenau.

Dalton, A. J.: "The Arena Viruses". In: "The Ultrastructure of Animal Viruses and Bacteriophages--An Atlas". Academic Press, New York, (in press). Editors, A. J. Dalton and F. Haguenau.

Schidlovsky, G., Ahmed, M., and Dalton, A. J.: "Syncytial (Foamy) Viruses". In: "The Ultrastructure of Animal Viruses and Bacteriophages--An Atlas". Academic Press, New York, (in press). Editors, A. J. Dalton and F. Haguenau.

Serial No. NCI - 4812

1. Office of the Associate Scientific Director, Viral Oncology, Division of Cancer Cause and Prevention
2. Virus Studies Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Studies on virus-host cell interactions

Previous Serial Number: Same

Principal Investigator: Dr. Ursula Heine

Other Investigators: Dr. D. V. Ablashi
Dr. G. S. Beaudreau
Dr. A. J. Dalton
Dr. G. H. Weber

Cooperating Units: (inside NIH) Microbiology Section, VBB, NCI

Cooperating Units: (outside NIH) Oregon State University, Department of Agricultural Chemistry, Corvallis, Oregon

Man Years:

Total	2.0
Professional:	1.0
Others:	1.0

Project Description

Objectives:

(A) To investigate the relationship of selected tumor viruses to their host cells by ultrastructural and other biological methods.

(B) To study and identify variations in fine structure of different tumor viruses and their components.

(C) To study the relationship between viral and cellular nucleic acids by biochemical and ultrastructural methods (in collaboration with Dr. G. Weber).

Methods Employed:

(A) Virologic methods and routine electron microscopic techniques are employed for cell studies at the ultrastructural level.

(B) and (C) RNA extractions, gradient sedimentations, spectrophotometry, negative staining, Kleinschmidt monolayer method, electron microscopic techniques, including ultracytochemistry, are used to study the fine structure of viruses and their components.

Major Findings:

(A) 1. Ten isolates from biopsies of solid human tumors have been established as continuously growing lymphoid blasts in suspension cultures. By electron microscopy and compliment fixation tests, eight of these cell lines showed readily the presence of EB virus, whereas two cell lines did not reveal virus by electron microscopic examination. The addition of 5-bromodeoxyuridine (BUDR) to these two cell cultures, either in high doses for a short period of time or in low doses for prolonged period of time, stimulated the cells to release mature virions of the EBV type in small amounts. Only minor morphological changes affecting mitochondria and nucleoplasm have been observed in cells treated with BUDR. In addition, characteristic differences have been found in polymerase activity, chromosome patterns and HLA distribution among these two cell lines. This work was done in collaboration with Drs. Dalton and Ablashi.

2. In a continuing study with Dr. Ablashi, we examined the relationship of different herpesviruses with their host cells. It was shown that heat-treated non-cytopathogenic herpesvirus saimiri is capable of inducing lymphomas in owl monkeys. In the pool of heat-treated virus only the naked particles showed abberations from the normal fine structure. They possessed capsids with barely recognizable capsomeres. Possibly, these structures collapsed during heat treatment.

(B) During previous years, discrepancies appeared repeatedly in the literature concerning the fine structure of different tumor viruses, especially the arrangement of their internal components in relation to each other. These discrepancies have been observed with RNA tumor viruses of the C-type and with DNA-containing viruses of the herpestype.

It is reasonalbe to assume that variations in preparative methods may be an important factor in providing different appearances of these viruses. Therefore, two investigations have been initiated to study systematically the fine structure of these viruses after they have been subjected to different preparative methods. Thus far, it has been shown that the appearance of fixed C-type particles is highly dependent on their physical state before preparation. Under optimal conditions, round particles can be obtained containing an icosahedral nucleoid, the latter being surrounded by a thin electron dense membranous layer. The distance between nucleoid and this thin layer appears to be of fixed size in contrast to the highly variable space between the same layer and the unit membrane which surrounds the virions.

(C) In collaboration with Dr. G. Weber, we are establishing methods to visualize viral nucleic acids in the electron microscope. Thus far we have shown that these nucleic acids are highly sensitive to manipulation and their appearance is dependent on the methods used for isolation.

Significance to Biomedical Research and the Program of the Institute:

(A) Herpestype viruses have been found to be closely associated with certain human tumors and they are the causative agents for a number of animal malignancies. Their detection in continuously growing cell lines of human origin underlines their importance for continuous growth and, possibly, tumor production. The search for herpestype viruses in human tumors and the study of these viruses in the animal are important for the understanding of the etiology of cancer. The appearance of lymphomas after inoculation of heat-inactivated virus of the herpestype (H. saimiri) demonstrates that oncogenicity of viruses and coding for complete virus may not be lost by this treatment, which destructs partly the viral fine structure.

(B) The widening of our knowledge concerning the fine structure of tumor viruses is of major importance in different aspects, since, i.e., it may help in clarifying the process of infection or the assembly of virions inside the cells.

(C) RNA tumor viruses are known to be the causative agents for a number of malignancies. The study of the relationship of their nucleic acids to cellular nucleic acids and proteins is necessary for the understanding of the infectious process.

Proposed Course:

The study of the fine structure of tumor viruses will be expanded using different techniques to elucidate their micromorphology. Investigations of the in vitro replication of RNA tumor viruses will be intensified, especially early events leading to nucleic acid transcription will be studied.

Note:

A number of investigators were supported in their search for oncogenic viruses. These are:

Dr. P. Gerber, BS, LVI (Herpesviruses in human material)
Dr. V. Dunkel, C, BGY (C-type particles in guinea pig cells)
Dr. C. Boone, C, VB (Herpesvirus at cellular membranes)
Dr. P. Neiman, University of Washington, Seattle (C-type particles in dog lymphoma cells)
Dr. K. Perk, Weizman Institute, Israel.

Honors and Awards:

Dr. Heine presented the following papers: "Herpesviruses and their relation to the infected cell, an electron microscopic study". June, 1972, Radiological Physics Division, Argonne National Laboratory, Argonne, Illinois.

"Herpesviruses and their host cells". October, 1972, Faculte' de Médecine, University of Sherbrooke, Canada.

Publications:

Heine, U.: The behavior of HeLa-S_3 cells under the influence of supranormal temperatures. Abstract in: Year Book of Cancer, 16th vol., 1972 (ed.: R. W. Cumley), Year Book Medical Publishers, Inc., Chicago.

Schaff, Z., Heine, U. I., and Dalton, A. J.: Ultramorphological and ultracytochemical studies on tubulorecticular structures in lymphoid cells. Cancer Res. 32: 2696-2706, 1972.

Heine, U.: Intranuclear Viruses, In: The Nucleus (ed.: H. Busch), Academic Press, New York. (in press)

Heine, U., and Dalton, A. J.: Ultrastructural analysis of herpestype viruses, In: 25th Ann. Symposium on Fundamental Cancer Research, M.D. Anderson Hospital and Tumor Institute, Houston, Texas, 1972. (in press)

Dalton, A. J., Heine, U. I., Kondratick, J. M., Ablashi, D. V., and Blackham, E. A.: Ultrastructural and complement-fixation studies on suspension cultures derived from human solid tumors. J. Nat. Cancer Inst. April, 1973. (in press)

Ablashi, D. V., Armstrong, G. R., Heine, U., and Adamson, R. H.: Establishment of a cell culture from an owl monkey tumor induced by Herpesvirus saimiri. (HVS) 63rd Annual Meeting of the American Association for Cancer Research, vol. 13: 124, 1972. (abstract)

Ablashi, D. V., Loeb, W. F., Pearson, G., Valerio, M. G., Armstrong, G. R., Rabin, H., Kingsburg, E. W. and Heine, U.: Induction of lymphoma in owl monkeys with heated, non-cytopathogenic Herpesvirus saimiri. Nature (New Biology), 1973. (in press)

Serial No. NCI - 4814

1. Office of the Associate Scientific Director, Viral Oncology, Division of Cancer Cause and Prevention
2. Virus Studies Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Autoradiographic and ultrastructural studies of the nucleus of chicken fibroblasts during the eclipse phase of infection with Rous sarcoma virus

Previous Serial Number: Same

Principal Investigator: Dr. R. Gerald Suskind

Other Investigators: Dr. Francoise Haguenau
(outside NIH) Dr. Giancarlo Rabotti
(previous) Dr. Susan C. Michelson-Fiske

Cooperating Units: Laboratoire de Medecine Experimentale,
(previous) College de France, Paris

Man Years:

Total	2.0
Professional:	1.0
Other:	1.0

Project Description

Objectives:

The determination of the ultrastructural sites of transcription of the viral genome of Rous sarcoma virus and the intracellular mechanism of infection in relation to the induction of the transformed state continues to be the underlying objective of our research efforts.

Methods Employed:

The main techniques employed are quantitative electron microscopic and

light microscopic autoradiography of tritiated RNA and DNA precursors in tissue culture cells exposed to various metabolic inhibitors and statistical evaluation of grain counts per unit area of organelle with the aid of planimetry. This requires growth of individual leukosis-free chick embryo cultures in serial passage and selection, freezing and storage of those susceptible to total transformation by the SR strain of RSV; electron-microscopic screening for C-particles and mycoplasma; the cloning, plaque purification and titration of virus pools; interference titration; assay of thermo-sensitive mutants; electron microscopic and cytochemical staining techniques.

Major Findings:

We have previously shown that RSV infected chicken fibroblasts have a reduced sensitivity to the action of Actinomycin D with respect to the nucleolus. Thus after a pulse exposure to Actinomycin D, quantitative differences in both nucleolar morphology and in the rate of RNA synthesis could be detected between newly infected, transformed and uninfected cells at a period within 80 minutes to 9 hours after infection. During this period concomitant differences in the binding of tritiated Actinomycin D to nucleolar sites were observed. The experimental hypothesis that a newly transcribed "proviral" DNA may have different binding affinities for Actinomycin and might thus be defined with respect to time and cellular sites of synthesis is under consideration. However, results of EM autoradiographic experiments with DNA precursors have failed so far to demonstrate statistical differences in the intranuclear and nucleolar distribution of tritiated thymidine in transformed cells. On the other hand the cytoplasm of transformed cells shows a small but consistent increment in the proportion of thymidine label per unit area already after a 10 minute pulse exposure.

This label has not been statistically localized to a specific cytoplasmic organelle but is seen frequently near the plasma membrane and virus particles. The labelling of virus plaques is, however, infrequent at exposures shorter than one to two hours. After pulse exposure to Actinomycin results indicate a persistence of extranuclear label in infected cells, but a significant shift in nucleolus-associated DNA in transformed cells at the periods during which nucleolar RNA synthesis is increased, has not been demonstrated. These results suggest that the plasma membrane or budding virus particles are rapidly labelled with DNA precursors and may be a site of transcription of proviral DNA but do not exclude a nucleolar precursor site. A quantitative shift in the distribution of nucleolus-associated DNA, if present, in transformed

cells may be smaller than present limits of statistical discrimination with standard errors of seven to ten per cent. The observed differences in binding affinities of tritiated Actinomycin to nucleoli of transformed cells at the level of the light microscope are perhaps more pertinent and may indicate conformational differences in nucleolar DNA of transformed cells. Further attempts to demonstrate a differential uptake of tritiated Actinomycin at the level of the electron microscope have shown that this requires a higher specific activity than currently available.

A long-term interruption in the supply of a suitable photographic nuclear track emulsion has substantially delayed the conduct and evaluation of these experiments.

Related experiments on the effect of inhibitors of reverse transcriptase and viral DNA dependent RNA polymerase (Rifamycin SV derivatives) and C particle formation and on the distribution of RNA and DNA precursors in transformed cells are in progress, but definite results can not, as yet, be reported.

Other experiments using various cytochemical techniques have been conducted with a view to finding morphologic differences in the plasma membrane of transformed cells and on virus particles, as a means of localizing lectin binding sites and the sites of group specific antigen at the ultrastructural level. So far qualitative differences have not been found. A thick mucopolysaccharide coat on the outer membrane of RSV, Herpes virus and on transformed cells, similar to that seen with other techniques has been demonstrated with fixatives containing Alcian blue, cetylpyridinium chloride and lanthanum salts.

Significance to Biomedical Research and the Program of the Institute:

Evidence consistent with the appearance of viral DNA in the cytoplasm near virus particles has been obtained after a 10 minute pulse label with tritiated Thymidine; the sites of synthesis and transcription remain unclear. The working hypothesis that previously observed differences in sensitivity and binding affinity of nucleoli of RSV infected cells to Actinomycin D might be the result of virus induced changes in the distribution of nucleolar DNA has not been proven by results so far obtained.

Proposed Course:

Further attempts to localize the sites of transcription and the sequence of labelling of the virus particle are either in progress or projected. These include:

(a) A morphologic and autoradiographic study of the effects of inhibitors

of reverse transcriptase on the maturation and labelling of C-particles with RNA and DNA precursors.

(b) A comparison of the effect of infection with transforming and non-transforming thermo-sensitive mutants of RSV on the rate of recovery of nucleolar RNA synthesis from inhibition by Actinomycin D.

(c) A study of the autoradiographic distribution of tritiated Actinomycin applied to frozen thin sections of RSV infected cells.

(d) In-situ hybridization and autoradiography of radioactively labelled viral RNA in infected and non-infected cells.

(e) A study of the localization of RNA and DNA precursors at varying sequences after infection with radioactively, labelled, purified RSV under conditions of inhibition of ribosomal RNA synthesis.

(f) The isolation and biochemical characterization of nuclear and nucleolar RNA in cells infected with RSV under improved conditions of inhibition of cellular RNA synthesis.

Honors and Awards:

None

Publications:

None

Serial No. NCI - 4815

1. Office of the Associate Scientific Director, Viral Oncology, Division of Cancer Cause and Prevention
2. Virus Studies Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Nucleic acids associated with RNA tumor virus replication

Previous Serial Number: None

Principal Investigator: Dr. George H. Weber

Other Investigators: Dr. U. Heine
Dr. G. S. Beaudreau

Cooperating Units: Oregon State University, Department of Agricultural Chemistry, Corvallis, Oregon

Man Years:

Total	0.5
Professional:	0.5
Others:	0.0

Project Description

Objectives:

To define the relationship between viral and host cell nucleic acids by biochemical and ultrastructural methods.

Methods Employed:

Biochemical and ultrastructural procedures which include virus and cell fractionation, purification of nucleic acids, and electron microscopy of RNA and DNA.

Major Findings:

Preliminary research has been centered about establishing a reproductive procedure for visualizing with the electron microscope a variety of sizes and types of nucleic acids. Double-stranded DNA, isolated from L-cells and E. coli, can easily be seen when using the spreading techniques of Kleinschmidt. RNA, on the contrary, has been more difficult to observe; its integrity being affected by methods of isolation and preparation for spreading. Under our conditions, single stranded RNA molecules can only be seen when they are complexed to large basic proteins. This work was done in collaboration with Dr. Heine and Dr. Beaudreau.

Significance to Biomedical Research and the Program of the Institute:

Clarifying the role of RNA tumor virus nucleic acid replication and its association with host cell chromatin is important in deciphering the steps involved in transformation of infected cells. A direct observation of virus specific nucleic acids within host cell DNA would lend further evidence to the viral etiology of malignant transformation by RNA tumor viruses.

Proposed Course:

Examination into the in vitro replication of RNA tumor viruses will be expanded to include the detection of early nucleic acid transcription and the interaction of newly synthesized nucleic acid with that of the host cell. Autoradiographic techniques are now being established to complement those findings.

Honors and Awards:

None

Publications:

None

Serial No. NCI - 4817

1. Office of the Associate Scientific Director, Viral Oncology, Division of Cancer Cause and Prevention
2. Virus Studies Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: (A) The role of mitochondria in the production of RNA-containing tumor viruses and in transformation by these viruses.
(B) A study of the morphological and biochemical changes occurring in CE cells after infection with Rous sarcoma virus.

Previous Serial Number: Same

Principal Investigator: Dr. Artrice V. Bader

Other Investigators: (Project B) Dr. John P. Bader
Dr. Bruce Wetzel

Cooperating Units: Cell Growth Regulation Section, Chemistry Branch, National Cancer Institute
Dermatology Branch, National Cancer Institute

Man Years:

Total	2.0
Professional:	1.0
Others:	1.0

Project Description

Objectives:

(A) To quantitate data obtained by electron microscopy showing virus production in the presence of degenerative mitochondria which were produced by treatment with ethidium bromide (EB).

To further investigate the effect of EB treatment on transformation of chick embryo cells (CE) infected with Rous sarcoma virus (RSV).

To compare effects of EB with other inhibitors to substantiate morphological results which indicate that intact mitochondria are not required for the production of RNA-containing tumor viruses.

(B) To determine the morphological and biochemical nature of the vacuoles which typify transformation of CE cells infected with the Bryan "high titer" strain of RSV.

To characterize the structural appearance of transformed cells compared to non-transformed cells based on surface topography.

Methods Employed:

(A) Electron microscopy, cell culture, radioactive labelling, polyacrylamide gel electrophoresis.

(B) Phase contrast microscopy, histological staining, scanning electron microscopy, transmission electron microscopy.

Major Findings:

(A) Earlier data obtained from treatment of either murine cells infected with Rauscher leukemia virus (JLS-V5 cells) or chick embryo cells infected with Rous sarcoma virus (CE-RSV) showed that viruses were produced in cells whose mitochondria were defective as a result of treatment with ethidium bromide. Quantitation of these results with electron microscopy showed that JLS-V5 cells which had been treated with EB for 7 days contained more than 90% degenerative mitochondria. After 21 days, all mitochondria observed within any given cell section were degenerative. Incorporation of uridine-^{3}H into virions was then investigated. Uridine-^{3}H was incorporated into virions which were released by cells which had more than 90% degenerative mitochondria per cell section. These results were evidence that synthesis of viral RNA and its incorporation into virions is not dependent upon intact mitochondria.

Deliberate infection of EB treated CE cells with RSV resulted in complete cycles of reproduction equivalent to the reproduction of RSV found in control cells. These cells by electron microscopy showed approximately 75% of the cell sections to have major degenerative effects in 3/4 or more of their mitochondria after 4 days exposure to EB. Virus production in CE cells in which infection with RSV had been established was investigated

during simultaneous experiments with EB and Actinomycin D. Over a 6 hour period, virus production was inhibited in the Actinomycin D treated cultures but not in the EB treated cultures. The results of this study are inconsistent with a mitochondrial function in RNA-containing tumor virus production.

Transformation was not inhibited in EB treated CE cells when high multiplicities of RSV were used. However, using low multiplicities of infection and nutrient agar overlays, development of transformed foci was inhibited. Attempts to resolve this discrepancy are in progress.

(B) Vacuoles which are easily identified under phase contrast microscopy can be used as a morphological marker of transformation when CE cells have been infected with the Bryan "high titer" strain of RSV, a mixture of Rous sarcoma virus$_0$ and Rous associated virus$_1$. The nature of these vacuoles has been examined from both morphological and biochemical aspects. Phase contrast microscopy reveals highly refractile bodies which appear to be membrane bound. Treatment of viable cultures with neutral red show that these vacuoles will take up the dye. Various specific histological stains for lipid, glycogen, and acid and neutral mucopolysaccharides failed to stain the vacuoles. It was found that ethanol fixed cells showed better preserved vacuoles then cells fixed with formaldehyde. Also, if neutral red was added to cells fixed with formaldehyde, the neutral red was released from the vacuoles but remained in the cells. Ethanol fixed cells released the neutral red immediately from both vacuoles and cells.

Transmission electron microscopy gave variable results depending upon the plane of sectioning and treatment of cells prior to fixation. Cells which were fixed directly on the plate and cut perpendicular to the plane of attachment to the plates showed the vacuoles as low electron dense areas which did not appear to be membrane limited. Cells which were fixed directly on the plates and cut parallel to the plane of attachment show slightly smaller low electron dense areas than those seen in perpendicular cuts and a limiting membrane was not observed. Others vacuoles did seem to be membrane bound. Higher magnifications of these areas and their relationship to the plasma membrane are in progress. Cells scraped prior to fixation showed vacuoles which were smaller in size and surrounded by a membrane which has the same "unit membrane" structure as the plasma membrane.

Scanning electron microscopy (collaboration with Bruce Wetzel) is being employed to study differences in surface topography which may be definitive for easy recognition of transformed cells as compared to

control cells. The vacuoles of transformed cells appear as holes when they are seen on the screening tube. This suggests that the vacuoles may contain a high degree of water. Other topographical changes which may be characteristic of transformed cells are under investigation.

Significance to Biomedical Research and the Program of the Institute:

(A) The results of these experiments demonstrate that intact mitochondria are not essential for the production of RNA-containing tumor viruses. The mitochondrial DNA template is not involved in the synthesis of Rous sarcoma virus and Rauscher leukemia virus. The reproduction of Rous sarcoma virus and Rauscher leukemia virus as representative of RNA-containing tumor viruses is important to the understanding of the infectious cycle and the role of these viruses in oncogenesis. The elimination of mitochondria as an organelle involved in RNA-containing tumor virus production is important in efforts to locate the intracellular viral RNA and viral DNA. Because mammalian and avian cells are able to grow with a deficiency or loss in energy provided by the Kreb's cycle, this system offers an attractive model for the study of the role of mitochondria in tumorigenesis.

(B) Vacuoles are formed as a result of virus infection and provide an excellent marker for morphological changes occurring in transformed cells. The elucidation of the role of these vacuoles in transformation will be significant in answering the question of what molecules are responsible for tumorigenesis.

If definite surface structural features can be differentiated between control cells and transformed cells, then scanning microscopy could be a potential instrument for rapid screening of transformed cells. This will be useful in systems where assays for transformation take a long time using in vitro test. Also surface changes in transformed cells may give some indication to cellular defects which cause tumorigenesis.

Proposed Course:

(A) The ethidium bromide system represents one which can be used to examine metabolic factors effecting tumorigenesis. Ethidium bromide appears to give certain cells a selective advantage for survival. This selective mechanism provides a way of obtaining cells which could be used for other projects such as somatic cell genetic studies.

(B) Other approaches to studying these vacuoles will be use of interference microscopy and more sampling at higher resolutions (transmission electron microscopy) of cell sections for the presence

or absence of membranes. Scanning electron microscopy will be continued and cell mapping techniques will be used to allow a known area in phase microscopy to be viewed under the SEM.

Honors and Awards:

Invited to present lecture, Howard University Medical School.

Invited to be session chairman, RNA Tumor Viruses, American Society for Microbiology, Annual Meeting, Miami Beach, Florida. May 6-11, 1973.

Publications:

Bader, A. V.: Mitochondrial function in RNA-containing tumor virus production. J. Cell Biol. 55: 11A, 1972.

Bader, A. V.: The role of mitochondria in the production of RNA-containing tumor viruses. J. of Virology 11: 314-324, 1973.

Serial No. NCI - 4818

1. Office of the Associate Scientific Director, Viral Oncology, Division of Cancer Cause and Prevention
2. Virus Studies Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: (A) Kinetics of virus adsorption and penetration.
(B) Identification of virus produced following chemical carcinogenesis and mutagenesis of guinea pig cells.
(C) Ultrastructural study of virus-cell interactions in the presence of viral antiserum.

Previous Serial Number: None

Principal Investigator: Dr. John E. Dahlberg

Other Investigators: None

Cooperating Units: None

Man Years:

Total	1.0
Professional:	1.0
Others:	0.0

Project Description

Objectives:

(A) To resolve conflicting observations on the mode of penetration of vesicular stomatitis virus (VSV) into L cells. To analyze the kinetics via electron microscopy of the penetration of VSV into L cells infected under varying conditions.

(B) To obtain information on the likelihood of C-type virus being produced from clones of guinea pig cells following *in vitro* treatment with carcinogens and mutagens. This may help to understand possible genetic controls exerted on the viral genome(s) by the cell.

(C) To determine how VSV is capable of spreading from cell to cell in the presence of large quantities of anti-VSV serum. This may aid in understanding the means by which virus may persist in animals for long periods.

Methods Employed:

(A) Electron microscopy; cell culture.

(B) and (C) Light and electron microscopy; cell culture.

Major Findings:

(A) At present, viruses delimited by membranes are thought to penetrate cells either by viralpexis or by fusion with the cell membrane. In the case of VSV infecting L cells, there are conflicting reports as to which is the dominant mechanism. It appeared possible that differences in technique and/or environmental factors, rather than strain differences in either the virus or cell, could account for these disparate reports.

Using conditions designed to minimize trauma to the cell prior to fixation, viralpexis is the predominant mechanism of virus entry for the strains of cell and virus tested. A kinetic analysis of penetration following synchronization of infection by adsorption in the cold showed that the following sequence of events occur early in infection:

1) Attachment of the virus to the cell membrane (in the cold) with the orientation being random;
2) entry of the virus into invaginations of modified ("thickened") cell membrane;
3) penetration into the cell via small vacuoles formed from the thickened cell membrane.
Under the conditions used, fusion occurred very rarely (less than 1% of the rate of viralpexis).

Attempts to duplicate the published procedures which led predominantly to fusion are now under way. The major technical difference is that synchronization of infection is achieved by high speed centrifugation of virus-cell mixtures. If fusion can be obtained by manipulation of experimental conditions, it seems possible that fusion in the VSV/L cell system may be an artifact.

(B) I am interested in studying aspects of the cellular genetic control of in vitro transformation. For example, it seems possible that certain

mutations of the cellular genome could derepress all or part of a set of genes (viral genome?) involved in transformation. Since guinea pigs have a remarkably low rate of spontaneous neoplasia, it should be possible to manipulate guinea pig cell cultures with a minimal risk of spontaneous transformation.

A series of transformed sub-lines of guinea pig embryo cells, following treatment with benz(α)pyrene and 3-methylcholanthrene, are currently being isolated. These cloned derivatives will be screened in the electron microscope for viruses before and after standard induction procedures. This work is in a preliminary stage, and screening has not yet been attempted.

(C) I have observed that when anti-VSV serum is incorporated in fluid medium overlaying L cells infected with a few infectious VSV, plaques are produced which enlarge with time but do not increase in number. This apparently indicates that infectious VSV may spread from cell to cell but does not spread through the medium.

Preliminary ultrastructural analysis of undisrupted monolayers have been complicated by the presence of large quantities of extracellular antibody-coated (and inactivated) virus, but it appears possible that the spread of infectivity may be due to the rapid passage of complete virus from cell to cell wherever adjacent cell membranes are close enough to prevent access of the emergent virus particle to antibody. This preliminary conclusion is not certain, however, and the transfer of infectivity could conceivably occur via a sub-viral particle.

Significance to Biomedical Research and the Program of the Institute:

(A) The major result of this project is the demonstration that a quantitative use of the electron microscope may permit the resolution of an often controversial issue that resisted qualitative approaches. In the particular case of VSV penetrating L cells, it is not yet possible to rule out fusion as a productive mode of entry, but it is clear that, under the conditions used by most virologists to infect monolayers, VSV penetrates L cells via viralpexis. It should be emphasized, however, that the evidence for fusion is more compelling in other virus systems, and it is entirely possible that either mechanism for penetration might lead to productive infection depending upon the particular cell and environment. If it becomes possible to manipulate the means by which VSV can be made to enter cells, it might be possible to manipulate other membrane-coated viruses in the same way. Fusion is an esthetically

satisfying mechanism, since a means is readily at hand for the entry of viral nucleic acid into the cell cytoplasm. If it turns out that virus present in small vacuoles is also infectious, as seems the case here, it will be necessary to explain exactly how viral nucleic acid becomes functional.

(B) At the early stage of this investigation, it is difficult to access its potential importance. It seems clear from work with mice and chickens that the cellular genome exerts an enormous influence upon the expression of indigenous oncogenic RNA and DNA viruses. Guinea pigs have posed something of a problem because it is relatively difficult to induce and work with their viruses. It seems possible that rather than approaching this problem from the standpoint of the virus, it should be possible to alter the genetic makeup of the cell in order that the virus genome(s) are readily expressed. The production of a variety of cell mutants varying in their ability to express virus genome functions is of obvious interest.

(C) This system may be a model for studying persistant and latent infections. A simple explanation of long term persistance of lytic virus in animals in the presence of circulating antibody is that the virus never has to enter the blood system. It is also likely, however, that some additional form of sequestering of the virus may be necessary to fully protect the virus from other types of immunological response. For example, it is possible that viral mutants that are unable to mature would occur in rare individuals, thus minimizing the occurrance of viral antigen on the surface of infected cells and permitting long term persistance. It does not seem excessively far-fetched to hypothesize that oncoronaviruses may have originated in such a manner.

Proposed Course:

(A) It is hoped that this project will be completed in the near future. It is desirable to extend the kinetic analysis to relatively low multiplicities of infection; hopefully approaching those used in normal biological techniques. In theory, this simply requires greatly increased scanning time to permit the counting of several thousand cell profiles per time point rather than several hundred. Some efforts to match the ultrastructural results with biological results, using normal multiplicities, will also be carried out.

(B) If it becomes apparent that virus can be induced in at least a fraction of chemically transformed guinea pig cells, an effort will be made to isolate a relatively large number of clones following treatment

with what are usually thought to be mutagens (rather than carcinogens). If indigenous viral genomes are under the control of the cellular genome, it should be possible to find evidence of virus production, or antigen synthesis, in a fraction of these sub-lines.

(C) The most reasonable approach to this problem would be to produce temperature-sensitive mutants incapable of synthesizing coat antigen at the non-permissive temperature. If such mutants are capable of spreading from cell to cell at the non-permissive temperature in the presence of antiserum, a subviral form of the virus might be responsible for such a type of persistance.

Honors and Awards:

None

Publications:

None

SUMMARY REPORT

Office of Program Resources and Logistics

The Office of Program Resources and Logistics within the Office of the Associate Scientific Director for Viral Oncology is responsible for the review and scientific management of collaborative research contracts providing resources and logistical support to intramural investigators within Viral Oncology and collaborating laboratories participating in the NCI Special Virus Cancer Program, and is responsible also for the day-to-day general management of resources distribution. This Office was established in June, 1972 to centralize the scientific administration and management of research resources and logistical functions and to unify these activities within the Office of the Associate Scientific Director. In this way individuals responsible for coordinating these support activities are able to provide to the entire Program with an awareness of the overall scope of activities. This has avoided any special consideration to a particular area, unnecessary duplication of effort, and the appearance of undesirable competition within the Program.

Many of the research investigations carried out in the Viral Oncology Area depend on the availability of clinical and laboratory materials of optimal purity, viability, and potency. Comparable studies in an integrated program of international scope, as encompassed in the SVCP, make more meaningful and rapid progress when adequate quantities of standardized reagents, cell cultures, and test animals are available. The Office of Program Resources and Logistics provides these supportive activities through contract operations representing four general areas of activities. These include:

1. Activities directed toward production and characterization of purified viruses and viral reagents.

2. Activities concerned with acquisition, collection, storage, inventory and distribution of normal and malignant human specimen material.

3. Activities concerned with animal resources, including production of pathogen-free and germ-free species of animals, breeding of primates, maintenance of animal colonies, and containment-type holding facilities.

4. Activities directed toward the provision of specialized testing services for the examination of experimental materials.

The overall requirement for research resources and the number and extent of requests received frequently exceed the availability of Program resources. This is due, to some extent, to the high cost of producing or preparing certain scarce reagents, to fiscal considerations which limit the ability to prepare every potentially necessary item, and to a valid

reluctance to prepare highly specialized and expensive materials which can be utilized by only a few laboratories. Because requests do exceed availability, the distribution of resources is influenced by the relative value of specific contract research in relation to overall Program goals. These relative values, or priorities, are determined by the joint Program Segment Chairmen, the individual Segment Working Groups, the OPR&L Advisory Group, and the VO Branch and Associate Branch Chiefs. The distribution of resources is patterned on the evaluations and recommendations of these groups.

During the past year, this Office has coordinated the distribution to intramural investigators and SVCP participants of a wide variety of biological, chemical, and materiel resources. The amount of material processed has been enormous. To illustrate the scope of activity, during this year the purified and concentrated products from over 25,000 liters of tissue culture-grown viruses were distributed to over 50 participating laboratories throughout the world. Additionally, approximately 500 grams of a single agent, not propagated in tissue culture, was sent to over 40 different investigators. Furthermore, the Program repositories handled or shipped out over 50,000 items. In addition to these activities, the Office has coordinated the distribution to U.S.S.R. scientists of a variety of resources in keeping with the Memorandum of Understanding signed in Moscow on November 18, 1972 covering the mutual exchange of cancer research materials.

Additionally, a recent analysis covering a twelve-month time period of the distribution of Program resources by type of recipient has revealed the extent to which collaborating investigators have been supplied by this Office with research resources. NIH intramural investigators received 18%, collaborating SVCP laboratories 71%, and non-Program participants 11%, respectively, of the total resources effort. For both the collaborating laboratories and the non-participating investigators, approximately one-half of the total resources were distributed to scientists affiliated with academic institutions.

To help assess the extent to which various types of resource management systems may apply to the management of the new National Cancer Program, a pilot study was conducted during this year to determine the acceptability of alternative resource management systems to the SVCP scientific community. This survey was independently performed by Auerbach Associates, Inc., and no NCI personnel were involved in the interviewing of SVCP participants, tabulation of the data, or analysis of the results. During the study 26 scientists and 19 administrators were interviewed from 19 different contract organizations. The tabulation and analysis of the interview data indicated an overwhelming preference by the scientific community for an NCI-SVCP operated centralized resource support system. Those interviewed believed that an SVCP centralized system would be the most effective, would be the most equitable, and would provide the most efficient overall utilization of scientific research time.

Another activity of the OPRL is the preparation of an annual catalog listing and describing the research resources available to collaborating laboratories within the Program. Usually the information provided for each item includes origin, processing procedure, degree of purity, and infectivity titer or other measures of biological activity. This year a completely revised new edition of the catalog was prepared and distributed to Program participants. Loose-leaf binding was adapted to permit efficient future updating of information, and the format and contents were revised to expedite the recovery of items or services and reflect current research interests. Additionally, in collaboration with the Office of Program Analysis and Communication, the OPRL is concerned with the development of a computerized central inventory for the sera, tumor tissue, cell outgrowths, and other human specimen materials continuously being acquired by the Program. The central inventory will greatly facilitate matching investigators requests for human materials with specimens available, regardless of the geographic location of the repository or laboratory at which it is stored.

Within the Office of the Chief, which is currently understaffed, support is provided by one staff scientist and a secretary who assist in responsibilities for the management of the extramural contract operation and the coordination of resources distribution.

SUMMARY REPORT

VIRAL LEUKEMIA AND LYMPHOMA BRANCH
July 1, 1972 - June 30, 1973

The Viral Leukemia and Lymphoma Branch conducts research designed to elucidate the role of viruses in the etiology of human neoplasms, particularly leukemias, lymphomas and sarcomas. A variety of scientific approaches are used which provide a broad base of knowledge applicable to the identification and isolation of human oncogenic agents and the prevention or control of the disease as it occurs in man. More specifically, the Branch encompasses a range of scientific disciplines including molecular biology, genetics, immunology, biochemistry, pathology and cell culture techniques. In the past year, the emphasis has been away from model systems to the more direct study of human materials.

The Section of Molecular Biology seeks to obtain comprehensive knowledge of the biology and biochemistry of sarcoma and leukemia viruses and conducts quantitative studies on the interaction of oncogenic viruses and cells to determine the mechanisms of viral replication and cellular transformation at the molecular level. The Section of Viral Pathology exerts a multidisciplinary approach towards the in vivo and in vitro study of viral oncogenesis. The areas of study include virology, immunology, pathogenesis and the interferon system, and are pursued emphasizing several viral induced and spontaneous leukemias and sarcomas. The Section of Immunology examines the antigenic nature of oncogenic viruses and the induced tumors as well as the immune response of the host to viral infection and tumor development. The Section of Tumor Viruses is concerned with defining in detail the biological and biochemical properties of tumor viruses in order to understand how they may be applied to the search for human tumor viruses. A "helper" assay to "rescue" oncogenic virus information is currently being applied to human cell systems. The Section of Genetics is concerned with genetic factors of both the tumor virus and the host it infects that are involved in the oncogenic process. Particular emphasis is placed on the viral genes involved in oncogenesis and cellular "susceptibility" genes, particularly those genes of man that predispose individuals to the development of cancer. The Office of the Chief coordinates the research of the various sections while recognizing the scientific freedom of the individual investigators. The office is responsible for establishing collaborative efforts with investigators in other areas of NIH and elsewhere such that information derived from studies within the Branch is constantly being applied in investigations leading to a better understanding of the etiology of human neoplasia.

Potential RNA containing tumor viruses have been recognized by a number of methods based on biological, biochemical, and immunological properties. The reverse transcriptase has provided another potentially extremely sensitive method for virus detection.

The discovery that certain RNA tumor viruses have an enzyme capable of transcribing the viral RNA back into DNA has lead to the possibility of using extremely sensitive biochemical probes to search for evidence of viral etiology of cancers, and especially, cancers in man. Some of the potential applications to the etiology and control of human cancers are:

1. The use of synthetic DNAs produced from the viral RNA to search for complementary RNA in human tumors by DNA-RNA hybridization techniques.

2. The use of highly effective synthetic templates and optimal enzymatic conditions to search for viral reverse transcriptase in human tumor cells.

3. The use of specific antiserum prepared against the purified viral enzymes to identify individuals that have been exposed to the viral enzyme. It is reasonable to expect that the antibodies to viral specific proteins may persist for much longer periods than the virus itself would persist.

Each of the above approaches are being actively followed by members of the Viral Leukemia and Lymphoma Branch.

Following the initial reports of RNA-dependent DNA polymerase in the virions of certain RNA tumor viruses, it was important to see if the enzyme was specifically restricted to tumor viruses and whether it was specifically restricted to tumor cells. All of the oncogenic RNA viruses tested so far have been found to have DNA polymerase, as indicated both by endogenous reaction using the viral RNA and by synthetic polymer-stimulated reactions, using such templates as poly rA.rU, poly rI.rC and poly rA.dT. The non-oncogenic RNA viruses have shown no evidence of this enzyme activity. Two apparent exceptions were found. The first, visna virus, produces a chronic, progressive, neurological disease of sheep but has, heretofore, not beeen associated with tumors in sheep. The second major exceptions are the group of "foamy" viruses. These RNA-containing viruses are frequently found in healthy as well as diseased monkeys, cattle and cats, and they have not yet been associated with any disease. Visna and "foamy" viruses, then, are apparent exceptions to the rule that only tumor viruses contain reverse transcriptase. Whether visna and the related viruses of sheep and the various "foamy" viruses are potentially oncogenic in their natural hosts remains to be resolved.

The polymerase, as an antigen, like the gs antigen has both species specific and interspecies characteristics. Tumor-bearing animals can make antibody to the viral polymerase and some sera appear to be more broadly reactive than others. The murine polymerase has been partially purified and used to produce an antibody in rabbits. The antibody, an IgG immunoglobulin, is directed against the enzyme and not against the template. The antibody to the mouse leukemia virus polymerase will also inhibit the enzymatic activity of hamster, rat and cat leukemia virus polymerase. Thus, the polymerases from different mammalian tumor viruses

are antigenically related. However, the crosses with other mammalian type C viruses are only partial crosses allowing precise identification of the species of origin of an unknown type C virus. The mouse mammary tumor virus, visna, and the avian leukemia virus polymerases are not inhibited at all by this serum. The antibody to the avian virus polymerase inhibits all the major avian type C viruses, but not any mammalian type C virus polymerase.

Two new type C viruses of primates have recently been described. One is from a woolly monkey fibrosarcoma; the other is from a gibbon ape lymphosarcoma. Both have a polymerase with the characteristic properties of tumor viruses and can be classified as type C viruses and also have a cross-reacting gs antigen. The polymerase antibody studies, however, show a very weak or absent cross reaction with antibody to rodent or cat virus polymerase. Both the murine and feline type C viruses can grow in primate cells without losing the immunologic specificity of their polymerase. These findings provide additional evidence that the polymerase coded for, at least in part, by the viral genome. An antiserum prepared to the gibbon type C polymerase inhibits gibbon and woolly virus enzyme well, but only poorly crosses with the previously described rodent and feline type C virus polymerase.

The isolation of type C viruses from both old world and new world monkeys from naturally occurring tumors greatly strengthens the possibility that related viruses will be directly isolated from human tumors. Several reports of type C viruses in human cells have been presented in the past year. While some of these are more reasonable candidates than others, no proven "human" type C is yet available. Until such viruses are found, the type C viruses of primate origin should provide the best probes for the detection of type C information in human cells.

In 1969, it was proposed that the cells of most or all vertebrate species contain type C RNA virus genomes that are vertically transmitted from parent to offspring. Depending on the host genotype and various modifying environmental factors, either virus production or tumor formation, or both, may develop at some time during the life of these animals or in their cells when grown in culture. The evidence for this concept was derived both from cell culture experiments and from a variety of seroepidemiologic studies and was presented as a unifying concept that would be consistent with the facts as they were known at the time.

In the two years that have followed, a great deal more evidence has accumulated that provides strong support for the general theory. One particular prediction that was made was that the genetic information for making an RNA tumor virus, being present in a repressed form in all cells, would be potentially inducible by carcinogenic and/or mutagenic agents. Recent evidence from single cell clones of mouse embryo cells of both the high susceptible strain, AKR, and the low susceptible strain, Balb/c,

indicate that every cell clone in culture does contain the information for producing a type C virus. Infectious virus can be induced from clonal lines of mouse, rat, and Chinese hamster cells, normal as well as transformed, which provides dramatic support for the original hypothesis.

One of the major advances in the past year has been the characterization of the endogenous viruses contained within most, and very likely all, vertebrate cells. These viruses differ in certain of their properties from the horizontally transmitted viruses isolated from the same species. The differences are most extreme in the case of the cat where there are two essentially unrelated type C viruses. The first (FeLV) is a virus which can be horizontally transmitted and can produce tumors. The second, the endogenous virus, can be isolated from cell lines and primary cultures from a variety of cat tissues such as, cat embryo cells, kidney cells, and lung cells. The endogenous type C virus is unable to grow in cat cells, but grows readily in primate cells. It will appear spontaneously from continuous lines of cat cells and the probability of appearance can be increased by treating the cells with thymidine analogs. Six independent cat cell cultures have yielded, either spontaneously or upon induction, a virus with properties indistinguishable from the prototype endogenous cat virus, RD-114. The cat, then, offers a unique species in which to test the relative contribution of endogenous and exogenously added viruses in the development of naturally occurring tumors in that species.

As a general rule, in outbred populations, the endogenous virus that is released cannot reinfect cells that release it. Thus, a type C virus is found in Chinese hamster cells, rat cells, Syrian hamster cells, cat cells and pig cells; in each case, clonal lines can be found that spontaneously produce virus. In all the above cases, except for the cat, a highly susceptible host has not been found. For the endogenous cat virus as described above, human and primate cells are permissive hosts. The isolation and characterization of the endogenous cat viruses during the past year by VLLB scientists represents a major advance. It suggests, however, greater complexity than was previously thought. If there are two unrelated type C viruses in a single species, there could be others. The following table shows those species in which it has now been shown that complete virogene information is present in normal somatic cells of the species.

Species Where a Complete Virogene is Known to be Present in Normal Cells

Chicken
Chinese hamster*
Mouse*
Rat*
Cat*
Pig*

*Single cell clones spontaneously produce virus in long term culture

The oncogene hypothesis makes several testable predictions. The first is that all somatic cells should contain the genetic information to produce a type C virus that can be detected by using the DNA product made from type C viral RNA of that particular species. For example, normal cat cells should have in their DNA a complete copy of cat leukemia type C virus RNA, and the hamster should have in its DNA the genetic information for making a hamster type C virus. A second and, perhaps, stronger prediction is that transformed cells, whether transformed by exogenously added tumor viruses or by radiation, chemical carcinogens or even "spontaneously," should contain new messenger RNA sequences that are not found in normal cells and that are common to all transformed and tumor cells of a particular species. This information should also be contained in the type C tumor virus of that species. The new messenger RNA would be the product of the oncogene and in turn code for the production of the transforming protein(s). A third prediction is that it should be possible to isolate cell mutants that, at the nonpermissive temperature, because of a temperature sensitive repressor, would be superinducible or would possibly spontaneously produce type C virus without exogenous inducer. A final prediction, if the general hypothesis is correct, and type C viral information has been a stable part of the evolution of vertebrates, would be that the type C viruses derived from closely related species would be closely related to one another in the antigenic properties of their characteristic proteins. Two such proteins are now available--the major group specific antigen and the reverse transcriptase. The isolation of type C viruses from reptiles, birds, as well as mammals would suggest that they have evolved as the organism has evolved for many millions of years and that the species specific proteins will have evolved in much the same way that serum albumins, globulins, and other proteins have evolved. The genetic relatedness of the group specific antigens and the reverse transcriptases may well, then, be used as an index of the genetic relatedness of the species from which the type C virus was derived. Obviously, those viruses derived from higher mammals, and especially primates, will be the most related to the viruses that come to be obtained from man. The VLLB is concentrating on the primate type C viruses, because they should have enough genetic relatedness so that an antiserum produced to the purified polymerase or the purified group specific antigen should show some ability to recognize type C viruses isolated from human tissues, unless, of course, a situation exists in primates similar to that in cats where the endogenous type C virogene is very different from a horizontally transmitted virus also in that species.

The system involving the activation of the type C virus has obvious superficial similarities to the lysogenic system in bacteria. However, in many ways virogene induction might be considered more analogous to the switching on of a differentiated function by vertebrate cells. Either spontaneously or after addition of a small molecule, the cell begins producing proteins whose genes are normally repressed and assemble a rather complex package for export from the cell. BrdU has been known

to greatly affect the differentiated state in culture and to both increase and decrease the rate of only partial expression of viral genetic information. However, upon induction, new viral RNA rapidly appears. Clearly, then, one major control in this system is at the level of transcription. Whether there will be additional controls at the level of translation remains to be resolved. The induction of a lysogenic prophage involves the excision of that genetic information from the bacterial chromosome and also the lysis of the cell. In the induction of type C virus, the cells produce virus but do not die; whether this, too, involves an excision mechanism is not clear. It is possible that the system works entirely by reading off cellular genes. So in a sense, the cell lines that produce endogenous virus would not be replicating the virus, but would, rather, be transcribing and translating information that is part of their natural genetic makeup.

Other Research Developments in the Branch

Characterization of continuous, contact-inhibited mouse cell lines from Balb/c and NIH/Swiss embryo cells has provided excellent model systems for study of the effects of tumorigenic viruses both in vitro and in vivo. These cell lines, and a wide variety of well-characterized subclones from them transformed by several different agents, are supplied to numerous investigators throughout the world, and have become standard cell lines for biochemical and biological investigations of cellular growth control mechanisms.

Human tumor material has been processed, and several lines of tumor cells have been established. These are being extensively investigated for evidence of tumor virus expression using techniques developed from work in model systems.

Cell strains derived from leukemic patients are generally more susceptible to SV40 transformation than those derived from normal individuals. This extends the previous observation that cells from patients with Fanconi's anemia or Down's syndrome, have increased SV40 transformation susceptibility.

Human S+L- cells were established by infection of a continuous cell line from human amnion cells with a virus stock containing MSV (FeLV) in excess of helper FeLV. A terminal focus was cloned and established into a transformed cell line demonstrating no release of infectious focus-forming virus until superinfected with either FeLV or RD-114 virus. These superinfecting helper viruses determined the envelope-associated properties of host range and interference for the rescued MSV pseudotypes. Murine gs-1 antigen was conclusively identified in these human S+L- cells indicating that the sarcoma genome present is from MSV and not a derepressed endogenous human sarcoma genome. No type C virus production from these human S+L- cells was demonstrable, either by electron microscopy or by release of polymerase into the culture supernatant fluid.

The HBT-39 cell line, previously established from a human breast tumor, was inoculated into immunosuppressed rats. Four of ten developed progressively growing tumors whose pathological appearance closely resembled that seen in the original biopsy of the patient.

The HBT-39 cell line contains an enzyme which behaves like a viral reverse transcriptase, while other human cell lines tested to date have been negative for such.

DNA polymerases purified from human milk by ion exchange and affinity chromatography have some but not all the characteristics of "viral" reverse transcriptase.

The American Burkitt Lymphoma Registry has been continued and expanded. Ninety-two patients have been confirmed and as many as 5 patients a week are being reported. Cell lines have been established from American Burkitt Lymphoma patients. Unlike African Burkitt patients, no EBV genome could be detected in American Burkitt tumors. Studies continue to demonstrate some correlation between EBV antibody titer and prognosis.

High EBV titers were found in normal individuals who are members of families with multiple first degree relatives with cancer; this suggests that elevated EBV titers may be an indication of susceptibility to cancer rather than a specific immune response to an oncogenic virus. Studies on EBV antibody levels on patients with spontaneous cryptococcosis, a disease strongly associated with poor cell-mediated immunity indicated normal EBV titers in these patients, again providing evidence that elevated EBV titers are not simply a non-specific finding in patients with poor cell-mediated immunity.

Owl monkeys with Herpesvirus saimiri (HVS) induced leukemias responded to several chemotherapeutic agents currently used in the treatment of human neoplasia. Heat-inactivated HVS, non-infectious in vitro, was capable of inducing in vivo tumors from which the complete virus genome could be obtained.

Using antisera to the viral reverse transcriptase, it was shown that the Mason-Pfizer monkey virus (MP-MV) was unrelated to either known type C viruses or the primate syncytium-forming ("foamy") viruses. Among the type C viruses, the woolly monkey and gibbon ape viruses were found to be very closely related immunologically, but the two were only distantly related to RD-114.

The major internal protein (gs antigen) from each of the following type C viruses--mouse, cat, woolly monkey, gibbon ape, and RD-114--was purified to homogeneity, and used in species specific radioimmunoassays. The close relationship of gibbon ape and woolly monkey virus, and their difference from RD-114 was demonstrated.

A clonal isolate of a primate sarcoma virus analogous to the S+L- strain of murine sarcoma virus has been obtained. It contains woolly gs antigen and woolly polymerase. Primate and murine helper viruses have equal ability to rescue primate or murine sarcoma viruses.

A focus-forming virus from a woolly monkey sarcoma has been shown to induce transformation of mammalian cells in the absence of infectious virus production. The morphologic alteration induced by this virus is strikingly different from that induced by MSV.

An immunoaffinity chromatography system using the covalent coupling to Sepharose of IgG from an immune serum directed against the gs-3 mammalian intraspecies determinant has been developed. Crude murine antigen containing extracts can be chromatographed on such columns to yield highly purified gs protein.

Radioimmunoassay for mammalian interspecies gs-3 antigen detected reactivity in purified pig type C virus, leukemic bovine cells and rabbit lymphosarcoma tissues. In human tumors the results are still not conclusive, but have generally been negative.

An endogenous virus (CCC) of a single cell clone of feline fibroblasts has been isolated. It has a reverse transcriptase, gs antigen and host range distinct from FeLV and similar to RD-114. CCC and RD-114 both grow well in human and primate cells and poorly on feline cells. FeLV, in contrast, grows well on feline cells and poorly on monkey and human cells. Several type C viruses with properties similar to RD-114 and CCC have been isolated from cat embryo cells and even from stocks of FeLV (Rickard and Theilen strains).

The relatedness of type C viruses in hybridization experiments shows that within one family of viruses there is usually greater than 50% homology (for example, between woolly and gibbon viruses or between Rauscher and Kirsten viruses) while between families there is little or no detectable homology. One exception is that the endogenous type C cat virus (CCC) and RD-114 while closely related to each other are not related to the Rickard or Thielen strains of feline leukemia virus.

MTV-associated soluble antigen (MSA), was detected in the plasma of MTV^+ mice by the indirect immunofluorescence test. Anti-mammary tumor serum, after preabsorption with G and other leukemic cells, specifically reacted with reference mammary tumor cells and MSA adsorbed onto the surface of indicator cells. MSA was positive in mice bearing either primary or serially transplanted spontaneous mammary tumors of syngeneic mice. The incidence of MSA increased progressively in normal C3H/He mice with age. No MSA was found in C57BL/6 mice (MTV^-) of any age.

Chemicals such as BrdU can transiently activate endogenous type C viruses from Balb/c mouse embryo cells, but not from NIH Swiss cells. A

single genetic locus responsible for inducibility of virus in Balb/c cells was detected and designated Ind. A second locus, previously described in studies of mouse cell susceptibility to exogenous virus infection, Fv-1, was found to be genetically nonlinked to Ind. This regulatory gene plays an important role in determining whether the induced viruses of Balb/c cells can persist after chemical activation. MSV-transformed nonproducer mouse and rat cells can be activated by BrdU to produce both MSV and MuLV.

Some spontaneously transformed clones of Balb/3T3 were found to spontaneously and continuously release high titers of the endogenous type C virus. Other transformed subclones were found to be virus-free, but treatment with BrdU induced production of the endogenous virus in large amounts. Non-transformed Balb/3T3 clones never spontaneously released type C virus, and could be induced to release very small quantities only with BrdU; in contrast, cells transformed by mouse sarcoma virus, radiation, methylcholanthrene and also spontaneously were "superinducible," i.e., BrdU treatment caused virus production within 8 hours and resulted in secretion of very large quantities of virus with exponential kinetics.

All murine cell lines examined so far have been found to contain RNA which is homologous to 35S RNA of murine leukemia virus. After purification of cytoplasmic RNA by dT-cellulose chromatography to remove RNA not containing poly-A sequences, and hybridizing this RNA to the ^{3}H-DNA product it was found that a "normal" murine cell line, A31, transcribes enough RNA to saturate 2-4% of the ^{3}H-DNA product. Murine cell lines transformed either spontaneously, by radiation, or by SV40 all transcribe an additional amount of RNA so as to saturate about 10% of the ^{3}H-DNA viral probe. Normal rat cell RNA, poly-A, or dT-cellulose purified RNA from an SV40 transformed human cell line do not hybridize to the ^{3}H-DNA murine probe.

Natural expression of the mouse gs antigen was found in all mouse tissues examined, strongly suggesting that a continuous synthesis of this polypeptide is an integral part of the murine cell macromolecular synthesis.

S+L- Balb/3T3 and S+L- NRK clones have been established in addition to the original S+L- 3T3 cells. All 3 classes of cells release noninfectious virus-like particles, contain murine leukemia virus gs antigens, and respond to superinfection with leukemia virus by lytic focus formation. Human S+L- cells, however, exhibit only some of these properties.

A virus has been isolated in immunosuppressed NIH Swiss mice bearing human rhabdomyosarcoma cells (RD). This virus, AT-124, has mouse virus polymerase and mouse virus gs-1 antigens, yet its host range is primarily human and primate. It grows in none of the known mouse cell systems. A pseudotype with this virus readily transforms human cells.

As a general rule, it has been found that endogenous viruses of several species are unable to reinfect the cells from which the virus is produced. For example, the endogenous Balb/c cell virus does not grow on Balb/c cells, but will grow in NIH Swiss mouse cells. The endogenous cat virus (CCC) does not grow in cat cells, but grows readily in primate cells. Similarly, endogenous viruses have been obtained in the past year from several other species; such as, Chinese hamsters, Syrian hamsters, rats, and pigs. In all these cases, the virus does not infect the clonal lines that release it. Further, in all these cases the virus will appear spontaneously in long-term spontaneously transformed cultures. It appears that spontaneously transformed cells more readily release their endogenous virus than do untransformed cells. Since the mouse, the chicken, and the cat clearly have recognizably different endogenous and horizontally transmitted type C viruses, the central question becomes which, if either, of these viruses is responsible for naturally occurring tumors in that species. In the cat, CCC and FeLV are easily distinguishable. The endogenous Balb/c virus by immunoelectron microscopy can be distinguished from both the viruses of the FMR group and the Gross leukemia viruses. They are, however, related to the viruses found in spontaneously occurring myelomas of Balb/c mice. Thus, methods are available even in the mouse system to distinguish the endogenous virus from some of the laboratory strains.

Spontaneous reversion of MSV transformed mouse cells to normal phenotype was observed at high frequency. The revertant cells were epithelioid, contact inhibited, grew to low density, and their low cloning efficiency in soft agar was similar to that of normal parental 3T3 cells. They generally contained murine leukemia (MuLV) group-specific antigen(s) without demonstrable virus production and reverse transcriptase activity. MSV could no longer be rescued from these flat revertant cells by superinfection with MuLV, by cocultivation with normal 3T3 cells or by transpecies rescue into cat cells. These revertants spontaneously gave rise to retransformed cells during extended cultivation. Morphology, saturation density, and cloning efficiency in soft agar of cloned spontaneous retransformed cell lines were similar to the original MSV-transformed cells. However, they failed to demonstrate MuLV gs antigen(s), virus production, reverse transcriptase activity and a rescuable MSV genome; both cocultivation with homologous or heterologous cells, and chemical induction with halogenated pyrimidines failed to detect an infectious sarcoma virus.

The spontaneous reversion rate of S+L- cells was increased by treatment with colcemid or fluorodeoxyuridine (FdU). Spontaneous retransformants of FdU revertants, however, were like the parental S+L- cells in that MSV was rescuable from them (which was not observed in the spontaneous or colcemid treated revertants).

The noninfectious particles produced by S+L- mouse cells do possess a viral reverse transcriptase activity which is indistinguishable from that of infectious leukemia particles. Most revertants of S+L- murine cells do not produce particles and cannot be chemically induced to produce particles.

MSV nonproducer cells have been found to lack any detectable transplantation antigens in contrast to RNA virus-producing transformed cells which are highly antigenic.

Twenty-seven temperature sensitive leukemia virus mutants have been isolated from clonal stocks of Kirsten and Rauscher leukemia viruses. Each mutant transmits to new cells with efficiency comparable to that of wild-type MuLV at the permissive temperature, but is at least 4-5 logs less efficient than wild-type at forming XC plaques at the non-permissive temperature. The mutants all have very low rates of reversion to wild-type. These have been separated into three distinct classes based on the stage at which viral replication is blocked at the nonpermissive temperature.

Using similar procedures, three separate murine sarcoma virus nonproducer cell lines have been isolated which are temperature sensitive for the maintenance of transformation. In each case, a viral rather than a cellular genetic mutation is the reason for the temperature-sensitive effect. Superinfection of one of the mutants with murine leukemia virus overcomes the temperature-sensitive change in the transformed state.

Morphologic revertants which contain avian or murine sarcoma viruses have been isolated from clonal lines of transformed mammalian cells. These lines are indistinguishable from nontransformed parent cell lines with respect to parameters such as saturation density and colony formation in depleted medium or on monolayers of contact-inhibited cells. The rate of glucose uptake had also reverted to normal. The malignant potential of one of the revertant lines was examined and found to be markedly reduced compared to that of the corresponding transformed cells. With these virus and cell mutants, it is hoped that the viral protein directly responsible for the maintenance of the transformed state can be studied.

A low molecular weight polypeptide (16,000 daltons) was isolated from Rauscher murine leukemia virus. A very sensitive and highly specific radioimmunoassay was developed for its quantitation. This polypeptide is virus-coded and antigenically distinct from another virion protein, the group-specific (gs) antigen. Different strains of MuLV contain antigens immunologically cross-reactive with the low molecular weight polypeptide. The presence of this polypeptide was detected in mouse and rat cells transformed by the S+L- sarcoma genome but not in the same cells transformed by the MSV nonproducer genome. The assay for the 16,000 dalton polypeptide was used in studies of temperature-sensitive mutants of MuLV. The polypeptide was detected in some but not

other ts mutant-infected cells at the permissive, but not the nonpermissive temperature.

MSV strains transform rat liver cell cultures. Both parent and transformed cells have multiple biochemical markers indicating liver origin. Rat tumor induced by inoculation of the transformed cells histologically resemble hepatomas.

There exists on the surface of MuLV virus particles a small (10-15,000 MW) component which was purified by two cycles of preparative gel electrophoresis, whose ability to enhance MuLV infectivity and to protect the virus from neutralizing antibody, was quite unexpected. The effect was on the MuLV virion and not on the cells, and only tissue culture derived virus was susceptible to its action.

Hamster tumors induced by Gz-MSV, and their cell cultures, release non-infectious virus-like particles (S+H- virus). The cells, and the virus, express mouse gs-1 antigen. After repeated in vivo or in vitro passage, some lines also express hamster type C virus (HaLV) gs-1 antigen. These cells release the HaLV pseudotype of Gz-MSV. A comparison of the Gz-MSV (HaLV) and S+H- viruses helps characterize the nature of defectiveness of Gz-MSV and the contribution of helper viruses. S+H- virus can express mouse gs-1 antigen but is non-infectious for mouse and hamster cells, and reverse transcriptase levels are very low. All of the virions have electron lucent nucleoids and many broken or incomplete virions are present. In contrast, Gz-MSV (HaLV) is positive for both mouse and hamster gs antigens, the levels of reverse transcriptase are relatively high, the virus is a hamster-tropic sarcoma virus, and its virions have both electron-dense and lucent nucleoids. The defectiveness of S+H- virus may be related to the small size of its viral RNA (53S RNA). While S+H- virus is non-infective, the expression of mouse gs antigen and presence of sarcoma genome in its virions makes it the least "defective" of the known murine sarcoma viruses.

Interferon inducers enhance the growth of transplanted and virus-induced tumors. Rat and mouse tumors releasing type C viruses grow slower than non-infected lines, perhaps as a result of increased antigenicity. The presence of type C virus is apparently necessary for interferon inducer mediated tumor enhancement. Treatment with potent interferon preparations also enhanced tumor growth. This effect was species specific, pH resistant, heat sensitive, and dose dependent, suggesting that it is a specific interferon action.

A clonal line of S+L- 3T3FL cells can produce exceptionally high yields of interferon. Concentrated preparations have interferon titers of up to 10^6 units/ml. Injection of mice with such preparations results in serum levels of interferon comparable to those induced by polynucleotides.

Murine mineral oil induced myeloma-associated virus (MuMAV) carries a specific viral envelope antigen, xVEA, different from those on MuLV. Endogenous type C viruses released from spontaneously transformed Balb/3T3 cells can be classified into at least two different populations: (a) type C viruses which carry xVEA envelope antigen, and (b) uncharacterized type C viruses which have neither xVEA nor the MuLV envelope antigens. Various anti-murine type C virus sera reacted differently with the envelope of various murine tumor-associated type C viruses, suggesting the presence of a variety of type C viruses in terms of envelope antigen specificities.

GSA in the body fluids of AKR and C58 mice were classified according to the known specificity of G-antigens in the murine Gross leukemia system. GSA showed the several specificities corresponding to G cell surface antigens, GCSAa, b, and c, and type-specific and group-specific viral envelope antigens, tsVEA and gsVEA, respectively. However, the plasma of C58 mice lacks GSAc. GSA corresponding to G_{IX} antigen was not detected in the body fluids at all. In addition, G soluble antigens were obtained from Gross leukemia cells by repeated washing with saline and by autolytic sulfate precipitation. Sephadex G-150 chromatography indicated heterogeneity in the molecular forms of the antigens. The specificity of these solubilized antigens contained both G cell surface antigens and viral envelope antigens.

Immunoelectron microscopy has demonstrated an MSV-associated cell surface antigen on the surface of nonproductively transformed cells infected by two different strains of MSV, Kirsten, and Moloney. This antigen is distinct from previously described antigens on the surfaces of cells infected by murine leukemia virus, on the viral envelope, and on the surfaces of cell lines transformed spontaneously or by x-irradiation.

The serum of aged NZB mice reacts mainly with the viral envelope but not with the surface of murine virus-induced leukemias. However, recently minute positive areas have been occasionally found on the cell surface of murine leukemias and myelomas by immunoelectron microscopy. These results suggest the formation of cell surface antigens common to those on the viral envelope. This has been supported by the results obtained in the avian sarcoma system; gs^+ and cellular helper factor$^+$ chick embryo fibroblasts absorbed out the activity against Rous sarcoma viral envelope antigens from the chicken anti-Bryon Rous sarcoma virus serum.

An affinity chromatographic procedure for the avian type C viral polymerase was developed. Using this procedure, viral enzyme can be selectively purified from extracts of virus producing avian cells. Extracts of Rous sarcoma virus transformed rat cells (XC cells) which synthesize no virus but do contain easily detected quantities of the avian gs antigen(s), failed to show any viral polymerase.

Other Activities within the Branch

In addition to their intramural research activities, several of the senior investigators within the Branch spent a substantial portion of their time in support of the Special Virus Cancer Program. The members serve as Chairmen, Vice Chairmen, Work Group members and Executive Secretaries of segments of the Program. They provide scientific guidance as Project and Assistant Project Officers on research contracts supported by the Special Virus Cancer Program.

The activities of the SVCP and the direction of the internal research program of the Branch are aimed at the common goal of the determination of the viral etiology of human cancer. It is apparent that the efforts of the Branch members have played a significant role in the progress of the SVCP to date. The broad scientific perspective developed by these investigators in their SVCP activities has also contributed significantly to the direction of the Branch program for the attainment of research goals.

The effective functioning of senior personnel in dual capacities, i.e., in-house research and program administration requires a delicate balance of effort. It must be realized through constant monitoring, that such a balance does exist and over-emphasis in either direction would be to the detriment of both programs. It has become clearer during the past year that an understanding of the suspected relationship between tumor viruses and human neoplasia requires an interaction between, among others, highly skilled molecular biologists, epidemiologists, cell biologists and physicians, along with sound and constructive administrative support; the answers will come from no one discipline alone.

Serial No. NCI-4821

1. Viral Leukemia and Lymphoma Branch
   OASDVO, Division of Cancer
   Cause and Prevention
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Genetic and environmental factors in susceptibility to endogenous and exogenous tumor virus information

Previous Serial Number: 4904, 4906, 4919

Principal Investigator: Dr. George J. Todaro

Other Investigators:
Dr. Raoul Benveniste
Dr. Isaac C. Henderson
Dr. Paul H. Levine
Dr. Michael M. Lieber
Dr. David M. Livingston
Dr. Edward M. Scolnick

Cooperating Units:

Inside NIH
Dr. Joseph Fraumeni, EPID, NCI
Dr. Robert Huebner, VC, NCI
Dr. Wade Parks, VC, NCI
Staff, VLL

Outside NIH
Meloy Laboratories, Springfield, Virginia
Bionetics Laboratories, Bethesda, Maryland
Electronucleonics Laboratories, Bethesda, Maryland
Naval Biological Research Laboratory, Oakland, California
Dr. John Kersey, University of Minnesota, Minneapolis, Minnesota

Man Years:

Total:	1.25
Professional:	1.00
Other:	0.25

Project Description

Objectives:

1. Determine the mechanism by which cells prevent and/or express endogenous oncogenic virus information.

2. Study how agents such as radiation, chemical carcinogens and viruses act alone or in concert to facilitate cell transformation.

3. Study the ability of RNA and DNA from tumor viruses to transform human cells.

4. Determine the basis for the difference in susceptibility of cells to exogenously added oncogenic RNA viruses.

5. Characterize endogenous and horizontllly transmitted type C viruses and their relationship to "spontaneous transformation" in cell culture.

6. Devise methods to find, isolate and characterize putative endogenous type C virus of primates and man.

7. Determination of the reason for the difference between normal and susceptible human cell strains with regard to transformation by oncogenic virus.

Methods Employed:

Standard techniques of tissue culture, biochemistry, and immunology, virus isolation and virus antigen detection are being used.

Major Findings:

1. Long term cell cultures which spontaneously transformed were found, with high probability to release endogenous type C virus. Spontaneously transformed subclones of the continuous cell line, Balb/3T3, have yielded virus-producing lines, and the properties of the endogenous virus have been compared with more conventional horizontally transmitted MuLV strains. Certain transformed cells not releasing virus were found to be "superinducible" for type C virus, making 5 to 15 times as much virus per cell as the untransformed Balb/3T3 parent. Endogenous type C virus can be detected in supernatent fluid within eight hours after the addition of thymidine analog inducers.

2. Spontaneously transformed subclones of Balb/3T3 were compared to clones transformed by DNA viruses, RNA viruses, chemical carcinogens and radiation with respect to their tumorigenicity, ability to release endogenous virus, and degree of expression of endogenous viral information. The transformed cells were tumorigenic under conditions where the normal cells were not. The viral specific gs antigen could

be found in all cells, normal as well as transformed. Viral specific information could also be found in all cells, although there seemed to be greater expression in the transformed cells as compared to the normal cells.

3. A tissue culture system using an SV40 transformed human cell line (SV cl 80) has been developed to study the transforming effect of various sarcoma virus pseudotypes. This continuous line offers several advantages compared to diploid cells; transformed foci are recognized within five days, and the system lends itself to easy quantitation of transformation. Pseudotypes with mouse sarcoma information have been produced with the various mammalian type C viruses, and the interference pattern, as measured by the ability to inhibit focus-formation on the SV40 transformed human cells, has been determined. A virus has been isolated in immunosuppressed NIH Swiss mice bearing human rhabdomyosarcoma cells (RD). This virus, AT-124, has mouse virus polymerase and mouse virus gs-1 antigens, yet its host range is primarily human and primate. It grows in none of the known mouse cell systems. A pseudotype with this virus readily transforms human cells.

4. As a general rule, it has been found that endogenous viruses of several species are unable to reinfect the cells from which the virus is produced. For example, the endogenous Balb/c cell virus does not grow on Balb/c cells, but will grow in NIH Swiss mouse cells. The endogenous cat virus does not grow in cat cells, but grows readily in primate cells. Similarily, endogenous viruses have been obtained in the past year from several other species; such as, Chinese hamster, Syrian hamster, rat, and pig. In all these cases, the virus does not infect the clonal lines that release it. Further, in all these cases the virus will appear spontaneously in long-term spontaneously transformed cultures. The probability of virus release, however, can be increased by treating the cells with thymidine analogs, such as iododeoxyuridine. The findings in the past year in mouse cell lines suggest the possibility that spontaneously transformed cells more readily release their endogenous virus than do untransformed cells. The endogenous Balb/c virus by immunoelectron microscopy can be distinguished from both the viruses of the FMR group and the Gross leukemia viruses. The endogenous viruses, however, are related to the viruses found in spontaneously occurring myelomas of Balb/c mice. Thus, methods are available even in the mouse system to distinguish the endogenous virus from some of the laboratory strains.

5. Human tumor cell lines have been developed in culture. These include carcinomas, sarcomas, melanomas, and brain tumors. In addition, diploid fibroblast strains have been transformed by SV40 and permanent cell lines have been established from them. Using these cultures, attempts have been made to develop evidence for type C virus expression. As of this writing, there is nothing conclusive. However, the evidence from the model systems, and in particular, the cat would suggest that one of the major problems would be to find a permissive host for the endogenous

human virus, and that permissive host may be cells from a species quite far removed, in an evolutionary sense, from man. Normal diploid cells, their transformed counterparts, and tumor cells are being compared with one another using various probes for evidence of type C virus activation.

6. Studies are continuing to characterize normal and susceptible human cell strains with respect to transformation by the oncogenic virus, SV40. Cell strains derived from leukemic patients are generally more susceptible to SV40 transformation than those derived from normal individuals. This extends the previous observation that cells from patients with Fanconi's anemia and cytogenetic diseases, such as Down's syndrome, have increased SV40 transformation susceptibility. Cells from patients with immunodeficiency diseases who are at high risk of developing cancer, however, were not more susceptible to SV40 transformation than normal controls. The results suggest thea one factor in susceptibility to transformation by a tumor virus can be studied in cell culture. High-susceptible and low-susceptible cell strains with respect to SV40 transformation are being tested for their susceptibility to transformation by RNA tumor virus and by chemical carcinogens.

Significance to Biomedical Research and the Program of the Institute:

These projects relate to the nature of the interaction between tumor viruses and the cells that contain them. Development of techniques in the model systems are frequently applicable to the search for human oncogenic viruses. The methods by which the Program can hope to detect or to prevent virus-induced tumors will depend on how the viruses produce their effect. In general, this may be via the addition of genetic information to the cell from the outside, or, via activation of previously repressed genetic information. Which of these is the more critical under natural conditions for cancer in man is of paramount importance in determining how best to interrupt or prevent this process.

Proposed Course:

1. To define the level at which cells prevent the expression of endogenous virus information and to determine where in the cell the virus information physically resides. In addition, the project will be directed towards finding specific repressor substances that may control type C viral expression and to develop techniques that utilize radiation, chemical carcinogens, and viruses to produce human cell transformation and/or activation of type C virus in tissue culture.

2. A combination of methods that have recently become available will be used to try to resolve whether type C viruses are present both in human tumor cells and in normal human cells. If they are, we will try to determine how they are transmitted and their importance as etiologic agents in human cancer. The factors responsible for both susceptibility and resistance to viral infection will be studied with the goal of characterizing these factors in biochemical terms. Simple in vitro

tests measuring a person's risk of developing cancer in reasonably large population samples will hopefully resolve whether major differences exist between normal and those that actually develop cancer.

3. To characterize the biology and immunology of the viruses that clonal cell lines spontaneously produce.

4. To resolve, in the cat, which of the two groups of type C viruses that are known are responsible for naturally occurring tumors.

Honors and Awards:

Gustav Stern Award for Virology, 1972.

Publications:

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Serial No. NCI-4822

1. Viral Leukemia and Lymphoma Branch
   OASDVO, Division of Cancer Cause
   and Prevention
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: (A) EBV Studies in Humans and Non-Human Primates
(B) The Role of RNA Viruses in Human Leukemia and Breast Cancer

Previous Serial Number: NCI-4811

Principal Investigator: Dr. Paul H. Levine

Other Investigators: None

Cooperating Units:

Inside NIH

Dr. Ronald Herberman, GL&C, NCI
Dr. Paul Gerber, DBS, NCI
Dr. Dharam Ablashi, VLLB, NCI
Dr. Costan W. Berard, LP, NCI
Dr. Gregory T. O'Conor, LP, NCI
Dr. Deward Waggoner, PAC, NCI
Dr. Joseph Fraumeni, EPID, NCI
Members of the Medicine, Viral Biology, Immunology-GL&C, and Epidemiology Branches, NCI

Outside NIH

Dr. E. Perlin, National Naval Medical Center, Bethesda, Maryland
Dr. L. Dabich, Simpson Memorial Inst., Ann Arbor, Mich.
Dr. Peter Ebbesen, University of Denmark, Copenhagen
Dr. Paul Terasaki, UCLA, Los Angeles, Calif.
Bionetics Research Laboratories, Bethesda, Maryland

Man Years:

Total:	1
Professional:	1
Other:	0

Serial No. NCI-4822

Project Description

Objectives:

Project A

1. To develop seroepidemiological studies using viral capsid antibody and antibody to the early antigen to determine whether EBV is etiologically related to human lymphoma.

2. To investigate the role of cellular immunity to EBV as a possible controlling factor of antibodies to the viral capsid antibody.

3. To correlate the immune response to EBV-associated antigens and lymphoma-associated antigens in order to determine if there is a relationship between the two.

4. To evaluate the genetic factors controlling the immune response to EBV and susceptibility to lymphoma.

Project B

1. To pursue preliminary studies indicating that there is an antigen in human tumor cells which cross-reacts with murine and/or feline leukemia viruses.

Methods Employed:

Project A

1. A registry of American Burkitt Lymphoma patients was continued in order to obtain a population to compare clinically, epidemiologically, and virologically with the African Burkitt patients. Blood samples were collected from a variety of lymphoma patients, particularly those with American Burkitt's Lymphoma and Hodgkin's disease, as well as normal controls. Cell lines known to contain EB virus, fresh tumor biopsies, and cell lines without evidence of EBV were used as a source of antigen in these studies. Sera were tested for antibody to the viral capsid antigen and the early antigen by immunofluorescence. Lymphocyte cytotoxicity, skin testing, and lymphocyte stimulation were employed as tests of cellular immunity.

Project B

1. An indirect immunofluorescence assay was developed using the Raji cell line infected with RLV as the test antigen and uninfected Raji cell line as a control. Sera from monkeys immunized with RLV and test human sera were assayed for fluorescent antibody in these studies.

Major Findings:

Project A

Evidence was accumulated that the elevated EBV titers in American lymphoma patients were a significant finding possibly related to the etiology of the disease and not just a non-specific reflection of poor cell-mediated immunity. A correlation of EBV serology and measurements of specific cell-mediated immunity to membrane antigens from EBV carrying lines actually showed a close parallel between humoral and CMI in lymphoma patients. Tumor-associated specificity was indicated by the lack of skin test response in solid tumor patients to the lymphoid lines. Furthermore, in the lymphoma patients, antigens from cell lines derived from lymphomas caused positive delayed hypersensitivity reactions while antigens from cell lines derived from a normal individual did not elicit a skin test response.

A major effort was placed on the development and utilization of the American Burkitt Lymphoma Registry. By April 1st, 102 patients had been confirmed and more than one new case per week was being reported. The apparent finding that a significant number of American Burkitt Lymphoma patients were available for laboratory studies resulted in the organization of a system for more rapid detection of cases and utilization of materials from the patients. Collaborative studies with other SVCP investigators resulted in the establishment of cell lines from American Burkitt lymphoma patients. Biochemical attempts to identify EBV genome demonstrated that, unlike in African Burkitt tumors, no EBV genome could be detected in American Burkitt tumors. Some evidence for an RNA virus cross-hybridizing with Rauscher leukemia virus was found. Studies of EBV antibody continued to demonstrate some correlation between titer and prognosis as well as indicating that young American Burkitts have higher titers than controls.

Another approach to evaluating the role of elevated EBV titers in cancer was taken by looking at normal individuals who are members of families with multiple first degree relatives with cancer. The studies demonstrated that high EBV titers are found in such normal individuals and suggest that elevated EBV titers may be an indication of susceptibility to cancer rather than a specific immune response to an oncogenic virus. Studies on EBV antibody levels in patients with spontaneous cryptococcosus, a disease strongly associated with poor cell-mediated immunity and a susceptibility to lymphomas, indicated normal EBV titers in these patients, again providing evidence that elevated EBV titers are not simply a non-specific finding in patients with poor cell-mediated immunity.

Project B

Studies on the possible relationship between RNA viruses analogous to Rauscher leukemia virus and human leukemia were de-emphasized in this year except for the development of a flourescent antibody technique

measuring immunity to Rauscher leukemia virus. This assay was used in a limited fashion to demonstrate that monkeys and humans inoculated with Rauscher leukemia virus do produce antibody against human adapted Rauscher leukemia virus but not the control cell lines.

Significance to Biomedical Research and the Program of the Institute:

1. The studies in American Burkitts are important since a closer scrutiny of the same disease in geographically distinct areas allows a more careful look at the etiology by separating various factors. If American Burkitt's has the same etiology as African Burkitt's, it can be evaluated without concern for the role of malaria as a complicating factor.

2. Studies in EBV have now linked the virus to a number of diseases, including nasopharyngeal carcinoma, Burkitt's lymphoma, Hodgkin's disease, chronic lymphocytic leukemia, and sarcoidosis. The evidence for etiology, however, is not clear. An inverse correlation of cell-mediated immunity and humoral antibodies was suspected because of the high titers in the lymphocyte depletion form of HD, and might indicate that high EBV antibody levels could be the result of poor cell-mediated immunity to the virus. In finding a direct correlation between humoral and CMI, however, these studies support a more specific role for EBV in the etiology of human lymphoma. The detection of lymphoma related antigens may not only lead to further information on the etiology of these tumors but may also lead to a rational form of immunotherapy.

3. The American Burkitt Lymphoma Registry is providing detailed information of great importance to oncologists. The geographical distribution and time-space clustering suggests an environmental contribution, perhaps a virus, to the etiology. Characterization of the clinical aspects of the disease is permitting chemotherapists to select drugs which prevent relapse more effectively.

Proposed Course:

Project A

A more intensive effort will be made to identify humoral and cell-mediated immunity against EBV and other potential oncogenic viruses. Additional patients will be entered into the ongoing studies to confirm whether the immunological reactivity against potential oncogenic, viral and tumor associated antigens is disease specific. Longitudinal studies will state whether or not the EBV titers and cell-mediated immunity tests can be used to predict changes in the clinical course of disease. Immunological assays will be associated with tests presumably measuring susceptibility to cancer (such as HL-A type and virus transformation of skin fibroblasts). An attempt will be made to permit immunological and laboratory studies to pinpoint as many factors relevant to the etiology and control of American BL as possible.

Project B

Studies to relate antigens associated with Rauscher leukemia virus and human leukemia will continue with more specific antisera. In collaboration with other SVCP investigators, it should be possible to indicate the nature of the specificity of the cross-reactivity between animal leukemia viruses and human leukemia and eventually lead to a better understanding of the etiology of this disease.

Honors and Awards:

Dr. Levine presented a talk entitled "Evidence for A Viral Etiology in Human Cancer" at M.D. Anderson Hospital and Tumor Institute in Houston, Texas, September 27, 1972.

Dr. Levine presented a talk entitled "EBV and Hodgkin's disease" at Walter Reed Army Institute of Research in October, 1972.

Dr. Levine presented a manuscript entitled "American Burkitt's Lymphoma: Current Status" at the Annual Meeting of the American College of Physicians in Chicago, Illinois on April 12, 1973.

Dr. Levine presented a manuscript entitled "Antibodies to Epstein-Barr Virus (EBV)-Associated Antigens in Familial Cancer" at the American Association for Cancer Research in Atlantic City, New Jersey on April 12, 1973.

Dr. Levine served Clinical Assistant Professor of Medicine at Howard University, Freedman's Hospital in January and February of 1973.

Dr. Levine received the Physician's Recognition Award from the American Medical Association.

Publications:

Rosenberg, E., Herberman, R.B., Levine, P.H., Wunderlich, J., and McCoy, J.: Lymphocyte cytotoxicity reaction to leukemia-associated antigens in identical twins. Int. J. Cancer 9: 648-658, 1972.

Levine, P.H., O'Conor, G.T., and Berard, C.W.: Antibodies to Esptein-Barr Virus (EBV) in American patients with Burkitt's lymphoma. Cancer 30: 610-615, 1972.

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Hesse, J., Anderson, E., Levine, P.H., Ebbesen, P., Halberg, P., and Reisher, J.I.: Antibodies to Epstein-Barr Virus and cellular immunity in Hodgkin's disease and chronic lymphatic leukemia. Int. J. Cancer, 11: 326-330, 1973.

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Levine, P.H., Sandler, S.G., Komp, D.M., O'Conor, G.T., and O'Connor, D.M.: Simultaneous occurrence of "American Burkitt's Lymphoma" in neighbors. NEJM: 562-563, 1973.

McCoy, J.L., Herberman, R.B., Rosenberg, E.B., Donnelly, F.C., Levine, P.H., and Alford, T.C.: ^{51}Cr Release cellular lymphocyte cytotoxicity in human leukemia and tissue culture systems. J. Nat. Cancer Inst., (In Press).

Levine, P.H.: Introduction to Workshop on Cell Mediated Immunity. J. Nat. Cancer Inst., 1973 (In Press).

Levine, P.H., and Reisher, J.I.: Relationship of titers of Epstein-Barr virus to cell-mediated immunity in patients with Hodgkin's disease. Proc. of the Hodgkin's Disease Sym, 1973 (In Press).

Serial No. NCI - 4824
1. Viral Leukemia and Lymphoma Branch
OASDVO, Dvision of Cancer
Cause and Prevention
2. Viral Pathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Studies with RNA oncogenic viruses

Previous Serial Number: Same

Principal Investigator: Dr. Adi F. Gazdar

Other Investigators: Dr. Yoji Ikawa
Dr. Herbert K. Oie
Dr. Richard Buswell

Cooperating Units: Inside NIH
Dr. Padman Sarma, VCB, NCI
Dr. Alfred Steinberg, LAR, NIAMD
Dr. Sam Baron, LBV, NIAID
Dr. Harish Chopra, VB, NCI
Dr. Ronald Herberman, LCBGY, NCI

Outside NIH
Dr. Gerald Spahn, Microbiological Associates, Walkersville, Maryland

Man Years:

Total:	3.5
Professional:	1.0
Other:	2.5

Project Description

Objectives:

1. To characterize the properties of "defective" sarcoma viruses.

2. To investigate the relationship between certain viruses, interferon inducers, autoimmune disease and neoplasia in New Zealand mice.

3. To investigate the relationship between the interferon

system, oncogenic viruses, and susceptibility to oncogenesis.

4. To investigate cross-immunity between murine sarcoma virus pseudotypes, non-producer cells and S+L- cells.

Methods Employed:

1. Routine tissue culture techniques, electron microscopy, reverse transcriptase assays, and complement fixation tests.

2. The progress of autoimmune disease is followed by radio-immunoassay tests for antibodies to RNA and DNA, as well as by light and electron microscopy.

3. Interferon assays are performed using an inhibition of hemagglutinin yield method. The effects of interferon inducers on the interferon system, immune system, tumor development, and virus yield are studied.

4. Mice are immunized with MSV(MLV), and when resistant, are challenged with MSV pseudotypes, spontaneous syngeneic tumors, MSV-induced non-producer cells, and S+L- cells.

Major Findings:

1. Hamster tumors induced by Gz-MSV, and their cell cultures, release non-infectious virus-like particles (S+H- virus). The cells, and the virus, express mouse gs-1 antigen. After repeated in vivo or in vitro passage, some lines also express hamster type C virus (HaLV) gs-1 antigen. These cells release the HaLV pseudotype of Gz-MSV. A comparison of the Gz-MSV (HaLV) and S+H- viruses helps characterize the nature of defectiveness of Gz-MSV and the contribution of helper viruses. S+H- virus can express gs antigen but is non-infectious for mouse and hamster cells, and reverse transcriptase levels are very low. All of the virions have electron lucent nucleoids and many broken or incomplete virions are present. In contrast, Gz-MSV(HaLV) is positive for both mouse and hamster gs antigens, the levels of reverse transcriptase are relatively high, the virus is a hamster-tropic sarcoma virus, and its virions have both electron dense and lucent nucleoids. The defectiveness of S+H- virus may be related to the small size of its viral RNA (53S RNA). While S+H- virus is non-infective, the expression of mouse gs antigen and presence of sarcoma genome in it virions makes it the least "defective" of the known murine sarcoma viruses.

2. The presence of antinuclear antibodies and immune complex

nephritis make NZB/NZW (B/W) mice a suitable animal model for human systemic lupus erythematosus. Because of the possibility of a viral etiology, the effects of long-term administration of various interferon inducers have been studied in B/W mice. Poly I·C, poly A·U, and COAM stimulated the appearance of anti-DNA and antiRNA antibodies and shortened longevity due to earlier onset of nephritis. In addition, intraperitoneal COAM injections caused a chronic chemical peritonitis. Tilorone had only a mild enhancing effect. NDV had no effect, perhaps because of the early development of NDV neutralizing antibodies. By contrast, statalon significantly delayed the appearance of antinuclear antibodies and increased longevity. The various effects of inducers may be explained by their different effects on the immune system.

3. Pretreatment with interferon inducers enhance oncogenesis by RNA tumor viruses, in vivo growth of syngeneic tumors, MSV focus formation, and spontaneous transformation. Pretreatment with poly I·C enhanced the growth of 10 out of 13 syngeneic mouse and rat tumors. All 10 "enhanceable" tumors released type C viruses. The 3 "non-enhanceable" tumors did not release detectable type C virus. Superinfection of these 3 lines with type C viruses resulted in greater antigenicity, slower growth rate, and poly I·C enhancement. Poly I·C pretreatment enhanced tumor growth in immunologically competent mice only. Poly I·C pretreatment is immunodepressive, and cytotoxic reactivity to MSV tumor cells is decreased in poly I·C treated mice. Potent mouse interferon preparations also enhanced tumor growth and MSV-induced tumors in mice. This action was dose dependent, species specific, heat inactivated and pH resistant, suggesting that it is due to interferon and not a non-specific effect.

4. Mice immune to MSV(MLV) are immune to challenge with FMR pseudotypes of MSV and leukemias induced by FMR viruses. They are not resistant to challenge with spontaneous tumors, whether these release virus or not, and Gross pseudotypes of MSV and AKR leukemias. Also, they are not resistant to MSV non-producer cells and S+L- cells unless these are superinfected with FMR viruses. In fact, immune mice appear more sensitive to challenge with S+L- cells.

Proposed Course:

1. The physical and antigenic properties of the sarcoma virus synthesized in the presence and absence of associated "helper virus" will be further compared and contrasted.

2. Studies on the effect of several interferon inducers and RNA oncogenic viruses on antinuclear antibodies, mortality, and renal disease in B/W mice will be completed.

3. The mechanism of enhancement of viral oncogenesis and tumor cell growth by interferon inducers will be studied.

4. The antigens necessary for MSV immunity will be characterized.

Significance to Biomedical Research and the Program of the Institute:

Investigations on the nature and mode of action of certain animal type C oncogenic viruses, especially as they affect species other than that of origin will contribute to a more complete understanding of certain human cancers. Indeed, prevention or control of cancer is dependent upon the elucidation of mechanisms by which viruses manipulate species barriers and further definition of host response to oncogenic agents. Characterizing the properties of "defective" oncogenic viruses will aid in the search for putative human sarcoma viruses.

Honors and Awards:

Dr. Gazdar presented a seminar entitled, "Etiology of RNA tumor viruses" at the Peter Bent Brigham Hospital, Boston, Mass., and at the University of Vermont Medical College, Burlington, Vt., April 1972.

Dr. Gazdar presented a paper entitled, "Interferon and polynucleotide mediated enhancement of tumor growth" to the American Society of Microbiology.

Dr. Gazdar was invited to participate and present his recent work at the Interferon Seminar organized by NIAID, at Williamsburg, Va., October 1972.

Publications:

Gazdar, A. F. and Ikawa, Y.: Synthetic RNA and DNA polynucleotides: *in vivo* and *in vitro* enhancement of oncogenesis by a murine sarcoma virus. Proc. Soc. Exp. Biol. Med. 140: 1166-1169, 1972.

Sarma, P. S., Gazdar, A. F., Turner, H. C., and Kunchorn, P. D.: Gazdar strain of murine sarcoma virus. Biologic and antigenic interaction with heterologous hamster host. Proc.

Soc. Exp. Biol. Med. 140: 928-933, 1972.

Oie, H. K., Gazdar, A. F., Buckler, C. E., and Baron, S.: High interferon producing line of transformed murine cells. J. Gen. Virol. 17: 107-109, 1972.

Gazdar, A. F.: Enhancement of tumor growth rate by interferon inducers. J. Natl. Cancer Inst. 49: 1435-1438, 1972.

Ikawa, Y., Gazdar, A. F., and Chopra, H. C.: Epithelial features of "nonproducer" BALB/3T3 cells transformed by murine sarcoma virus. J. Natl. Cancer Inst. 49: 1449-1453, 1972.

Gazdar, A., Hatanaka, M., Herberman, R., Russell, E., and Ikawa, Y.: Effects of dibutyryl cyclic adenosine phosphate plus theophylline on murine sarcoma virus transformed non-producer cells. Proc. Soc. Exp. Biol. Med. 141: 1044-1052, 1972.

Gazdar, A. F., Russell, E. K., and Herberman, R. B.: Mouse-strain related differences in the biologic and immunologic responses to a murine sarcoma virus. J. Natl. Cancer Inst. (In press).

Sarma, P. S., Log, T., and Gazdar, A. F.: Control of group specific antigen synthesis by the defective Gazdar murine sarcoma genome. Virology (In press).

Price, P. J., Suk, W. A., Zimmerman, E. M., Spahn, G. J., Gazdar, A. F., and Baron, S.: Enhancement of *in vivo* transformation by poly I·poly C. J. Natl. Cancer Inst. (In press).

Gazdar, A. F., Sims, H., and Spahn, G. J.: Interferon and polynucleotide mediated ennancement of tumor growth. Bacteriol. Proc. (In press).

Gazdar, A. F., Sarma, P. S., Peebles, P., and Chopra, H. C.: Properties of a defective mammalian sarcoma virus. Proc. Amer. Assoc. Cancer Res. 13: 55, 1972.

Serial No. NCI-4825

1. Viral Leukemia and Lymphoma Branch
   OASDVO, Division of Cancer
   Cause and Prevention
2. Tumor Virus Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Physico-chemical studies of viral nucleic acids and enzymes

Previous Serial Number: Same

Principal Investigator: Dr. Daniel K. Haapala

Other Investigators: Dr. Peter J. Fischinger
Dr. Robert H. Bassin
Dr. Brenda I. Gerwin
Dr. Shigeko Nomura
Dr. Paul T. Peebles
Dr. Leo A. Phillips

Cooperating Units: Inside NIH
Staff, VLLB, NCI

Outside NIH
None

Man Years:

Total:	2.00
Professional:	1.00
Others:	1.00

Project Description

Objectives:

To characterize viral nucleic acids and enzymes for use as diagnostic tools and to gain insight into the mechanism of viral oncogenesis.

Methods Employed:

Standard biochemical and biophysical methods were used.

Major Findings:

Murine sarcoma virus:

1. Infectious murine sarcoma viruses contain both unique RNA information and some information common to murine leukemia viruses.

2. No infectious particles from MSV transformed cells back certain RNA sequences present in infectious MSV particles.

3. Murine sarcoma virus shares very little information with avian sarcoma viruses.

Leukemia viruses:

1. Each leukemia virus isolate of mice and cats can be distinguished from other isolates by DNA-RNA hybridization techniques. This permits the identification of unknown virus isolates from any cell type to date.

2. Similar techniques permit the identification and characterization of viruses isolated from human material.

Significance to Biomedical Research and the Program of the Institute:

The physico-chemical properties of viral nucleic acids and enzymes provide tools with which to detect, characterize and possibly inhibit neoplastic changes of viral etiology.

Proposed Course:

Studies of human tumor material will be emphasized based on the findings reported above.

Honors and Awards

Participant in a symposium entitled "Role of the Scientist in Society" at the 72nd Annual Meeting of the American Society for Microbiology, Philadelphia, Pa., April 24 to 28, 1972.

Presented seminar entitled "Molecular Relatedness of RNA Tumor Viruses" at the Department of Microbiology, Howard University.

Publications

Fischinger, P. J., Nomura, S., Peebles, P. T., Haapala, D. K., and Bassin, R. H.: Reversion of murine sarcoma virus transformed mouse cells: Variants without a rescuable sarcoma virus. Science 176: 1033-1035, 1972.

Bassin, R. H., Plata, E. J., Gerwin, B. I., Mattern, C. F., Haapala, D. K. and Chu, E. W.: Isolation of a continuous epithelioid cell line, HBT-3, from a human breast carcinoma. Proc. Soc. Exp. Biol. Med. 141: 673-680, 1972.

Haapala, D. K., Jasmin, C., Sinoussi, F., Chermann, J. C., Mathe, G., and Raynaud, M.: Inhibition of tumour virus RNA-dependent DNA polymerase by the heteropolyanion, silicotungstate. Europ. J. Clin. Biol. Res. 19: 5-9, 1973.

Serial No. NCI-4826

1. Viral Leukemia and Lymphoma Branch
   OASDVO, Division of Cancer
   Cause and Prevention
2. Tumor Virus Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: A. The nature, mechanisms, and stability of cell transformation induced by murine sarcoma virus (MSV)

B. Nonproductive infection of various mammalian cells with transforming sarcoma genomes: Isolation of endogenous prototype viruses

C. Antigenic and molecular structure-function relationships of purified type C virus components

Previous Serial Number: Same

Principal Investigator: Dr. Peter J. Fischinger

Other Investigators: Dr. Robert H. Bassin
Dr. Daniel Haapala
Dr. Shigeko Nomura
Dr. Paul T. Peebles
Dr. Leo A. Phillips

Cooperating Units: Inside NIH
Staff, VLLB, NCI

Outside NIH
Dr. Werner Schafer, Max Planck Institut fur Virusforschung, Tubingen, Germany
Dr. Jens Lange, Max Planck Institut fur Virusforschung, Tubingen, Germany

Man Years:

Total:	4.0
Professional:	1.0
Others:	3.0

Project Description

Objectives:

A. To determine the manner of association of the MSV genome with cellular genetic mechanisms, specifically to enhance the rate of phenotypic reversion from the transformed state.

B. To exploit the ability of the various pseudotypes of MSV to obtain nonproductively infected heterologous species cells and to rescue from these the natural type C virus of the species.

C. To isolate specific RNA and protein molecules and substructures of type C viruses to identify their antigen content, to determine their relatedness, and to clarify the nature of their oncogenic information.

Methods Employed:

A. and B. Standard virological assays such as sarcoma virus focus formation, leukemia virus helper assay and focus induction in S+L- cells, viral interference, and virus antigen induction, are being used. Cloning of cells in microtiter dishes and as soft agar colonies with the usual biochemical modes of particle and protein isolation are employed routinely.

C. Biochemical methods such as sephadex or hydroxyapatite column chromatography, velocity, and isopycnic density gradient centrifugation, radioactive tracer studies, reverse transcriptase activity, gel immunodiffusion, preparative and analytical polyacrylamide gel electrophoresis, and the usual methods of extraction for proteins and nucleic acids are being employed.

Major Findings:

A. Reversion of MSV transformed mouse cells to the normal phenotype has been further analyzed. Various MSV transformed cells have different reversion rates and the revertant lines have been compared to each other. Generally they contain group specific antigenicity of MuLV although some exceptions have been found. The phenotypic state of reversion is unstable, and spontaneous back transformation has been observed in all revertant lines. In contrast, no reappearance of a rescuable MSV genome was ever found in the spontaneous back transformants. No evidence of inducible MSV remained in revertants; both cocultivation with homologous or heterologous cells, and chemical induction with halogenated pyrimidines failed to detect an infectious sarcoma virus. Chromosomal analysis showed that revertants were usually hyperdiploid and that back transformants had a chromosomal content roughly analogous to the parental S+L- cells. No clear cut criteria were established because all normal, transformed or revertant cell sublines had multiphasic populations of cells containing hypotetraploid or hyperploid chromosome numbers.

A second cycle of transformation of revertants was possible with different MSV genomes, and these cells were again found to be typical S+L- cells. Occasionally some of these released a low quantity of MSV which had little or no attending MuLV; on analysis the coat of this virus was immunologically identical to the initial MSV isolate.

B. Human S+L- cells have been isolated after an infection of human amnion cells with MSV(FeLV). These contained a rescuable MSV genome after infection with FeLV or RD-114 virus. The helper virus nature of RD-114 was clarified. The S+L- cell had gs-1 of the mouse but not of the cat or RD-114 virus. No reverse transcriptase or particles were apparent.

Infection of a clonal subline of feline cells (CCC) with MSV alone yielded at first cells which were apparently S+L- but later released infectious MSV which was not coated with FeLV. These MSV yielding cells and the untransformed subline were found to contain a type C virus which was immunologically very similar if not identical to RD-114. To obviate the contention of lateral transmission, CCC cells which were frozen prior to the existence of RD-114 were induced by iododeoxyuridine to yield the same virus anew.

C. Molecular studies on the function of antigens of MuLV revealed that apparently on the surface of the virus particle there exists a small (10-15,000 MW) component which was purified by two cycles of preparative gel electrophoresis, whose ability to enhance MuLV infectivity and to protect the virus from neutralizing antibody, was quite unexpected. The effect was on the MuLV virion and not on the cells, and only tissue culture derived virus was susceptible to its action. This activity and site correlated well with many observed phenomena of infectivity and immunogenicity of MuLV.

The technique of hydroxyapatite chromatography as a measure of nucleic acid hybridization was found to be useful for comparing the relatedness of various type C viruses. An endogenous DNA was made by the reverse transcriptase and hybridized to its own and heterologous virus RNA. The degree of relatedness was measured by the extent of hybridization and the thermal stability of the hybrid. The method is sensitive enough to differentiate among MuLV viruses which are difficult to resolve from each other even by neutralization. RD-114 was found to be quite different from the standard FeLV isolates.

The S+L- cells produce noninfectious particles whose RNA was found to be a 28S molecule or possible multimeric structure up to 80S. This RNA like viral RNA contained a poly A stretch, had a buoyant density characteristic of RNA tumor viruses; and possessed sequences which hybridize with the endogenous reverse transcriptase derived DNA from MSV-MLV preparations.

Significance to Biomedical Research and the Program of the Institute:

A. To understand the mechanisms of cell transformation is important; to induce reversion from the transformed to the normal state is to subvert oncogenesis. It is clear that some MSV genomes are more or less stable in their maintenance of transformation. Any procedure or drug which would accelerate the rate of reversion from the already transformed state has obvious potential curative value.

B. The existence of a heterologous species cell transformed by MSV alone resulted in the detection of what is presumably the natural virus of cats and which is different from previously known FeLV isolates. One must therefore consider the possibility of multiple, apparently little related, type C viruses in a single species. The extension of such a model system to human S+L- cells may lead to the isolation or induction of a human type C virus(es).

C. The dissection of the MuLV structure serves as a fundamental model for other mammalian oncogenic viruses with relevance to common structures and functions. New isolates from human sources have engendered controversy regarding the true nature of these altered viruses. At this time it is clear that a given species can contain more than one immunologically and molecularly distinct C virus type. Characterized reagents from model systems will readily define whether human, or contaminating known animal leukemia viruses, or interacting hybrids are involved. Molecular techniques can now be used to differentiate the informational content of the RNA of these various viruses. The specific oncogenic content of the virus can then be examined.

Proposed Course:

A. Further analyses of the reversion process with testing of suggestive chemicals to increase the reversion rate. Multiple cycles of transformation and reversion will be attempted to understand the basic cellular controls of reversion.

B. The isolation of the suspected human endogenous, prototype C virus.

C. To analyze other structure-function relationships of type C virus RNA and antigens. To isolate the informational moiety responsible for cell transformation.

Honors and Awards:

Dr. Fischinger presented a seminar at Howard University, Washington, D. C. to the Department of Microbiology on November 7, 1972 "Antigens and Functions of Isolated Murine Leukemia Virus Components".

Dr. Fischinger was an invited speaker at the M.D. Anderson Lecture Series at the University of Texas at Houston on November 30, 1972. "Interaction of Murine Sarcoma Virus with Homologous and Heterologous Cell Systems".

Publications:

Fischinger, P. J., Nomura. S., Peebles, P. T., Haapala, D. K., and Bassin, R. H.: Reversion of murine sarcoma virus transformed mouse cells: Variants without a rescuable sarcoma virus. Science 176: 1033-1035, 1972.

Fischinger, P. J., Lange, J., and Schafer, W.: Activating and protective capacities of a purified electrophoretic fraction of murine leukemia virus for murine leukemia virus infectivity. Proc. Natl. Acad Sci. USA 69: 1900-1904, 1972.

Schafer, W., Bauer, H., Bolognesi, D. P., Fischinger, P., Frank, H., Gelderblom, H., Lange, J., and Nermut, M. V.: Studies on structural and antigenic properties of C-type viruses. The 25th Annual Symposium on Fundamental Cancer Research, "Molecular Studies in Viral Neoplasia", Houston, Texas, 1972.

Lee, K. M., Nomura, S., Bassin, R. H., and Fischinger, P. J.: Use of an established cat cell line for investigation and quantitation of feline oncornaviruses. J. Natl. Cancer Inst. 49: 55-60, 1972.

Peebles, P. T., Fischinger, P. J., Bassin, R. H., and Papageorge, A. G.: Isolation of human amnion cells transformed by rescuable murine sarcoma virus. Nature, In press.

Nomura, S., Fischinger, P. J., Mattern, C. F. T., Peebles, P. T., Bassin, R. H., and Friedman, G. P.: Revertants of mouse cells transformed by murine sarcoma virus. I. Characterization of flat and transformed sublines without a rescuable murine sarcoma virus. Virology 50: 51-64, 1972.

Bassin, R. H., Phillips, L. A., Kramer, M. J., Haapala, D. K., Peebles, P. T., Nomura, S., and Fischinger, P. J.: Properties of 3T3 cells transformed by sarcoma virus in the absence of replicating murine leukemia helper virus. In Chieco-Bianchi, L. and Dutcher, R. M. (Eds): Proc. Vth International Symposium on Comparative Leukemia Research. In press.

Serial No. NCI-4827
1. Viral Leukemia and Lymphoma Branch
OASDVO, Division of Cancer Cause and Prevention
2. Immunology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The isolation, purification and characterization of viral and tumor antigens

Previous Serial Number: Same

Principal Investigator: Dr. Vincent W. Hollis, Jr.

Other Investigators:
Dr. Tadao Aoki
Dr. Ernest J. Plata
Dr. Toshio Kudo
Dr. Fujiro Sendo

Cooperating Units:

Inside NIH
Dr. Leo Phillips, VLLB, NCI
Dr. Arnold Fowler, OBEC, NCI
Dr. Alfred Hellman, OBEC, NCI

Outside NIH
Dr. Norman Weliky, TRW Systems, Los Angeles, California

Man Years:

Total:	2.0
Professional:	1.0
Other:	1.0

Project Description

Objectives:

The basic aims of this project are to isolate and purify the various antigenic components of the murine leukemia viruses, and later other viruses and tumors; (a) to determine the amino acid sequence of the surface antigens; (b) to test these components for the determination of antigenic properties; (c) to analyze which of the purified fractions elicits the production of protective antibody when used for immunization; and (d) to develop new and/or adapt known biochemical methods for the isolation and characterization of viruses and/or tumor-associated antigens.

Methods Employed:

Standard techniques of differential centrifugation, molecular-sieve (gel) and ion-exchange chromatography, rate zonal, isopynic and density gradient ultracentrifugation, and analytical dis-gel electrophoresis are used in the isolation and purification of the viral enzymes and antigens. The enzyme, i.e., RNA-directed DNA polymerase is identified by a standard assay procedure. A combination of two procedures are being used to isolate and solubilize surface antigens of C57BL leukemia EL4 cells. A radioimmunoassay is being used to measure cyclic AMP in tissues of mice treated with different hormones.

Major Findings:

Six protein peaks have been isolated from Rauscher leukemia virus by means of agarose chromatography in quanidine hydrochlorides. The membrane proteins, which have incorporated ^{14}C-glucosamine, are being accumulated for further physical studies.

Baseline values for cyclic AMP in normal BALB/c mice have been established for the uterus, kidney, spleen, thymus, ovary and liver.

Preliminary work with cell surface antigens solubilized from EL4 leukemia cells have been partially successful. Further work is in progress.

Significance to Biomedical Research and the Program of the Institute:

The isolation, purification, and chemical characterization of the antigens of a murine leukemia virus contribute to the elucidation of the structure of individual viruses and will assist in determining definite inter-relationships among other murine leukemia viruses. These and other procedures, when applied to the control or prevention of human leukemia may aid in the development of methods for immunological detection of this disease.

Proposed Course:

Studies will continue in an effort to elucidate the chemistry of viruses that induce leukemia and solid neoplasms in the murine and feline species. More specifically, it is planned to: (1) continue the studies on the isolation, purification, and chemical identification of viral and tumor specific antigens (murine and feline various tumors); (2) elute antigen-antibody complexes from AKR tissue and check for anti-polmerase activity; (3) continue collaborations with Dr. Phillips on the characterization of $S^{+}L^{-}$ virions; and with Drs. Fowler and Hellman on cyclic AMP levels during the induction of gs antigens.

Honors and Awards:

None

Publications:

None

Serial No. NCI-4828

1. Viral Leukemia and Lymphoma Branch
   OASDVO, Division of Cancer Cause and Prevention
2. Immunology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Immunology of animal and human tumors, to include studies on viral neoplasia, and detection and characterization of the host's response to tumor associated antigens

Previous Serial Number: Same

Principal Investigator: Dr. Ernest J. Plata

Other Investigators:
Dr. Tadao Aoki
Dr. Fujiro Sendo
Dr. Luigi Chieco-Bianchi

Cooperating Units:

Inside NIH
Dr. Brenda Gerwin, VLLB, NCI
Dr. Elizabeth W. Chu, LP, NCI
Dr. Ronald B. Herberman, LCBGY, NCI
Dr. Paul Levine, VLLB, NCI

Outside NIH
Dr. Donald Mashburn, Washington Sanitarium & Hospital, Takoma Park, Maryland
Dr. Paul Terasaki, University of California, Los Angeles, California
Dr. Evan M. Hersh, M.D. Anderson Hospital and Tumor Institute, Houston, Texas
Dr. F. Pflugy, Malcolm Grow Memorial Hospital Andrews Air Force Base, Camp Springs, Maryland
Dr. Joseph G. Sinkovics, The University of Texas, Houston, Texas
Dr. L.M. Boot, Netherlands Cancer Institute, Amsterdam C, The Netherlands

Man Years:

Total:	2.50
Professional:	1.00
Other:	1.50

Project Description

Objectives:

1. To develop new or to adapt current methods for the detection and characterization of viral or cellular antigens associated with neoplasia.

2. To study the humoral and cellular immune responses of the host to autochthonous and syngeneic tumors.

3. To establish well-characterized cell culture lines from human and animal tumors, both *in vitro* and *in vivo*, to serve as tools in the etiological and immunological studies of the respective tumors.

4. To explore applicable models for immunological diagnosis, immunotherapy, or possible prophylaxis of cancer.

Methods Employed:

1. Detection of viruses or antigens associated with neoplasia.

a. Murine mammary tumor studies. Detection of a circulating substance associated with MTV, like the G(Gross) soluble antigens (GSA), can aid significantly to (1) the early diagnosis of MTV infection, (2) the understanding of relationships among host, MTV, and mammary tumors, (3) the analysis of genetic influence on MTV infection, and (4) the evaluation of potential methods of therapy. MTV-associated soluble antigen (MSA), not precipitated by centrifugation at 100,000 xg for 1 hour, were detected in the plasma of MTV^+ mice by the indirect immunofluorescence test. Anti-mammary tumor serum was obtained from hyperimmunized allogeneic and syngeneic mice. After preabsorption with G and other leukemic cells to remove antibodies against G and other unrelated antigens, this antiserum specifically reacted with reference mammary tumor cells and MSA adsorbed onto the surface of indicator cells. Incidence of MSA in mice was as follows: (i) MSA was positive in all C3H/He, BALB/c, and ICR/Ha mice bearing either primary or serially transplanted spontaneous mammary tumors of syngeneic mice. (ii) The incidence increased progressively in normal C3H/He mice with age from less than 1% in 6-month-old ♀ virgins to approximately 26% in 14- to 16-month-old multiparous ♀ exbreeders. (iii) C3H/He ♂ mice showed very low incidence (less than 1%) even in 14- to 18-month-old exbreeders. (iv) No MSA was found in C57BL/6 mice (MTV^-) of any age. Current studies will resolve the relationship of the detected antigens to various classes of MTV- and mammary tumor-associated antigens.

b. BALB♂RL leukemia studies. In collaboration with Dr. F. Sendo (see NCI-4862) the transplantable BALB/c irradiation-induced leukemia BALB/c cells were established *in vitro* cultures. These cells produce type C virus whose envelope antigen differs from antigens either on MuLV or on murine myeloma-associated viruses (Aoki *et al.*, in press). Current studies include base line determinations on the biological characteristics of the cell line

in vitro and on the production and characterization of this virus by biophysical, biochemical, infectivity, and immunological techniques.

c. Human breast tumor studies.

1) Cell cultures. For the years 1971-72, the culture of 32 benign and 68 malignant tumors was reported. This year we concentrated in culturing biopsies from 36 selected malignant breast tumors and completing the characterization studies of three cell lines previously established from human breast tumors. The characterization data will be published in JNCI, April, 1973. The new series of culture has yielded one additional candidate cell line, which is at passage 17; in contrast to the previous lines, grows as a suspension culture. Selected tumors will continue to be cultured to provide additional cell lines for study and for direct immunological testing of freshly explanted tumors.

Cells from the HBT-39 human breast carcinoma cell line at passages 21 and 62 were inoculated into immunosuppressed CD rats. Four of 10 rats treated developed progressively growing tumors. Histological examination showed epithelial, polygonal, hyperchromatic cells with prominent nuclei. Tumor cells grew in clusters or infiltrated neighboring connective tissue. The transplanted cells were actively growing and showed increased mitotic activity. The pathological appearance closely resembled that seen in the original biopsy from the patient. These results indicate that HBT-39 cell line is most probably an outgrowth of malignant cells derived from the original human breast adenocarcinoma. Previously reported data has confirmed the human species origin of HBT-39. Work in progress will compare these tumor cells before and after animal passage for any cultural, immunological, or virological differences.

2) Virus detection. Fresh tumors and cultured cells at each 5 to 8 passage interval were monitored for the presence of viruses resembling the known RNA oncogenic viruses. To date, no viruses have been detected in any cultured by either ^{3}H-uridine pulse labeling, electron microscopy, or reverse transcriptase enzymatic techniques. Current studies include mixed cultivation of tumor cells with various apparently virus-free or virus-infected cells and subsequent monitoring for virus production by the human tumor cells.

2. The study of the immune response of the host to the autochthonous, syngeneic, or xenogeneic tumor.

a. Natural antibodies. Apparently healthy mice of MTV^{+} strains were shown to develop spontaneously with age, antibodies of at least two distinct specificities. Such antibodies were absent in the MTV^{-} strains of mice tested. Identical antibodies were detected in mice bearing either spontaneous or transplanted mammary tumors. Studies in progress aim to determine the significance of this system and any association of these antigens with those of MTV and mammary tumors. The methods employed in these experiments include the ^{51}Cr releasing humoral antibody cytotoxicity and the indirect

immunofluorescence techniques.

b. Seroepidemiology of breast cancer. One hundred-seventy-seven samples of sera were obtained from patients with malignant breast tumors, nonmalignant breast diseases, other tumors, and healthy people. These sera were tested by the indirect membrane immunofluorescence technique using primary and established human breast tumor cultured cells. No significant levels or patterns of antibodies were detected by these tests.

c. Response of cancer patients to a type C virus. In collaboration with Dr. E. Hersh, selected samples of serum obtained from patients immunized with RLV were tested for specific antibodies against RLV. Cytotoxic release of ^{51}Cr was used to detect antibodies that reacted with RLV-infected and RLV-free JLS-V9 cells (established cell line derived from BALB/c mice) and normal BALB/c mouse thymocytes. The sera were tested before and after in vivo absorption in 6-week-old normal BALB/c mice. Anti-mouse hetero-antibodies were detected in all sera but no significant anti-RLV activity was detected.

Major Findings:

1. Establishment and characterization of HBT-39A and B cell lines as a human breast adenocarcinoma continuous culture.

2. Detection of a soluble antigen associated with MTV-infection which is different from but similar to that described for the murine viral leukemias.

3. Detection of natural antibodies of two common specificities in healthy mice of MTV^+ strains and mice bearing transplanted on spontaneous mammary tumors.

Significance to Biomedical Research and the Program of the Institute:

The research approach outlined here is specifically oriented to perform experiments using animal model systems as well as materials derived from tumor patients. This approach will yield results which are directly applicable to the solution of problems of the diagnosis, etiology, treatment, or prevention of human malignancy. The experiments performed fit well within the programs of the DCCP, Viral Oncology, and the SVCP of NCI.

Proposed Course:

The goals of this project for the next fiscal year are:

1. To advance significantly in the study of soluble antigens associated with solid tumors to determine their incidence, diagnostic and immunological implications.

2. To continue the study of the relationship of naturally occurring

antibodies to the susceptibility, incidence and genetics of tumor incidence.

3. To identify one or more tumor or viral specific antigens from human breast tumors.

4. To continue to study the question of whether animal oncogenic viruses play a discernible role in human leukemia or breast cancer.

Awards and Honors:

None

Publications:

Plata, E.J. and Murphy, W.H.: Growth and hematologic properties of the BALB/Wm strain of inbred mice. Lab. Anim. Sci. 22:712-720, 1972.

Bassin, R.H., Plata, E.J., Gerwin, B.I., Mattern, C.F., Haapala, D.K., and Chu, E.W.: Isolation of a continuous epithelioid cell line, HBT-3, from a human breast carcinoma. Proc. Soc. Exptl. Biol. Med. 141:673-680, 1972.

Plata, E.J., Aoki, T., Robertson, D.D., Chu, E.W., and Gerwin, B.I.: Established cultured cell lines from human breast carcinoma (HBT-39). J. Natl. Cancer Inst. (in press).

Oldham, R., Suvarski, D., McCoy, J., Plata, E., and Herberman, R.: Evaluation of a cell-mediated cytotoxicity assay utilizing I-125 iododeoxyuridine labeled tissue culture target cells. J. Natl. Cancer Inst. Monogr. (in press).

Serial No. NCI - 4831

1. Viral Leukemia and Lymphoma Branch, OASDVO, Division of Cancer Cause and Prevention
2. Viral Pathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Studies on murine leukemia

Previous Serial Number: Same

Principal Investigator: Dr. Ruth Merwin

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	2.0
Professional:	1.0
Other:	1.0

Project Description

Objectives:

Previous observations on the transplantation for many generations of reticulum cell neoplasms type B in BALB/c mice revealed differences between the tumors in the amount of local growth at the inoculation site as compared to generalized growth in the spleen and nodes. It seemed likely that these differences had an immunologic basis. My objective was to transplant many spontaneous reticulum cell tumors in order to obtain a group of tumors that would show a large range in the amount of local growth. Another objective was to observe the effect of changing the immunologic conditions on the occurrence of local growths.

Methods Employed:

Standard methods of transplantation and immunological procedures were employed.

Major Findings:

A. Twenty-three spontaneous tumors were carried for many

generations under standard conditions of transplantation, that is, the intraperitoneal inoculation of a suspension of approximately 5 X 10^7 cells. The gross pathology and rate of growth as indicated by the length of the latent periods was studied in these lines and changes in the latent periods and in the degree of local growth were the main characteristics that seemed to reflect immunologic changes.

B. For the 23 tumors, the latent periods shortened from an average of 5 months (range 1 to 12 months) to an average of 8 days (range 4 to 20 days) in 3 to 7 generations in most cases. The latent periods for tumors inoculated subcutaneously was slightly longer in some lines and much longer in others. However, in one line subcutaneous inoculations grew better.

C. Among 25 tumors, the amount of local growth was characteristic for any one line and if under certain circumstances it became much more local, it gradually returned in most cases to the original mixture of local and general growth. Some tumors yielded no detectable local growth but others produced extremely large growths. Large locals were for some tumors associated with considerable generalized growth in the spleen and nodes, but for other tumors there was almost no spleen involvement. In some of the sublines, large locals were accompanied by a few scattered nodules of growth in the spleen, liver and kidney. Whether these differences are due to different emigration rates from the inoculation site or to differences in concommitant immunity has yet to be determined. One tumor characterized by no local growth when transplanted to normal mice grew locally when inoculated into a mouse that had received several immunizing doses of irradiated cells. This suggests that the immune response under the usual conditions of transplant was just too weak to be effective. In order to determine the role of humoral immunity in causing local growths, a tumor showing very slight amounts of local growth was placed in diffusion chambers in mice that had been effectively immunized by treatment with irradiated cells. The cells in the diffusion chamber were exposed to humoral immunity without being destroyed by cellular immunity. However, when the chambers were opened and the tissues transplanted into new hosts, only generalized growth resulted. Either the immunization of the host of the diffusion chamber was cellular and not humoral or the cells of this tumor are not affected by humoral immunity so as to give local growth, or possibly both immune cells and sera are needed to cause local growth. Another tumor placed in diffusion chambers in nonimmune hosts gave very localized tumor growth when tested in new hosts. This finding suggests that the long interaction of the tumor cells and the other cells carried along with it in the diffusion chamber may have

caused the marked local growth.

D. Two tumors, one showing slight and one moderate amounts of local growth, were transplanted for a number of generations into antilymphocytic serum (ALS) treated hosts. ALS is supposed to inhibit particularly the action of T lymphocytes. No effect was evident until 3 or 4 generations of passage. In both lines the subcutaneous growth was suppressed or seemed to grow and then regress while the growth of the cells inoculated intraperitoneally did not seem to be affected. Cells transplanted from the ALS treated mice to untreated ones continued to behave like cells in ALS-treated mice for at least one generation. This slow change with the ALS treatment and with the removal of this treatment and the slow change in latent periods in the first transplant generation from a spontaneous tumor all suggest that these changes are the result of immunological action and reaction. Tumor cells or accompanying lymphocytic or macrophage cells are changed in one host so that they cause a different immunological stimulation in the next host which in turn affects the cells differently, thus leading to a progressive change.

Significance to Biomedical Research and the Program of the Institute:

Since immunity may play an important role in destroying the last remaining tumor cells after treatment of tumors with chemotherapy, it is important to understand all the possible immunologic interactions between tumors and their hosts. This series of reticulum cell tumors provides quite a variable group although most seem to be of relatively low antigenicity. It is particularly important in the treatment of tumors in humans to avoid enhancement and again this series of tumors provides a considerable range in enhancement. Human leukemias show late recurrence after therapy, but leukemias in animals showing such recurrences are rare. The reticulum cell tumors, however, do show late recurrences and may be used as an animal model for determining how to prevent them.

Proposed Course:

To try to determine the basis of the differences of the immunological nature between these lines. To determine the role of cellular and humoral immunity in the development of local growths by passive transfer of cells and serum from immunized donors. To try to obtain unaltered cells like those of the early transplant generation by trypsin treatment. Also, to freeze tumor tissue from early generations to have unaltered cells for comparison with altered ones. To obtain pure tumor cells so that carry-over from one generation to the next of lymphocytes and macrophages can be prevented. To analyze those

conditions that lead to late recurrences.

Honors and Awards:

None

Publications:

None

Serial No. NCI - 4834
1. Viral Leukemia and Lymphoma Branch
OASDVO, Division of Cancer
Cause and Prevention
2. Viral Pathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: A. Herpesvirus saimiri

B. Further characterization of Rauscher leukemia virus propagated in human cells

C. Activation of virus in cell cultures established from human tumors

Previous Serial Number: Same

Principal Investigator: Dr. Dharam V. Ablashi

Other Investigators: None

Cooperating Units: Inside NIH
Drs. A. J. Dalton and U. Heine, Ultrastructure Studies Unit, DCCP, NCI
Drs. G. Pearson and H. C. Chopra, VBB, DCCP, NCI
Drs. H. K. Oie, J. M. Easton, and P. H. Levine, VLLB, DCCP, NCI
Dr. S. S. Yang, Laboratory of Cell Biology, NCI
Dr. D. Twardzik, VCB, DCCP, NCI
Dr. R. H. Adamson, Laboratory of Chemical Pharmacology, DCT

Outside NIH
Drs. H. Rabin and W. F. Loeb, Bionetics Research Laboratories, Kensington, Maryland
Dr. Clyde R. Goodheart, BioLabs, Inc., Northbrook, Illinois
Dr. G. Klein, Karolinska Institute, Stockholm, Sweden

Man Years:

Total:	3.0
Professional:	1.0
Other:	2.0

Project Description

Objectives:

A. In certain human tumors (Burkitt's lymphoma, Hodgkin's disease, nasopharyngeal carcinoma) EBV has been implicated as a possible etiological agent. The object of the project is to use Herpesvirus saimiri findings as related to the EBV role in these human tumors.

B. More recent molecular and immunologic studies with murine Rauscher leukemia (RLV) virus indicate hybridization of RLV with certain human tumors as well as a possible common antibody detectable in sera from human patients. The object of this project was to further characterize the RLV propagated in human cells so that its oncogenic potentials may be later tested in other animals beside mice and hamsters.

C. The search for viruses (DNA and RNA) in the human tumors is still in progress. In some tumors, viral genome activation is required to produce complete virion and antigens directed to the virion by use of IdU and BrdU.

Methods Employed:

A, B, and C. Primary and continuous cell lines of human and nonhuman origin were used for virus isolations, biological assays and in vivo experiments. The tumors of human and nonhuman origin were employed for establishment of cell cultures. For in vivo experiments, owl, marmoset, and rhesus monkeys were used.

Major Findings:

A. In vitro and in vivo findings suggest that there are analogies between HVS and EBV. Owl monkeys bearing HVS-induced tumors (lymphoma and leukemia) responded to chemotherapeutic agents used for humans. Repeated heat-inactivated (56°C) HVS produced tumors in owl monkeys. The same inoculum was noncytopathogenic in vitro. The oncogenicity of the heat-treated virus suggests that the in vivo system is more susceptible to infection than the in vitro system. Transfer of HVS genome has been demonstrated by inoculating the virus-

free tumor in owl monkeys. A modified method of methyl alcohol concentration was found to be effective for the preservation of the infectivity of oncogenic herpesviruses.

The development of a continuous virus production system from an infected rhesus monkey transformed cell line offers an important research tool.

A reduction in cellular DNA-dependent DNA polymerase activity was observed after infection of vero cells with HVS. Partial purified HVS showed DNA-dependent DNA polymerase.

A non-producer lymphoid cell line established from an HVS-induced tumor in its 11th passage produced endogenous RNA-dependent DNA polymerase, which banded at 1.16. The cell extracts did not react with mouse gs-3 antibody.

B. Human cell adapted murine Rauscher virus (RLV) will infect monkeys of certain species, but not mouse cells. BrdU treatment enhances virus yields and CF titers. Monkey cell adapted RLV is infectious for human as well as monkey cells but not the mouse.

C. BrdU and IdU treated solid human tumor lines produced EBV. Similar treatment of American Burkitt cell cultures did not produce any virus and no EBV genome could be detected by hybridization.

Significance to Biomedical Research and the Program of the Institute:

A. Even though EBV may be implicated in the etiology of some human neoplasia, as yet there have been no susceptible animals found to be experimental hosts for EBV. Herpesvirus saimiri, including its nonhuman primate host, is proving to be a most suitable model system for the evaluation and understanding of the relation of EBV and human tumors. The HVS model is also very valuable for preclinical immunotherapy and chemotherapy studies.

B. The evidence of RLV infecting cells other than mouse, for example, cells of monkey origin, may be useful for studying the changes in viral coat antigens and its leukogenicity in monkeys.

C. The activation of EBV in cell cultures of human tumor origin suggests that either EBV plays a significant role in the establishment of these cultures or is a passenger virus in

these tumors.

Proposed Course:

A. Studies of biochemical, immunological, biological, and chemotherapeutic natureswill be continued to understand more about HVS and its similarities in tumors of Burkitt's lymphoma.

B. The *in vitro* and *in vivo* monkey cell-adapted RLV studies will be continued.

C. The culturing of tumors of human and nonhuman origin will be continued for isolation and identification of tumor viruses.

Honors and Awards:

Dr. Ablashi presented a seminar entitled,"Animal models for viral associated tumors of man" at the International Agency for Cancer Research, Lyon, France, June, 1972.

Dr. Ablashi presented a seminar entitled,"*Herpesvirus saimiri* *in vivo* and *in vitro* studies" at the Delta Regional Primate Center, Tulane University, School of Medicine, December, 1972.

Dr. Ablashi presented a seminar entitled,"*Herpesvirus saimiri* infection on monkeys as a model for the study of human lymphoma and leukemia" at Howard University, College of Medicine, February, 1973.

Publications:

Ablashi, D. V., Armstrong, G. R., and Blackham, E. A.: Certain characteristics of *Herpesvirus saimiri* cultured in subhuman primate cell cultures. *Am. J. Vet. Res.* 33: 1689-1694, 1972.

Rangan, S. R. S., Wong, M. C., Ueberhort, P. J., and Ablashi, D. V.: Mixed culture cytopathogenicity induced by virus preparation derived from cultures infected by simian sarcoma virus. *J. Natl. Cancer Inst.* 49: 571-577, 1972.

Pearson, G., Ablashi, D., Orr, T., Rabin, H., and Armstrong, G.: Intracellular and membrane immunofluorescence investigations on cells infected with *Herpesvirus saimiri*. *J. Natl. Cancer Inst.* 49: 1417-1424, 1972.

Ablashi, D. V., Loeb, W. F., Pearson, G., Valerio, M. G., Armstrong, G. R., Rabin, H., Kingsbury, E. W., and Heine, U.: Induction of lymphoma in owl monkeys with heated, non-cytopathogenic Herpesvirus saimiri. Nature (In press).

Loeb, W. F., Valerio, M. G., Ablashi, D. V., and Armstrong, G. R.: Lymphoma induction and viral isolation from owl monkey inoculated with lymphoma cells. Am. J. Vet. Res. (In press).

Ablashi, D. V., Armstrong, G. R., and Turner, W.: Production and characterization of human cell-adapted murine Rauscher virus pseudotype of murine sarcoma virus. J. Natl. Cancer Inst. 50: 381-385, 1973.

Ablashi, D. V., Loeb, W. F., Armstrong, G. R., Yang, Y. S., Valerio, M. G., and Adamson, R. H.: Oncogenicity of Herpesvirus saimiri induced lymphoma and DNA polymerase of the lymphoma derived cell line and Herpesvirus saimiri. Monograph, 3rd Conf. Exp. Med. & Surgery in Primates. (In press).

Rabin, H., Pearson, G., Klein, G., Ablashi, D., Wallen, W., and Cicmanec, J.: Herpesvirus saimiri antigen and virus recovery from cultured cells and antibody levels and virus isolations from normal squirrel monkeys. Am. J. Phys. Anthropol. (In press).

Dalton, A. J., Heine, U., Kondratick, J. M., Ablashi, D. V., and Blackham, E. A.: Ultrastructure and complement fixation studies of suspension cultures derived from human solid tumors. J. Natl. Cancer Inst. (In press).

Chopra, H. C., Lloyd, B. J., Ablashi, D. V., and Armstrong, G. R.: Morphologic studies of a cytomegalovirus isolated from an owl monkey. J. Natl. Cancer Inst. 48: 1333-1340, 1972.

Ablashi, D. V., Armstrong, G. R., Heine, U., and Adamson, R. H.: Establishment of a cell culture from an owl monkey tumor induced by Herpesvirus saimiri (HVS). Proc. Am. Assoc. Cancer Res. 13: 124, 1972.

Armstrong, G. R., Goodheart, C. R., Ablashi, D. V., Pearson, G., and Orr, T. W.: Concentration of oncogenic herpesviruses by methyl alcohol. Proc. Am. Soc. Microbiol. (In press).

Wallen, W. C., Rabin, H., Neubauer, R. H., and Ablashi, D. V.:

Depression of general mitogen response in Herpesvirus saimiri infected owl monkeys (Aotus trivirgatus). Proc. Am. Soc. Microbiol. (In press).

Wallen, W. C., Rabin, H., Neubauer, R. H., and Ablashi, D. V.: Functional characteristics of a lymphoblastoid tumor cell line derived from a Herpesvirus saimiri. Am. Assoc. Cancer Res. (In press).

Ablashi, D. V., Loeb, W. F., Pearson, G., Valerio, M. G., Armstrong, G. R., Rabin, H., Kingsbury, E. W., and Heine, U.: Induction of lymphoma in owl monkeys with heated, non-cytopathogenic Herpesvirus saimiri. Proc. Vth International Conf. on Cancer. (In press).

Armstrong, G. R., Ablashi, D. V., Easton, J. M., and Adamson, R. H.: Suppression of cytopathic effects (CPE) of Herpesvirus saimiri (HVS) by cytosine arabinoside (CA) in 1° owl monkey cells. Proc. Am. Soc. Microbiol. 246: 226, 1972.

Ablashi, D. V.: Review of Virology Monographs, Volume 11: Canine distemper virus; Marburg virus. ASM News 39: 161-162, 1973.

Serial No. NCI-4835

1. Viral Leukemia and Lymphoma Branch
   OASDVO, Division of Cancer
   Cause and Prevention
2. Tumor Virus Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: A. Studies of cell lines infected with murine sarcoma virus in absence of MuLV: Rescue phenomenon and evidence for production of a defective virus-like particle

B. Virus search in pediatric solid tumors

C. Development of in vitro human quantitative tissue culture assay systems for detection of potential human RNA tumor viruses

Previous Serial Number: Same

Principal Investigator: Dr. Paul T. Peebles

Other Investigators: Dr. Robert H. Bassin
Dr. Peter J. Fischinger
Dr. Brenda I. Gerwin
Dr. Daniel K. Haapala
Dr. Shigeko Nomura
Dr. Leo A. Phillips

Cooperating Units: Inside NIH
Staff of the VLLB, NCI

Outside NIH
None

Man Years:

Total:	2.50
Professional:	1.00
Others:	1.50

Project Description

Objectives:

A. To study rescue phenomena from cell lines infected with murine sarcoma virus (MSV) in the absence of murine leukemia virus (MuLV) and to characterize defective virus-like particles produced from these lines and clonal cell lines of nontransformed revertants.

B. To search for viruses in pediatric solid tumors.

C. To develop sensitive biological human tissue culture assay systems for the detection of possible human type C viruses.

Methods Employed:

A. Sarcoma and leukemia virus production in cell cultures were measured by known tissue culture assays, tritiated uridine labelling, immunological tests, and electron microscopy. Virus production from S+L- revertant cell lines was measured by reverse transcriptase activity in their supernatant fluids.

B. Recently developed human tissue culture detection systems have been utilized in an attempt to find evidence for a viral etiology in pediatric tumors.

C. Human tissue culture cell lines are being investigated for their potential use as detection systems for human oncorna viruses using techniques previously employed for investigation of MSV and MuLV.

Major Findings:

A. The isolation of clonal cell lines infected with MSV in the absence of detectable MuLV (S+L- cells) provided the opportunity to study the defectiveness of MSV. S+L- mouse 3T3 cells, which yield sarcoma virus after superinfection with murine leukemia virus, spontaneously give rise to flat variants from which murine sarcoma virus can no longer be rescued. The revertants support leukemia virus growth and show an enhanced sensitivity to murine sarcoma superinfection and, like normal cells, do not release RNA-dependent DNA polymerase activity. Because revertants could be obtained with high frequency from progeny of single transformed cells, each cell that contains the sarcoma virus genome seems to have the capacity to suppress or eliminate an RNA tumor virus native to its species of origin.

Such variant cells were epithelioid, contact inhibited, and grew to low density, and their low cloning efficiency in soft agar was similar to that of normal parental 3T3 cells. However, they contained murine leukemia (MuLV) group-specific antigen(s) without demonstrable virus production and

reverse transcriptase activity. MSV could no longer be rescued from these flat variant cells by superinfection with MuLV, by cocultivation with normal 3T3 cells or by transspecies rescue into cat cells. An enhancement of sensitivity to MSV and MuLV infection was observed in all flat variant cultures. Flat variant clones spontaneously gave rise to retransformed cells during extended cultivation. Morphology, saturation density, and cloning efficiency in soft agar of cloned spontaneous retransformed cell lines were similar to the original MSV-transformed cells. However, they failed to demonstrate MuLV gs antigen(s), virus production, reverse transcriptase activity and a rescuable MSV genome. The spontaneously retransformed cells were susceptible to MSV and MuLV infection. After treatment with 5-iododeoxyuridine (IdU) reverse transcriptase activity and virus particles were only rarely induced in flat variant or spontaneously retransformed clones. These particles were not infectious for the original host cells and were not induced in normal 3T3 cells or a majority of the variant clones.

Chromosome studies of these variants suggested that the partial or complete loss of expression of transformation in variants might have been associated with an imbalance in the number of chromosomes mediating expression or suppression in these cells.

B. Studies are in progress with fresh tumor material from pediatric patients with solid tumors using the recently described techniques outlined below.

C. Human in vitro tissue culture systems are needed for the isolation, assay, and analysis of potential human RNA tumor viruses. Infection of continuous cell line AV-3 from human amnion cells with a virus stock containing MSV (FeLV) in excess of helper FeLV resulted in small discrete foci of transformed cells and a "one-hit" dose-response curve suggesting that terminal foci were infected with MSV in the absence of FeLV. A terminal focus was cloned and established into a transformed cell line demonstrating no release of infectious focus-forming virus until super-infected with either FeLV or RD-114 virus. These superinfecting helper viruses determined the envelope-associated properties of host range and interference for the rescued MSV pseudotypes. The fact that the RD-114 virus pseudotype of MSV preferentially infected human cells did not necessarily indicate that RD-114 was a human virus, but it did indicate that RD-114 virus was at least a type C virus well-adapted for growth in human cells.

The apparent absence of RNA tumor virus information in the normal parental human amnion cell line made it unlikely that the human S+L- cells contained simply a derepressed endogenous human sarcoma virus genome. To assure that the sarcoma virus genome present in S+L- human cells was murine and not a putative endogenous human sarcoma virus, "S+L-" type MSV was used for transformation because of its distinctive genetically stable marker of murine specific gs-1 antigen. Murine gs-1 antigen was conclusively

identified in S+L- cells by both CF assay and by immunodiffusion techniques. Preliminary experiments in this laboratory have also failed to demonstrate type C virus particle production from S+L- human cells examined by electron microscopy and by release of viral-type RNA-dependent DNA polymerase into culture supernatant fluids.

These human cell lines have proved of value for the isolation and characterization of a new virus isolate (CCC virus) from cat cells which has biological and immunological properties identical to RD-114 virus.

Significance to Biomedical Research and the Program of the Institute:

A. The exploration of murine oncogenic RNA viruses, their defectiveness, and the variance between their capacity to replicate and their capacity to transform has great implications in that their existence makes probable the viral etiology for human cancer.

B. The tumors from pediatric patients are of particular interest for an oncogenic virologist because of the possibility of vertical transmission on oncogenic viruses. Also clinically, outside of automobile and ingestion accidents, cancer is responsible for most of the deaths in children in the United States.

C. For the first time, the interrelationship between a sarcoma virus and human cells has been demonstrated to be identical to that in known animal models. "Defective" MSV is able to infect and transform human amnion cells but is unable to replicate infectious progeny focus-forming virus in the absence of replicating helper virus. This relationship may be operating in some human tumors and the inability to find infectious virus may be due to the presence of defective transforming sarcoma genomes in the absence of detectable replicating leukemia viruses.

The isolation of S+L- human cells has already proved of practical significance in identifying the biological helper nature of the human candidate type C virus RD-114. RD-114 had previously been characterized as being a nontransforming infectious type C virus biochemically analogous to, but immunologically different from, all known animal type C virus isolates. Superinfection of S+L- human cells with RD-114 now provides high titers of the RD-114 pseudotype of MSV and experiments are in progress to determine if this MSV (RD-114) pseudotype is useful in sero-epidemiological investigation for neutralizing antibodies in patients, especially those who have had rhabdomyosarcoma. Studies are also in progress to determine if S+L- human cells derived from single cell clones revert to normal nontransformed variants in a manner comparable to reversion in the mouse system.

Most important is the possibility that the combination of the normal uninfected and the S+L- human amnion cell lines may provide a unique in vitro method in a human system for the isolation, assay, and identification of sarcomagenic and leukemogenic RNA tumor virus from human tumors. Assays for sarcomagenic-type virus may be performed by focus formation on F-49-1 cells, and assays for leukemogenic-type viruses may be by induction of CF antigen, interference, or reverse transcriptase in normal human amnion cells, or, more simply, by focus-induction or virus rescue from S+L- human cells.

Proposed Course:

A. We are attempting to characterize the phenotypic expression of MSV defectiveness in the heterologous human host S+L- cells in comparison to mouse S+L- cells

B. We are continuing to attempt to demonstrate a viral etiology in pediatric solid tumors.

C. The quantitative aspects of the tissue culture assays using human cell lines susceptible to MSV transformation are being studied in order to improve the chances for detection of human sarcoma viruses. Rapid tissue culture assays are being developed using human S+L- cells for possible detection of replicating nontransforming human type C viruses.

Honors and Awards

Dr. Peebles presented a talk entitled "Isolation of Human Amnion Cells Nonproductively Transformed by Murine Sarcoma Virus: Potential Use in Detecting Human Type C Viruses" at the Seventh Annual Joint Working Conference, Special Virus Cancer Program, Hershey, Pa., November 1, 1972.

Dr. Peebles presented a special seminar entitled "An Approach to Isolation of RNA Viruses (Type C) From Human Tumors" at the School of Medicine, University of California, San Diego, October 2, 1972.

Publications

Fischinger, P. J., Nomura, S., Peebles, P. T., Haapala, D. K., and Bassin, R. H.: Reversion of murine sarcoma virus transformed mouse cells: Variants without a rescuable sarcoma virus. Science 176: 1033-1035, 1972.

Nomura, S., Fischinger, P. J., Mattern, C. F. T., Peebles, P. T., Bassin, R. H., and Friedman, G. P.: Revertants of mouse cells transformed by murine sarcoma virus. I. Characterization of flat and transformed sublines without a rescuable murine sarcoma virus. Virology 50: 51-64, 1972.

Peebles, P. T., Fischinger, P. J., Bassin, R. H., and Papageorge, A. G.: Isolation of human amnion cells transformed by rescuable murine sarcoma virus. Nature. In press.

Bassin, R. H., Phillips, L. A., Kramer, M. J., Haapala, D. K., Peebles, P. T., Nomura, S., and Fischinger, P. J.: Properties of 3T3 cells transformed by sarcoma virus in the absence of replicating murine leukemia helper virus. In Chieco-Bianchi, L. and Dutcher, R. M. (Eds.): Proc. Vth International Symposium on Comparative Leukemia Research. In press.

Serial No. NCI-4836

1. Viral Leukemia and Lymphoma Branch
   OASDVO, Division of Cancer
   Cause and Prevention
2. Molecular Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Viruses in experimental oncogenesis and human cancer

Previous Serial Number: NCI-4914

Principal Investigator: Dr. Stuart A. Aaronson

Other Investigators: Dr. John R. Stephenson
Dr. Steven R. Tronick

Cooperating Units: Inside NIH
Dr. Joel S. Greenberger, VC, NCI
Dr. Robert J. Huebner, VC, NCI
Dr. Malcolm Martin, LBV, NIAID
Dr. Kenneth K. Takemoto, LVD, NIAID
Dr. Robert H. Bassin, VLL, NCI
Dr. Tadao A. Aoki, VLL, NCI
Dr. Wallace P. Rowe, LVD, NIAID

Outside NIH
Dr. Garth R. Anderson, Hazleton Laboratories,
Vienna, Virginia
Dr. Robert C. Good, Hazleton Laboratories,
Vienna, Virginia
Dr. David Lytle, Environmental Health Bureau,
Rockville, Maryland
Dr. Harvey Dosik, Maimonides Hospital,
Brooklyn, New York
Dr. John Kersey, University of Minnesota,
Minneapolis, Minnesota
Dr. Leonard Hayflick, Stanford University,
Stanford, California
Naval Biological Research Laboratory,
Oakland, California
Dr. Roger E. Wilsnack, Huntingdon Research Labs.,
Baltimore, Maryland

Man Years:

Total:	2.0
Professional:	1.0
Other:	1.0

Project Description

Objectives:

1. To study the mechanisms of action of RNA and DNA-containing tumor viruses.

2. To apply knowledge gained from experimental systems to search for a viral etiology to human neoplasia and to develop rational approaches to prevention and treatment of human tumors.

Methods Employed:

Standard cell culture procedures of this laboratory. Immunological and biochemical techniques such as enzymology and nucleic acid homology studies.

Major Findings:

1. Continuous lines of Balb/c and NIH Swiss embryo cells have been developed which are very useful for study of normal growth control mechanisms and the effects of RNA and DNA tumor viruses on growth regulation.

2. A genetic approach to the study of RNA tumor viruses has led to the development of rapid methods for screening large numbers of cultures for temperature-sensitive (ts) mutants. Ts mutants of both murine leukemia (MuLV) and sarcoma virus (MSV) have been isolated, and some have been partially characterized.

3. Sarcoma virus transformed cells have been isolated which show normal cell morphology but contain the typical sarcoma viral genome. These cellular mutants are currently being studied to determine the cellular functions involved in the expression of transformation.

4. Type C RNA viruses have been found to be inducible from numerous clonal lines of Balb/c embryo cells by treatment with chemical agents such as BrdU. These studies demonstrate that all Balb/c cells contain a latent type C viral genome in a genically stable form. In addition, MSV-transformed nonproducer mouse and rat cells isolated in this laboratory can be activated by these same chemicals to produce both MSV and MuLV. The mechanisms of action of various chemical inducers are currently being investigated.

5. Genetic studies have revealed the presence of genetic loci for virus induction in mouse cells of different strains. Biologically distinguishable viruses can be activated at distinct loci. These findings, along with biochemical evidence, indicates that type C viruses are normally integrated within the mouse cell genome. Genetic factors affecting inducibility and persistence of endogenous viruses are currently being studied.

6. MSV nonproducer cells have been of particular interest because they provide a model system that shows many similarities to the human tumor cell. Nonproducer cells have been found to lack any detectable transplantation antigens in contrast to RNA virus-producing transformed cells which are highly antigenic. They have, however, recently been shown to contain an MSV-associated cell surface antigen which may be involved in the transformation process.

7. The reverse transcriptases of RNA tumor viruses have been extensively investigated. Normal mouse cells have been found to contain two enzymes which can utilize RNA-RNA or RNA-DNA synthetic templates. These enzymes can be distinguished, however, by physical properties, template preferences, and antigenicity from type C viral polymerase. While viral enzyme can be readily found in virus-producing cells, MSV nonproducer cells lack detectable virus-specific enzyme. Antibodies have been developed which specifically inhibit murine type C viral polymerases. Antibodies to other type C viral enzymes have also been obtained. These are of use in the identification of type C virus isolates and in studying the reverse transcriptase enzymologically.

8. Primate isolates of type C viruses are being characterized biologically, immunologically, and biochemically. A focus-forming virus from a woolly monkey sarcoma has been shown to induce transformation of mammalian cells in the absence of infectious virus production. The morphologic alteration induced by this virus is strikingly different from that induced by MSV.

9. Biochemical and immunologic techniques have been developed to detect RNA virus-specific information in normal and transformed cells. Sensitive radioimmunologic procedures are being used to detect the expression of a number of virion antigens. DNA-DNA and DNA-RNA hybridization techniques have also been utilized. The integration of viral DNA has been demonstrated using such techniques.

10. Human tumor material has been processed, and several lines of tumor cells have been established. These are being extensively investigated for evidence of tumor virus expression using techniques developed from work in model systems.

Significance to Biomedical Research and the Program of the Institute:

The systems that are being intensively investigated have provided a much better understanding of the biology and biochemistry of viral transformation. It is felt that a clear understanding of these phenomena will significantly speed the progress in our search for a viral etiology of human cancer.

Proposed Course:

To continue research already in progress in the following major areas: (1) mechanisms of action of murine sarcoma and murine leukemia viruses; (2) induction of type C viruses from virus-negative cells; (3) determination of the role of viruses in human neoplasia; and (4) to develop new research areas in model systems that pertain to human disease.

Honors and Awards:

None

Publications:

Aaronson, S.A., Bassin, R.H., and Weaver, C.: Comparison of murine sarcoma viruses in nonproducer and S+L- transformed cells. J. Virol. 9: 701-704, 1972.

Aaronson, S.A. and Stephenson, J.R.: Genetic factors involved in C-type RNA virus expression. Day, S.B. and Good, R.A. (Eds.): Membranes and Viruses in Immunopathology. New York, Academic Press, 1972, pp. 355-366.

Aaronson, S.A.: Immunologic detection of C-type RNA viral reverse transcriptase. JNCI Monograph Series. (in press).

Aaronson, S.A. and Stephenson, J.R.: Endogenous RNA C-type viruses of mammalian cells. Amsterdam, The Netherlands, North-Holland Publishing Co. (in press).

Aaronson, S.A.: Biologic properties of mammalian cells transformed by a primate sarcoma virus. Virology. (in press).

Aaronson, S.A. and Stephenson, J.R.: Independent segregation of loci for activation of biologically distinguishable RNA C-type viruses in mouse cells. Proc. Natl. Acad. Sci. USA. (in press).

Serial No. NCI - 4840

1. Viral Leukemia and Lymphoma Branch, OASDVO, Division of Cancer Cause and Prevention
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Non-human primate surgery - development of special techniques and procedures in addition to routine surgery

Previous Serial Numbers: Same

Principal Investigator: Dr. Sergio A. Leiseca

Other Investigators: None

Cooperating Units:

Inside NIH
Dr. Roy F. Kinard, VLLB, NCI
Dr. Paul H. Levine, VLLB, NCI
Dr. Jack Gruber, VB, NCI

Outside NIH
Litton Bionetics Research Laboratories
Kensington, Maryland

Man Years:

Total:	1.0
Professional:	1.0
Other:	0.0

Project Description

Objectives:

1. To provide to the National Cancer Institute, and other cooperating institutes, embryonic and fetal material from the primate colony maintained at Litton Bionetics Research Laboratories (BRL).

2. To provide surgical procedures required to maintain and improve the health of primate colony inhabitants.

Methods Employed:

1. Cesarean sections technique performed on primates (primarily by Macaca mulatta).

2. Developed and applied procedures for cannulation of thoracic lymph ducts.

3. Hysterotomy and biopsy of fetal skin and muscle.

4. Intra-uterine surgery in nonhuman primates the 65th day of gestation.

5. Many operative procedures are done to repair various clinical conditions.

Major Findings:

1. C-section techniques are also used for the following: to obtain fetal material for large amounts of primary cell cultures used in NCI; to provide most of the infants for the S.V.C.P. virus inoculation program at the Litton Bionetics Research Laboratories project; to study the effect of carcinogenic materials on fetuses inoculated in utero; to provide through amnioncestesis recovery of drug in the amniotic fluids for the determination of possible inducement of anomalies or tumors, and to salvage all placentae for protein studies in relation to malignancy and birth control.

2. Cannulation of thoracic lymph ducts is used in rhesus monkeys for the continuous collection of in the study of the malignant tumors.

3. The C-section technique is used to present the fetuses and neonatal animals for immunosuppressive studies and for inoculation of leukemia or other cancerous materials as well as some viruses. Biopsies of fetuses are used for tissue cultures for later infection with cancerous materials and reinjection into the donor.

4. Cannulization of placental fetal vessel and maternal saphenous vein allowed continuous recovery of blood specimens. These specimens are used in investigations of the transfer of drugs, hormones, glucose, etc.

By fetal exsanguination intra-utero blood is obtained for alpha fetal protein studies. This study is a part of the leukemia program.

5. Surgical procedures are routinely performed, repair injuries resulting from accidents that occur in the primate breeding colony. Some of these procedures are:

a. suture and debridement of various wounds, and lacerations of head, body, extremities, tongue, tail

b. drainage of abscess

c. amputations of digits traumatized and tail

d. open reduction of fractures

e. herniorrhaphy-umbilical, vential, or inguinal

f. closure of wounds

g. C-section to obtain fetuses in breech presentation, bleeding due to premature placental separations, toxemia, protracted labor, prolonged or overdue pregnancy, dead fetus, etc.

h. prolapse of rectus

i. prolapse of vagina and cervix

j. D & C to remove retained decidua following abortion or delivery

k. tumor biopsy

l. laparotomy for ovarian cyst, ruptured uterus, splenomegaly, etc.

Significance to Biomedical Research and the Program of the Institute:

Non-human primates have proven very useful in research on human viral diseases. They are man's closest phylogenetic relative and are the most desirable model system in research projects which cannot utilize humans directly. Much of the work in primate cancer research is dependent on the highest quality surgical procedures for the provision of sterile fetal tissue and healthy young free of pathogenic organisms. Surgical intervention for lymph and other tissued requires great professional skill for maximum animal health and comfort in addition to experimental validity.

Proposed Course:

Continue to provide the best possible surgical procedures for primate cancer research.

Honors and Awards:

None

Publications:

Martin, D., Leiseca, S. A., and Darrow, C.: Methods of anesthesia in subhuman primates. Anesthesiology 22: 837-843, 1972.

Serial No. NCI-4842
1. Viral Leukemia and Lymphoma Branch
OASDVO, Division of Cancer
Cause and Prevention
2. Tumor Virus Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The biochemistry and biophysics of leukemia and sarcoma viruses

Previous Serial Number: Same

Principal Investigator: Dr. Leo A. Phillips

Other Investigators: Dr. Robert H. Bassin
Dr. Peter J. Fischinger
Dr. Daniel K. Haapala
Dr. Shigeko Nomura
Dr. Paul T. Peebles

Cooperating Units: Inside NIH
Dr. Adi F. Gazdar, VLLB, NCI
Dr. Harish C. Chopra, VBB, NCI

Outside NIH
Dr. Robert P. Perry, The Institute for Cancer Research
Philadelphia, Pa. 19111

Man Years:

Total:	2.00
Professional:	1.00
Others:	1.00

Project Description

Objectives:

These studies are concerned with the biochemical and biophysical properties of RNA tumor viruses. Our objectives are as follows:

1. The characterization of the nucleic acids and the structural polypeptides of purified virions released from cells which have been transformed by sarcoma viruses.

2. The isolation and characterization of the viral specific nucleic acid and polypeptides of cells infected with leukemia and sarcoma viruses.

3. The determination of which of the viral specific polypeptides is the reverse transcriptase of the virions.

4. The determination of what makes a sarcoma virus either competent or defective.

5. The determination of the state of the sarcoma genome in S+L- cells which are not releasing particles; a determination of whether the genome exists as a plasmid, an episome, or whether it is integrated into the cellular DNA.

6. The determination of what makes one cell line permissive to oncogenic virus infection and another cell line nonpermissive; therefore, trying to answer this vital question by in vitro protein synthesis.

7. The examination of human cancer materials (neoplasias) for viral specific nucleic acids.

Methods Employed:

The focus assay for sarcoma viruses and the helper assay for leukemia viruses are employed for the quantitation of the viruses. [^{3}H]-uridine incorporation in particles with a density of 1.16 g/cm^3, RNA-dependent DNA polymerase assays on supernatant fluids and electron microscopy are utilized to determine if virus is released from transformed cells. For characterization of the viral nucleic acids and polypeptides, standard biochemical and biophysical techniques (analytical and preparative ultracentrifugation, column chromatography, isotopic labeling, polyacrylamide gel electrophoresis, peptide fingerprinting, electrofocusing, in vitro protein synthesis, enzymology, etc.) are bieng employed.

Major Findings:

The RNA from noninfectious virions produced by two established clonal lines of sarcoma positive-leukemia negative (S+L-) transformed 3T3 cells has been characterized. RNA from virions or nucleoids of S+L-(C243) cells consisted of 3 to 4 sizes: ±44S(6%), 28S (17%), 18S (38%) and less than 18S (39%). The 28S virion RNA was found to contain virus specific information demonstrable by RNA·DNA hybridization with a DNA probe derived from the murine sarcoma-leukemia virus reverse transcriptase product. The RNA from virions or nucleoids of S+L-(D56) cells consisted of 5 sizes: 80S (6%), 68S (8%), 56S (5%), 28S (28%), and <28S (53%). Comparative parallel studies with rRNA from 60S subunits of transformed and nontransformed 3T3 cells determined that the 28S S+L- virion RNA was most probably the monomeric genome because it contained virus specific information and poly (A) sequences at the 3'-terminus. The 56S, 68S, and 80S RNA are most probably tetrameric, hexameric, and octomeric genome aggregates.

Serial No. NCI-4842

Significance to Biomedical Research and the Program of the Institute:

These biochemical and biophysical studies of RNA tumor viruses contain three avenues for obtaining pertinent knowledge for the possible cure of cancer: (1) studies which are being conducted on the viral nucleic acids and structural polypeptides could possibly determine if their mode of replication is by means of transcriptional-translational complexes, and if so, it may be possible to effect a cure for cancer by specifically blocking the replication of these RNA tumor viruses either at the transcriptional or translational level; (2) studies which are being done to determine the disposition of the viral genome in sarcoma transformed, nonproducer cells could possibly enable the excision of the viral genome with interfering with the cellular genome; (3) studies which are being conducted to ascertain the property which makes one cell line permissive, while another cell line is nonpermissive, to infection by RNA tumor viruses will be invaluable in effecting a cure for cancer.

Proposed Course:

These critical biochemical and biophysical studies of RNA tumor viruses will be continued in order of their complexity.

Honors and Awards

Invited to participate in the 1972 Gordon Research Conference on "Nucleic Acids" at the New Hampton School, New Hampton, New Hampshire, June 11-16, 1972.

Invited participant and speaker in the 1972 Gordon Research Conference on "Animal Cells and Viruses" at the Tilton School, Tilton, New Hampshire, August 27 to September 1, 1972.

Elected unanimously and served as Chairman of the National Cancer Institute Equal Employment Opportunity Advisory Group.

Publications

Bassin, R. H., Phillips, L. A., Kramer, M. J., Haapala, D. K., Peebles, P. T. Nomura, S., and Fischinger, P. J.: Properties of 3T3 cells transformed by sarcoma virus in the absence of replicating murine leukemia helper virus. In Chieco-Bianchi, L. and Dutcher, R. M. (Eds.): Proc. Vth International Symposium on Comparative Leukemia Research. In press.

Phillips, L. A. and Bussell, R. H.: Buoyant density of canine distemper virus. Arch. Gesamte Virusforsch. In press.

Serial No. NCI - 4845

1. Viral Leukemia and Lymphoma Branch, OASDVO, Division of Cancer Cause and Prevention
2. Viral Pathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: A. Studies on the effect of melatonin on the growth of selected murine tumors

B. Studies on the effect of melatonin on human affective disorders

C. Studies on the effect of melatonin on plasma levels of luteinizing hormone

Previous Serial Number: Same

Principal Investigator: Dr. Richard S. Buswell

Other Investigators: Dr. Adi F. Gazdar

Cooperating Units: Inside NIH
Dr. Frederick K. Goodwin, LCS, NIMH
Dr. Judith Vaitukaitis, RRB, NICHD

Outside NIH
Flow Laboratories, Rockville, Maryland

Man Years:

Total:	2.0
Professional:	1.5
Other:	0.5

Project Description

Objectives:

A. To assess the effect of parenterally administered melatonin on tumor growth.

B. To assess the effect of orally and parenterally administered melatonin on the affect of patients with affective disorders.

C. To assess the effect of melatonin on plasma levels of luteinizing hormone.

Serial No. NCI - 4845

Methods Employed:

A. Melatonin, a methoxyindole, was administered subcutaneously to the laboratory animal. Animals were challenged with MSV or MSV-induced non-producer cells after melatonin pretreatment. The treatment was continued daily. Animals were sacrificed at serial intervals and tumor weights determined. Longevity studies were also performed.

B. Different doses of melatonin solution were infused in patients with affective disorders and their subsequent affect and cognition were monitored, using standardized testing methods.

C. During these above mentioned melatonin infusions, samples of blood were drawn at fixed intervals and the plasma levels of luteinizing hormone were measured by radioimmunoassay.

Major Findings:

A. Melatonin inhibits the growth of MSV tumors in mice. A T.I.D. 80-100 was used. Spleen weight was also less in treated animals, suggesting inhibition of virus replication. Longevity studies are in progress.

B. Melatonin infusions seem to sedate and lessen depressive affect in patients with depression.

C. Thus far, the melatonin infusions have not altered the plasma luteinizing hormone levels in humans.

Significance to Biomedical Research and the Program of the Institute:

A. Several data suggest that pinealectomy enhances the growth of tumors. Since melatonin is produced almost exclusively in the pineal gland, it is relevant to investigate their effect on tumor growth.

B. A decrease in brain serotonin has been incriminated in the pathogenesis of depression. Data suggests that melatonin raises total brain serotonin. It is pertinent to investigate the antidepressant potential of melatonin.

C. Animal experiments repeatedly demonstrate that parenteral administration of melatonin is associated with a decrease in plasma luteinizing hormone. It is of interest to investigate whether this phenomenon holds true in humans.

Proposed Course:

A. Investigate the mechanism whereby melatonin inhibits MSV tumor growth. Further, it is possible that inhibitory action on other tumor types will be researched.

B. Continued evaluation of effect of oral and parenteral melatonin on the course of affective disorders.

C. Continued evaluation of effect of melatonin infusions on plasma levels of luteinizing hormones in humans.

Honors and Awards:

None

Publications:

None

Serial No. NCI-4846

1. Viral Leukemia and Lymphoma Branch
   OASDVO, Division of Cancer Cause
   and Prevention
2. Immunology Section
3. Bethesda, Maryland

PHS-NIH

Individual Project Report

July 1, 1972 through June 30, 1973

Project Title:

A. Development of a technique for identification and purification of a viral antigen - the enzyme, RNA-dependent DNA polymerase

B. Biochemical studies of a human breast carcinoma cell line

C. Purification of RNA-dependent DNA polymerase from non-infectious particles produced by sarcoma positive leukemia negative mouse cells. Characterization of other S+L- cells

D. Analysis of the DNA polymerase activities in human cells

E. Biochemical studies on human milk

Previous Serial Number: Same

Principal Investigator: Dr. Brenda I. Gerwin

Other Investigators: Dr. Tadao Aoki

Cooperating Units: Inside NIH

Dr. Robert Bassin, VLLB, NCI
Dr. Daniel Haapala, VLLB, NCI
Dr. Paul Ebert, VBB, NCI
Dr. Harish Chopra, VBB, NCI
Dr. Ernest J. Plata, VLLB, NCI
Dr. George Todaro, VLLB, NCI
Dr. Paul Peebles, VLLB, NCI
Dr. Shigeko Nomura, VLLB, NCI

Man Years:

Total:	2.0
Professional:	1.0
Other:	1.0

Serial No. NCI-4846

Project Description

Objectives:

A. To develop a technique which will identify and purify the internal antigen of RNA tumor viruses: RNA dependent polymerase (reverse transcriptase).

B. To search an available breast carcinoma cell line for evidence of enzyme activity characteristic of known tumor viruses.

C. To determine whether the noninfectious particles produced by sarcoma positive leukemia negative mouse cells possess a reverse transcriptase activity and to determine whether this activity can be distinguished from that of infectious leukemia particles. To analyze revertants of S+L- murine cells for their ability to produce particles or to be chemically induced to produce particles. To determine whether newly established human S+L- lines contain viral reverse transcriptase.

D. To determine whether the technique developed in 'A' will find viral type enzyme in human cells.

E. To determine whether enzyme activities reported in human milk are specific for a virus and/or related to a viral etiology for human breast carcinoma.

Methods Employed:

A. A primer which facilitates RNA copying by viral reverse transcriptase was covalently attached to cellulose and used for affinity chromatography of supernatant and cellular extracts.

B. The affinity chromatography technique developed in 'A' was used to search for a reverse transcriptase activity in the breast tumor cell line.

C. Supernatants of virions were centrifuged to concentrate any particles which might be present and screened for the presence of virus particles by assaying for the viral enzyme reverse transcriptase. The enzyme from particles produced by murine S+L- cells was purified by column chromatographic techniques. The affinity chromatography technique developed in 'A' was used to search human S+L- lines for reverse transcriptase.

D. Several human cell lines have been examined for the presence of reverse transcriptase using the technique developed in 'A'.

E. DNA polymerases have been purified from human milk by ion exchange and affinity chromatography.

Major Findings:

A. The affinity chromatography technique is useful for purification of viral reverse transcriptase. In addition, the technique appears to retain only viral DNA polymerase from infected cells while the DNA polymerases of noninfected cells pass through the column.

B. The breast carcinoma line contains an enzyme which binds to the affinity column and appears like a viral enzyme in other respects.

C. Sarcoma positive leukemia negative particles do contain a viral reverse transcriptase which is indistinguishable from that present in leukemia viruses. Most revertants of murine S+L- cells do not produce particles and cannot be chemically induced. Data on human lines is still premature.

D. To date all human cell lines with the exception of the breast carcinoma line in 'B' have been negative for viral reverse transcriptase by affinity chromatography.

E. DNA polymerases purified from human milk by ion exchange and affinity chromatography have some but not all the characteristics of "viral" reverse transcriptase.

Significance to Biomedical Research and the Program of the Institute:

A. The availability of a new technique for purification and identification of a viral antigen adds new possibilities for searching human material for RNA tumor viruses.

B. The finding of a possible virus specific component in a human tumor line supplies a marker which can be studied in various model systems to attempt to rescue a human RNA tumor virus.

C. The characterization of noninfectious S+L- particles and cells will further our understanding of the interactions of sarcoma and leukemia virions in model systems relevant to human cancer.

D. The use of a new technique to search human material for evidence of the presence of RNA tumor viruses attempts to examine the question of the viral etiology of human cancer.

E. Our findings in human milk suggest that, if viral specific components are present, they differ from those found in mammalian model systems.

Proposed Course:

A. The technique will be applied to search human cell lines and primary tissues.

B. The enzyme found in the breast tumor cells will be characterized

further and compared to "normal" cellular enzymes and known viral enzymes.

C. Experience gained in murine S+L- systems will enable us to study the effect of human cells on the S+L- genome.

D. Primary tumors and normal tissues will be examined.

E. This project is completed.

Honors and Awards:

None

Publications:

Gerwin, B.I. and Milstein, J.B.: An oligonucleotide affinity column for RNA-dependent DNA polymerase from RNA tumor viruses. Proc. Natl. Acad. Sci. U.S.A. 69:2599-2603, 1972.

Gerwin, B.I., Ebert, P.S., Chopra, H.C., Smith, S.G. and Kvedar, J.P.: DNA polymerase activities of human milk. Science (in press).

Bassin, R.H., Plata, E.J., Gerwin, B.I., Mattern, C.I., Haapala, D.K., and Chu, E.W.: Isolation of a continuous epithelioid cell line, HBT-3, from a human breast carcinoma. Proc. Soc. Exp. Biol. Med. 141:673-680, 1972.

Plata, E.J., Robertson, D.D., Aoki, T., Chu, E.W., and Gerwin, B.I.: An established cultured cell line from human breast carcinoma (HT-39). J. Natl. Cancer Inst. (in press).

Serial No. NCI - 4847

1. Viral Leukemia and Lymphoma Branch
OASDVO, Division of Cancer
Cause and Prevention
2. Viral Pathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Morphologic studies on virus induced tumors

Previous Serial Number: Same

Principal Investigator: Dr. Yoji Ikawa

Other Investigators: Dr. Herbert K. Oie
Dr. Adi F. Gazdar

Cooperating Units: Inside NIH
Dr. Harish C. Chopra, VBB, NCI
Dr. Paul S. Ebert, VBB, NCI
Dr. Abraham Goldin, DR&D, NCI
Dr. Philip Leder, LMG, NICHD
Dr. Akira Niwa, LBH, NICHD

Outside NIH
Dr. A. E. Bogden, Mason Research Institute, Worcester, Massachusetts
Dr. T. N. Fredrickson, University of Connecticut, Storrs, Connecticut
Dr. A. Hackett, Naval Biomedical Research Laboratories, Oakland, California
Dr. M. Hatanaka, Flow Laboratories, Inc., Rockville, Maryland
Dr. H. Sugano, Cancer Institute, Toshima-Ku, Tokyo, Japan

Man Years:

Total:	2.0
Professional:	1.0
Other:	1.0

Project Description

Objectives:

A. To study the relationship between differentiation arrest

and neoplastic characters in virus induced leukemia cells.

B. To study the biological behavior in vitro of virus-transformed, non-producer cells and their revertants.

C. To study replication and transformation kinetics of several murine sarcoma virus strains in rat and mouse liver cell lines.

D. To isolate and characterize type C viruses from a transplantable rat mammary carcinoma line.

Methods Employed:

A. Erythrocytic differentiation of a Friend leukemia line was induced by dimethyl sulfoxide, erythrocyte membrane antigens and globin appearance were studied by immuno-fluorescence, mRNA and ALA-synthetase and hemoglobin were determined biochemically.

B. Animals inoculated with Ki-MSV non-producer cells were treated with chemotherapeutic agents.

C. A rat liver cell line was transformed by MSV strains. Routine focus assays and complement fixation techniques were used for MSV detection.

D. Routine tissue culture and virus isolation techniques were employed.

Major Findings:

A. A clonal Friend leukemia line, negative for mRNA for globin, was switched on to produce the same mRNA by dimethyl sulfoxide, and was found to be controlled at the transcriptional level. A slight degree of macrophagic activity was induced in the RLV-induced myelomonocytic line.

B. A Kirsten MSV-transformed BALB/3T3 non-producer clone was morphologically epithelial and behaved like a spontaneous carcinoma in syngeneic hosts. This line is sensitive to cytoxan and BCNU and resistant to MTX and 5FU. This system appears to be useful in screening anti-carcinoma drugs. A flat, favorably contact-inhibited line was isolated from the above clone, and showed less tumorigenicity, but still had MSV genome.

C. Kirsten and Gazdar strains of MSV transformed established rat liver cell cultures. Both parent and transformed cell lines express liver cell membrane antigens, ornithine carbamyl transferase, and dexamethasone-inducible tyrosine transaminase activities. The transformation was 3,000-fold less efficient than transformation of mouse 3T3 cells and rat NRK cells. Transplantation of the transformed cells produced tumors histologically resembling hepatomas.

D. Both epithelioid and spindle-shaped lines have been isolated from R-35 tumors frozen in 1963, 1967, and 1972. The spindle-shaped lines propagated more virus and produced in vivo medullary tubular adenocarcinoma with marked mesenchymal reaction in the surrounding tissue. The epithelioid lines produced scirrhous carcinomas.

Significance to Biomedical Research and the Program of the Institute:

It may be possible to treat human leukemias by induction of maturation. Experimental non-producer tumors may resemble spontaneous tumors more closely in biological behavior than virus producing tumors. A study of type C viruses present in carcinomas may lead to understanding the role (if any) of viruses in the development of human epithelial neoplasms.

Proposed Course:

The present investigator will finish two years and three month's of research training in the Viral Leukemia and Lymphoma Branch in April, 1973, and will return to his home institute in Tokyo to continue the present projects as Head, Viral Oncology Section, Department of Pathology, Cancer Institute, Tokyo.

Honors and Awards:

Dr. Ikawa is an awardee of the Fogarty International Fellowship (January 4, 1971 to January 3, 1973).

Dr. Ikawa presented a seminar entitled, "Viral Transformation of Epithelial Cell Lines" at the Medical College of Pennsylvania, Philadelphia, Pennsylvania on February 22, 1972.

Dr. Ikawa presented a seminar entitled, "Erythrocytic Differentiation of Clonal Leukemia Lines" at NIH under the sponsorship of NICHD on March 1, 1973.

Serial No. NCI - 4847

Dr. Ikawa will present a paper entitled, "Transformation of a Rat Liver Cell Line by Murine Sarcoma Virus" at the 64th annual meeting of the American Association for Cancer Research, Atlantic City, New Jersey on April 12, 1973.

Publications:

Ikawa, Y., Sugano, H., and Furusawa, M.: Pathogenesis of Friend virus-induced leukemia in mice. GANN Monograph 12: 33-45, 1972.

Furusawa, M., Ikawa, Y., and Sugano, H.: Phenotypic changes in Friend tumor cells. GANN Monograph 12: 231-239, 1972.

Ida, N., Ogawa, K., Ohba, Y., Takada, M., Yokoguchi, E., and Ikawa, Y.: Vertical transmission of MLV-Moloney and MSV-Moloney and related problems. GANN Monograph 12: 11-31, 1972.

Ikawa, Y., Furusawa, M., and Sugano, H.: Erythrocytic membrane-specific antigens on Friend virus-induced leukemia cells. Bibl. Haemat. 39: 955-967, 1973.

Sugano, H., Furusawa, M., Kawaguchi, T., and Ikawa, Y.: Enhancement of erythrocytic maturation of Friend virus-induced leukemia cells in vitro by substances. Bibl. Haemat. (In press).

Ikawa, Y., Gazdar, A. F., and Chopra, H. C.: Epithelial features of "nonproducer" BALB/3T3 cells transformed by murine sarcoma virus. J. Natl. Cancer Inst. 49: 1449-1453, 1972.

Sugano, H., Furusawa, M., Kawaguchi, T., and Ikawa, Y.: Differentiation of tumor cells: Induction of erythrocyte membrane-specific antigens in the Friend cells. In Lettre, H. (Ed.): Recent Results in Cancer Research. Frankfurt, West Germany, Springer Verlag. In press.

Gazdar, A. F. and Ikawa, Y.: Synthetic RNA and DNA polynucleotides: In vivo and in vitro enhancement of oncogenesis by a murine sarcoma virus. Proc. Soc. Exp. Biol. Med. 140: 1166-1169, 1972.

Gazdar, A. F., Hatanaka, M., Herberman, R., Russell, E. K., and Ikawa, Y.: The effects of dibutyryl cyclic adenosine phosphate plus theophylline of murine sarcoma virus transformed non-producer cells. Proc. Soc. Exp. Biol. Med. 141: 1044-1050, 1972.

Serial No. NCI - 4847

Ikawa, Y., Fredrickson, T. N., Sims, H. L., Ortego, H. J., and Chopra, H. C.: Rauscher virus-induced myelomonocytic leukemia culture lines. Proc. Amer. Assoc. Cancer Res. 13: 41, 1972.

Ikawa, Y.: In vivo morphology of a clonal cell line transformed by different oncogenic factors. J. Cell Biol. 55: 121a, 1972.

Ikawa, Y., Niwa, A., Tomatis, L., Baldwin, R. W., Chopra, H. C., and Gazdar, A. F.: Transformation of a rat liver cell line by murine sarcoma virus (MSV). Proc. Amer. Assoc. Cancer Res. 14: 434, 1973.

Ross, J., Ikawa, Y., Gielen, J., Packman, S., Aviv, H., and Leder, P.: Globin mRNA induction during differentiation of cultured leukemia cells. Fed. Proc. (In press).

Serial No. NCI-4848
1. Viral Leukemia and Lymphoma Branch
OASDVO, Division of Cancer
Cause and Prevention
2. Tumor Virus Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: A. Studies with murine sarcoma virus (MSV)-transformed mouse 3T3 cells which are negative for leukemia virus

B. Isolation and characterization of a new human breast tumor cell line

Previous Serial Number: Same

Principal Investigator: Dr. Robert H. Bassin

Other Investigators: Dr. Peter J. Fischinger
Dr. Brenda I. Gerwin
Dr. Daniel Haapala
Dr. Shigeko Nomura
Dr. Paul T. Peebles
Dr. Leo A. Phillips
Dr. Ernest J. Plata

Cooperating Units: Inside NIH
Staff, VLLB, NCI
Dr. Elizabeth W. Chu, LP, NCI
Dr. Peter Fishman, LNC, NINDS
Dr. Carl F. T. Mattern, LVD, NIAID
Dr. Peter Mora, LCBGY, NCI

Outside NIH
None

Man Years

Total:	2.00
Professional:	1.00
Others:	1.00

Project Description

Objectives:

A. To characterize clonal isolates of 3T3 cells transformed by murine sarcoma virus in the absence of replicating leukemia virus.

B. To isolate and characterize a continuous cell line of human breast tumor cells.

Major Findings:

A. The interactions of defective MSV with a variety of host cells were examined. S+L- BALB/3T3 and S+L- NRK clones have been established in addition to the original S+L- 3T3 cells. All 3 classes of cells release noninfectious virus-like particles, contain murine leukemia virus gs antigens, and respond to superinfection with leukemia virus by lytic focus formation. Human S+L- cells, however, exhibit only some of these properties. Both the RNA and polymerase of the noninfectious virus particles produced by S+L- cells have been characterized. MSV stocks recovered from S+L- cells superinfected with concentrated leukemia virus can be used to transform mouse cells quantitatively in a short period of time (48 hours), and this system may be used to study the biochemical events associated with transformation by MSV. In an initial study, an altered ganglioside synthesis in freshly transformed 3T3 cells was detected, although kinetic studies suggest this change may be an effect rather than a primary cause of cell transformation.

B. A continuous cell line, HBT-3, originally derived from a human breast adenocarcinoma, was examined by a variety of techniques for evidence of virus particles either before or after induction with chemicals or hormones, with negative results. Evidence for reverse transcriptase indistinguishable from some of the known RNA tumor viruses was obtained using column chromatography.

Significance to Biomedical Research and the Program of the Institute:

A. The interactions of MSV with a variety of cells is of use in helping to determine the mechanics of cell transformation by viruses both biologically and biochemically. Quantitative transformation by MSV may eventually allow a detailed analysis of the events which precede and follow morphological transformation.

B. Recent evidence from several sources has shown an association of viral activities with human breast tumor material. The results make it imperative that additional continuous lines of human breast tumor cells in culture be developed and characterized for use in virological, biochemical and immunological studies. The finding of polymerase activity in the HBT-3 cell line is of obvious significance.

Proposed Course:

A. Quantitative infection with MSV will be used to study the kinetics of appearance of various parameters of transformation. Additional isolates of MSV-3T3 cell interactions will be studied in an effort to obtain mutants of wild-type MSV lacking some of the properties of the MSV genome in S+L- cells.

Serial No. NCI-4848

B. HBT-3 cells will be further characterized especially with respect to their reverse transcriptase and immunological activity with serum from breast cancer patients.

Honors and Awards

None

Publications

Bassin, R. H., Plata, E. J., Gerwin, B. I., Mattern, C. F., Haapala, D. K., and Chu, E. W.: A human breast carcinoma cell line, HBT-3, in culture. Proc. Soc. Exp. Biol. Med. 141: 673-680, 1972.

Bassin, R. H., Phillips, L. A., Kramer, M. J., Haapala, D. K., Peebles, P. T., Nomura, S., and Fischinger, P. J.: Properties of 3T3 cells transformed by murine sarcoma virus in the absence of replicating murine leukemia helper virus. In Chieco-Bianchi, L. and Dutcher, R. M. (Eds): Proc. Vth International Symposium on Comparative Leukemia Research. In press.

Chesterman, F. C., Harvey, J. J., Branca, M., Phillips, D. E. H., Hallowes, R. C., and Bassin, R. H.: Tumors and other lesions induced by murine sarcoma viruses. In Homberger, F. (Ed.): Prog. Exp. Tumor Res. Vol. 16, 1972

Lee, K. M., Nomura, S., Bassin, R. H., and Fischinger, P. J.: Use of an established cat cell line for investigation and quantitation of feline tumor viruses. J. Natl. Cancer Inst. 49: 50-55, 1972.

Fischinger, P. J., Nomura, S., Peebles, P. T., Haapala, D. K., and Bassin, R. H.: Reversion of murine sarcoma virus transformed mouse cells: Variants without a rescuable sarcoma virus. Science 176: 1033-1035, 1972.

Nomura, S., Fischinger, P. J., Mattern, C. F. T., Peebles, P. T., Bassin, R. H., and Friedman, G. P.: Revertants of mouse cells transformed by sarcoma virus. I. Characterization of flat and transformed sublines without a rescuable murine sarcoma virus. Virology 50: 51-64, 1972.

Peebles, P. T., Fischinger, P. J., Bassin, R. H., and Papageorge, A. G.: Isolation of human amnion cells transformed by rescuable murine sarcoma virus. Nature. In press.

Serial No. NCI - 4849

1. Viral Leukemia and Lymphoma Branch, OASDVO, Division of Cancer Cause and Prevention
2. Viral Pathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Studies on cell lines from humans and non-human primates

Previous Serial Numbers: NCI-4888, 4889, 4891, 4897, 4843

Principal Investigator: Dr. John M. Easton

Other Investigators: Dr. Dharam V. Ablashi
Mr. Gary R. Armstrong

Cooperating Units:

Inside NIH
Dr. H. Chopra, VBB, NCI
Dr. J. Peng, HTCB, NCI
Dr. H. Oie, VLL, NCI
Dr. D. Twardzik, VC, NCI
Dr. S. Aaronson, VLL, NCI
Dr. P. Levine, VLL, NCI

Outside NIH
Dr. Vance Yates, Univ. of Rhode Island, Kingston, R. I.
Dr. Louise Miller, Spelman College, Atlanta, Ga.
Dr. Jeff Anderson, Univ. of Rhode Island, Kingston, R. I.

Man Years:

Total:	1.5
Professional:	1.5
Other:	0.0

Objectives:

To study cell lines, in culture, which have been derived from owl monkey kidney, American Burkitt's lymphoma, and a tumor from the face of an African green monkey. The owl monkey lines had been treated in various ways: one with DNA from Herpesvirus saimiri; one with CELO (a chicken oncogenic adenovirus); and the third with Herpesvirus saimiri followed

by cytosine arabinoside. None of the lines are carrying overt virus, and attempts are being made to bring out covert virus. The cell lines are also being examined from the point of view of whether their cells are transformed. Herpesvirus saimiri is an important oncogenic virus to study because it has characteristics quite similar to those of the Epstein-Barr virus of man.

Methods Employed:

Cell cultures are split at appropriate intervals. Cultures are being treated with IdU and BrdU, both in short-term high dose (20-50γ/ml) and in long-term low dose (5γ/ml) regimens. Treated and control cells are being examined by electron microscopy and also for V and T antigens with fluorescence microscopy. Karyotype studies are done on cells showing evidence of transformation. Cells are being studied for viral derangement of host cell DNA metabolism.

Major Findings:

Preliminary results suggest that IdU treatment can cause CELO virus to replicate after 28 passages of the owl monkey cells in culture. Owl monkey kidney cells treated with Herpesvirus saimiri and cytosine arabinoside have begun to show characteristics of transformed cells, beginning at the thirteenth passage. These properties include growth out of the monolayer, and increased acid production. Early karyotype revealed normal owl monkey chromosomes. Karyotype analysis of these later passages reveals many breaks and chromatid exchanges. The main chromosome number is between 56 to 60 up from the diploid number of 54. It is interesting to note here that untreated owl monkey kidney cell cultures do not survive beyond the seventh or eighth passage. Cell monolayers derived from tissues of Burkitt lymphoma patients have not persisted beyond the seventh subculture.

Significance to Biomedical Research and the Program of the Institute:

Studies with transformation of treated owl monkey kidney cells are important because Herpesvirus saimiri can induce lymphomas in these animals in vivo and more knowledge is needed about the biology of tissues from this species in vitro. Information about the fate of Herpesvirus saimiri DNA in owl monkey kidney cells in vitro may provide clues as to the action of Herpesvirus saimiri in vivo. It is also important to know whether subsequent treatment of owl monkey kidney cells with

cytosine arabinoside has eliminated all vestiges of Herpesvirus saimiri, or whether virus remains in the owl monkey kidney cells in a cryptic form. Establishment of lymphoid cell lines from American Burkitt patients is important in order to try to determine what role Epstein-Barr virus plays in the pathogenesis of this disease.

Proposed Course:

Studies will continue in attempts to derive lymphoid cell lines from tissues of American Burkitt patients. Studies will be made to study similarities and differences between African Burkitt's tumor and Burkitt's lymphoma in other parts of the world. Studies will be continued on owl monkey kidney cell lines and their possible transformation. These cells ultimately will be studied in regard to their replication in soft agar. Transformed cells will be inoculated into various recipient animals in attempts to induce tumors. Co-cultivation experiments will also be performed in attempts to elicit virus replication.

Honors and Awards:

None

Publications:

Stevens, D. A., Easton, J. M., Levine, P. H., Waggoner, D. E., Manaker, R. A., and Schidlovsky, G.: Antilymphocyte serum and lymphoid cell-virus carrier culture: Cell kinetics, morphology, EB virus replication, interferon. Cell Immun. 33: 629-643, 1972.

Armstrong, G. R., Ablashi, D. V., Easton, J. M., and Adamson, R. H.: Suppression of the cytopathic effects of Herpesvirus saimiri by cytosine arabinoside in primary owl monkey cells. Proc. Amer. Soc. Micro. 246: 226, 1972.

Serial No. NCI-4850
1. Viral Leukemia and Lymphoma Branch
OASDVO, Division of Cancer
Cause and Prevention
2. Tumor Virus Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: A. Effect of chemical agents on the reversion of MSV-transformed mouse cells (S+L- cells) to flat variants

B. Influence of a resident and/or endogenous MSV genome in flat variant cells on exogenous MSV infection

Previous Serial Number: Same

Principal Investigator: Dr. Shigeko Nomura

Other Investigators: Dr. Peter J. Fischinger
Dr. Robert H. Bassin
Dr. Brenda I. Gerwin
Dr. Daniel K. Haapala
Dr. Paul T. Peebles
Dr. Leo A. Phillips

Cooperating Units: Inside NIH
Staff, VLLB, NCI
Mr. Paul R. Hill, VC, NCI
Dr. Carl F. T. Mattern, LVD, NIAID

Outside NIH
None

Man Years:

Total:	2.00
Professional:	1.00
Other:	1.00

Project Description

Objectives:

A. To study the effect of chemicals on the frequency of reversion of the S+L- cells to flat variants, and biological characteristics of these flat variants isolated spontaneously or chemically.

B. To study the influence of an endogenous and/or resident MSV genome in flat variants on the re-infection of these cells with MSV.

Methods Employed:

A. Routine tissue culture methods and standard virological techniques were used for the quantitation of cell transformation and focus formation by mouse sarcoma and leukemia viruses. Chemical treatments were carried out by exposing cells to 10 µg/ml of 5-fluorodeoxyuridine (FdU) for 1 hour or 0.04 µg/ml of colcemid for 24 hours. Induction of MSV genome was performed by treating cells with 20 µg/ml of 5-iododeoxyuridine (IdU for 30 hours and by cell fusion with Sendai virus. The presence of virus and reverse transcriptase activity (RTA) were determined by ^{3}H-uridine and [^{3}H-methyl] triphosphate incorporation into acid precipitable material, respectively.

B. Routine tissue culture methods and standard virological techniques were employed for quantitation of cell transformation and focus formation by mouse sarcoma and leukemia viruses. IdU treatment and assay for RTA were carried out as described above.

Major Findings:

A. Two clonal sublines of the S+L- cells, 3197-3-6-10 and 3197-3B-1-1 demonstrated frequencies of spontaneous reversion of 1 in 70 and 1 in 4300, respectively. Treatment of 3197-3-6-10 cells with FdU (FdU-variant) or colcemid (colcemid-variant) resulted in frequency of reversion of approximately 1 in 30. Frequency of reversion in 3197-3B-1-1 cells after treatment with both chemicals increased 50- to 100-fold. As observed in spontaneous variants, they were epithelioid, contact inhibited, and contained some MuLV gs antigens, but demonstrated no virus production, no RTA and no rescuable MSV genome. Colcemid-variants were indistinguishable from spontaneous variants by the characteristics described above, and also by an increase in chromosome number, similarly observed in spontaneous variants. In contrast, FdU-variants demonstrated no increase (decrease in some sublines) in chromosome number, and spontaneous retransformation of FdU-variants returned them to the parental S+L- cell condition (rescuability, RTA) which has not been observed in spontaneous and colcemid-variants.

B. Sublines N-101 and N-102 were established by cloning from a terminal focus of Moloney MSV on a flat variant (2-14-3). The N-101 cells demonstrated neither virus production nor RTA, but contained MuLV gs antigens and a rescuable MSV genome. RTA and small amount of infectious MSV were induced from N-101 cells by IdU treatment. Frequency of reversion in N-101 cells was far lower than that (1 in 70) of the parental S+L- subline. The N-102 cells did not produce virus in the early passages. At the third level, infectious MSV became demonstrable, and later infectious MuLV was also detected. These viruses were neutralized by antiserum against Moloney IC virus. MSV from N-102 cells appeared to be in a 10- to 100-fold excess

over MuLV in these cultures. However, this MuLV virus may be defective since they formed no foci or vague foci when inoculated onto S+L- cells. Approximately 10^3/ml MuLV was detected eventually in S+L- cells, but it was obtained only after 11 weekly passages of infected S+L- cells, as was seen in the case of radiation-induced MuLV. MSV produced from N-102 cells transformed 3T3 mouse cells to the S+L- type of cell. Studies of characterization of viruses from N-101 and N-102 and transformation of 2-14-3 by Kirsten MSV are under way.

Significance to Biomedical Research and the Program of the Institute:

A. Studies of the effect of chemical agents on reversion and the behavior of the viral genome in transformed and flat variant cells are not only of general biological interest but also provide new approaches to both the search for etiologically significant latent viruses in tumors as well as possible therapeutic measures.

B. Studies of the influence of an endogenous MSV on exogenous viral genetic material may provide a more efficient approach to understanding of viral oncogenesis and host cell control mechanisms.

Proposed Course:

A. Further studies on chemical and physical agents which induce reversion of transformed cells, the state of integration of the viral genome, possible functional changes in membranes of variant cells.

B. Further studies on the nature and the influence of an endogenous MSV genome on host control mechanisms and exogenous viral genetic material.

Honors and Awards

None

Publications

Nomura, S., Fischinger, P. J., Mattern, C. F. T., Peebles, P. T., Bassin, R. H., and Friedman, G. P.: Revertants of mouse cells transformed by murine sarcoma virus. I. Characterization of flat and transformed sublines without a rescuable murine sarcoma virus. Virology 50: 51-64, 1972.

Fischinger, P. J., Nomura, S., Peebles, P. T., Haapala, D. K., and Bassin, R. H.: Reversion of murine sarcoma virus transformed mouse cells: Variants without a rescuable sarcoma virus. Science 176: 1033-1035, 1972.

Lee, K. M., Nomura, S., Bassin, R. H., and Fischinger, P. J.: Use of an established cat cell line for investigation and quantitation of feline oncornaviruses. J. Natl. Cancer Inst. 49: 55-60, 1972.

Serial No. NCI-4850

Bassin, R. H., Phillips, L. A., Kramer, M. J., Haapala, D. K., Peebles, P. T., Nomura, S., and Fischinger, P. J.: Properties of 3T3 cells transformed by sarcoma virus in the absence of replicating murine leukemia helper virus. In Chieco-Bianchi, L. and Dutcher, R. M. (Eds.): Proc. Vth International Symposium on Comparative Leukemia Research. In press.

Serial No. NCI-4853

1. Viral Leukemia and Lymphoma Branch
OASDVO, Division of Cancer
Cause and Prevention
2. Genetics Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Detection of oncogenic virus specific information by molecular hybridization in mammalian cells

Previous Serial Number: Same

Principal Investigator: Dr. Raoul Benveniste

Other Investigators: Dr. Isaac C. Henderson
Dr. Michael M. Lieber
Dr. David M. Livingston
Dr. George J. Todaro

Cooperating Units: Inside NIH
None

Outside NIH
Meloy Laboratories, Inc., Springfield, Virginia

Man Years:

Total:	1.0
Professional:	1.0
Other:	0.0

Project Description

Objectives:

To examine various cell lines for the presence of type C viral information and to isolate transformed non-producer cell lines from cells infected with non-homologous sarcoma and leukemia type C viruses.

Methods Employed:

Nucleic acid hybridization is the principal method that is being used. Typically, a single-stranded ^{3}H-DNA copy of the RNA genome of the virus is hybridized to cellular or 70S viral RNA. A nuclease from Aspergillus oryzae that degrades only single-stranded nucleic acids is being used to detect RNA-DNA and DNA-DNA hybrids. $CsSO_4$ equilibrium centrifugation is also being used to detect RNA-DNA hybrids.

Major Findings:

1. Transcription of viral information in murine lines: All murine cell lines examined so far have been found to contain RNA which is homologous to the 35S RNA isolated from Kirsten murine leukemia virus or from an endogenous murine leukemia virus, S_2Cl_3. After purification of cytoplasmic RNA by dT-cellulose chromatography to remove RNA not containing poly-A sequences, and hybridizing this RNA to the ^{3}H-DNA product, it was found that a "normal" murine cell line, A31, transcribes enough RNA to saturate 2-4% of the ^{3}H-DNA product. Murine cell lines transformed either spontaneously, by radiation, or by SV40 all transcribe an additional amount of RNA so as to saturate about 10% of the ^{3}H-DNA viral probe. Normal rat cell RNA, poly-A, or dT-cellulose purified RNA from an SV40 transformed human cell line do not hybridize to the ^{3}H-DNA murine probe. It is not known if the additional amount of RNA transcribed by the transformed cells is related to transformation, since 35S RNA could contain cellular RNA sequences.

2. Homology of type C viruses: We have examined the relatedness of type C viruses by hybridizing a ^{3}H-DNA copy of viral RNA to 70S or 35S RNA isolated from various viruses. Exogenous and endogenous feline and murine viruses, endogenous rat viruses, and avian and primate viruses have been tested. The endogenous viruses of the various species are less than 5% homologous to each other. Within one family of viruses there is usually greater than 50% homology (for example, between woolly and gibbon viruses or between Rauscher and Kirsten viruses) with one exception: the endogenous cat viruses are not related to the Rickard or Thielen strains of feline leukemia virus.

3. Control of induction of endogenous leukemia virus: Viral-specific RNA can be detected soon after induction of spontaneously transformed murine cell lines. Experiments are currently being performed to determine if this expression of viral information can be blocked with known inhibitors of RNA and protein synthesis.

4. Relatedness of sarcoma and leukemia viruses: A ^{3}H-DNA copy of Kirsten leukemia virus RNA has been shown to hybridize with the RNA extracted from Kirsten sarcoma virus transformed non-producer normal rat kidney cells. These data suggest that there might be some homology between Kirsten leukemia and sarcoma viruses; alternatively, it is possible that there is a low level of leukemia virus information being transcribed in the non-producer cells. Therefore, non-producers are currently being isolated after infection of a cell with non-homologous viruses (for example, feline leukemia virus and Kirsten sarcoma virus) so that transcription of either virus can be detected in these non-virus producing cells. These non-producers will also be induced to see

if the exogenous leukemia virus (which is not homologous to the endogenous leukemia virus) can be produced. Attempts are also being made to isolate these non-producers in human cells so as to have a sensitive assay for the induction of endogenous human leukemia viruses.

Significance to Biomedical Research and the Program of the Institute:

The finding of type C viral information in "normal" cell lines has strong implications for a viral etiology for cancer.

The data concerning the homology of known endogenous and exogenous type C viruses does not reveal any obvious choice of probes to detect human type C virus information in human cells - indeed such a virus may be unrelated to other known type C viruses. The primate viruses now available (gibbon ape or woolly monkey) or perhaps an endogenous primate virus are the best candidates for relatedness to a human type C virus.

Proposed Course:

1. The induction of endogenous viruses from primate cell lines is especially important in order to isolate a virus that could be used to detect related type C viral information in human tumors.

2. Since the endogenous and exogenous cat viruses are essentially non-homologous, it becomes possible to ask which virus is present in normal feline tissue and in spontaneous feline tumors.

3. By taking advantage of the non-homology of some type C viruses, it is possible to isolate sarcoma-specific viral RNA and investigate the homology of the sarcoma viruses of different species.

Honors and Awards:

None

Publications:

Benveniste, R.E. and Scolnick, E.M.: RNA in mammalian sarcoma virus transformed non-producer cells homologous to murine leukemia virus RNA. Virology. (in press).

Scolnick, E.M., Aviv, H., Benveniste, R.E., and Parks, W.P.: Purification by oligo(dT)-cellulose of sarcoma specific RNA from transformed mammalian non-producer cells. J. Virol. (in press).

Parks, W.P., Livingston, D.M., Todaro, G.J., Benveniste, R.E., and Scolnick, E.M.: Detection of viral antigen by radioimmunoassay. J. Exp. Med. (in press).

Serial No. NCI-4854

1. Viral Leukemia and Lymphoma Branch
   OASDVO, Division of Cancer
   Cause and Prevention
2. Genetics Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: A. Isolation of RNA tumor virus specific proteins from non virus-producing cells

B. Studies on the mechanism of host cell control of transforming viral gene expression

C. Isolation of SV40 specific non-virion proteins

D. Isolation of an intact human tumor virus

E. Isolation and characterization of endogenous feline viruses

Previous Serial Number: Same

Principal Investigator: Dr. David M. Livingston

Other Investigators: Dr. Raoul Benveniste
Dr. Isaac C. Henderson
Dr. Michael M. Lieber
Dr. Wade Parks
Dr. Edward M. Scolnick
Dr. George J. Todaro

Cooperating Units: Inside NIH
None

Outside NIH
Meloy Laboratories, Inc., Springfield, Virginia
Dr. Roger Wilsnack, Huntingdon Research Lab., Baltimore, Maryland

Man Years:

Total:	1.0
Professional:	1.0
Other:	0.0

Serial No. NCI-4854

Project Description

Objectives:

A. To isolate type C virus specific proteins from cells which are not infected exogenously by a replicating RNA tumor virus.

B. To understand what cellular functions modulate the oncogenic expression of RNA and DNA tumor viruses.

C. To isolate, purify and characterize the SV40 T and U antigens-proteins which are virus specific but are not represented in the SV40 virion. These proteins have been hypothesized for some time to play a role in the viral transformation reaction. In particular, we would like to gain insight into the function of these two proteins.

D. To isolate an endogenous human tumor virus of the type C variety with a view toward using it to study the potential role of such putative agents in the development of human neoplasia.

E. To gain further insight into the role of type C viruses in feline oncogenesis.

Methods Employed:

A. Immunoaffinity chromatography for the type C viral reverse transcriptase and group specific antigen; standard biochemical separatory methods applicable to proteins.

B. Cell biological, virological and genetic methods.

C. Immunoaffinity chromatography and standard biochemical protein purification procedures.

D. Cell biological, virological and genetic methods.

E. Cell biological and virological methods.

Major Findings:

A. An immunoadsorbent specific for the avian type C viral polymerase was prepared and an affinity chromatographic procedure for this enzyme developed. Using this procedure, viral enzyme can be selectively purified from extracts of virus producing avian cells. In controlled experiments, we sought the enzyme in extracts of Rous sarcoma virus transformed rat cells (XC cells) which synthesize no virus but do contain easily detected quantities of the avian gs antigen(s). Multiple experiments with these extracts failed to yield any viral enzyme even when an amount of extract containing as much viral gs antigen(s) as the producer chicken extract was chromatographed. This immunoadsorbent contains additional determinants for the avian viral gs antigen(s) and, as a control, the amount of antigen

eluted from the column (compared to a non-immune control IgG column) was measured. When comparable amounts of gs antigen containing avian virus producer cell and XC cell extract were chromatographed, viral enzyme was eluted in the former but not the latter case, but nearly equivalent amounts of gs antigen were eluted in both cases. However, following addition of limiting quantities of RSV polymerase to the XC cell extracts, viral enzyme could be specifically eluted from the anti-polymerase column in excellent yield. Moreover, chromatography of a mixture of an avian virus producing cell extract with that from XC cells also resulted in the positive isolation of the avian viral enzyme in good yield. Thus, it appears that this avian transformed rat cell is relatively deficient in soluble, active viral polymerase compared to gs antigen.

An immunoaffinity chromatography system for the mammalian type C viral major internal protein - the gs protein - has been established. The system is based on the covalent coupling to Sepharose of IgG from an immune serum directed against the gs-3 intraspecies determinant of the mammalian gs protein. Crude murine antigen containing extracts can be chromatographed on such columns with approximately 50% yield of highly purified gs protein - in one step. This separatory method has been used to purify gs antigen from crude extracts of NIH-3T3 cells, a non-virus producing cell. The protein which elutes specifically from the anti-gs column is immunologically identical in terms of its gs-1 determinant with the 30,000 MW gs protein of known mouse type C virus. It also contains a readily detectable gs-3 determinant.

Recently, we have begun to look in the high speed pellet fraction of various non-virus producing subclones of Balb/c 3T3 cells for the presence of viral reverse transcriptase. Experiments to date with high speed pellet extracts reveal the presence in Balb/3T3 clone A31 and KA31 (an MSV non-producer transformant of A31) of a reverse transcriptase activity associated with a molecule with some of the chemical properties, including size and some degree of immunologic relatedness, of the known murine viral enzyme. Further investigation is underway to fully establish the nature of this protein.

B. During the year, we have begun to search for mutants of NRK cells which can be normally transformed by murine sarcoma virus at 32°C but not at 40°C. The wild type cell is equally well transformed at both temperatures. A simple scoring system is used. Two potential mutants have been recently isolated. Both appear to be reduced in transformability by 100-fold compared to the wild type NRK clone. The general nature of the block is being investigated, and more mutants are being sought.

C. Together with Dr. Craig Henderson, we have begun purifying the SV40 T and U antigens using conventional and affinity chromatographic techniques. More details of this project are described in Dr. Henderson's report. Basically, following three chromatographic steps, a partially purified aliquot of T antigen can now be prepared, which has a markedly increased

specific activity relative to the crude extract and, in analytical disc-gel electrophoresis experiments, contains fewer than 10 bands of protein.

D. We are attempting isolation of an endogenous replicating human type C virus from cultured cells. Induction experiments of various types with various lines have been tried and are being continued. To date, no such virus has been identified.

E. We have recently isolated an endogenous virus (CCC) of a single cell clone of feline fibroblasts, the reverse transcriptase and gs antigen of which have antigenic properties distinct from those of FeLV and similar to those of RD-114. The host range of this virus is similar to that of RD-114, and different from that of FeLV. The former agents both grew well on human and primate cells and poorly on feline cells. FeLV, in contrast, grows well on feline cells and poorly on monkey and human cells. Presently, we are attempting to identify other isolates of CCC-like virus from various feline cell cultures in an attempt to determine how prevalent this agent is.

Significance to Biomedical Research and the Program of the Institute:

A. This project is directed at detecting type C viral gene products in non-productive mouse cells as a model system for their potential detection in human cells.

B. This project is directed at understanding the cellular contribution to the mechanism of viral transformation.

C. This project is directed at understanding the function of these two proteins and thus to determine whether they play a role in the transformation mechanism.

D. The goal of this project is the isolation of an intact human type C virus.

E. This project is directed at understanding the role of endogenous type C viruses in natural feline oncogenesis.

Proposed Course:

A. Further experiments directed at determining the structural relationship of the polymerase isolated from the non-producer and uninfected Balb/3T3 derivatives to the known mouse viral and cellular polymerases will be undertaken. Experiments with monkey cells have also been initiated with a view towards looking for a polymerase in the high speed pellet fraction which is structurally homologous with the known monkey type C reverse transcriptases.

B. Experiments designed to define whether the conditional defect in the two cellular mutants already available are in sarcoma virus entry, initiation, or maintenance of transformation are underway. A major effort to isolate more cell mutants of the temperature sensitive type is underway.

C. The proposed course in this project is to isolate and characterize chemically homogenous T antigen and U antigen and develop sensitive, specific radioimmunoassays for each.

D. Efforts to isolate an intact human type C virus will be continued.

E. Further experiments directed at isolating intact feline type C viruses of the CCC/RD type will be performed.

Honors and Awards:

None

Publications:

Livingston, D.M., Parks, W.P., Scolnick, E.M., and Ross, J.: Affinity chromatography of avian type C viral reverse transcriptase: Studies with Rous sarcoma virus transformed rat cells. Virology 50: 388-395, 1972.

Parks, W.P., Livingston, D.M., Scolnick, E.M., and Todaro, G.J.: Radioimmunoassay of type C viral proteins. III. Identification of a protein with group specific antigenic reactivity from normal mouse cells. J. Exp. Med. (in press).

Livingston, D.M. and Todaro, G.J.: Endogenous type C virus from a cat cell clone with properties distinct from previously described feline type C viruses. Virology. (in press).

Todaro, G.J., Scolnick, E.M., Parks, W.P., Livingston, D.M., and Aaronson, S.A.: Detection of type C viruses in normal and transformed cells. Beers, R.F., Jr. and Tilghman, R.C. (Eds.): Cellular Modification and Genetic Transformation by Exogenous Nucleic Acids, Sixth International Symposium on Molecular Biology, Baltimore, Johns Hopkins University Press. (in press).

Serial No. NCI-4856

1. Viral Leukemia and Lymphoma Branch
   OASDVO, Division of Cancer
   Cause and Prevention
2. Molecular Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Genetic studies of induction and replication of RNA tumor viruses

Previous Serial Number: NCI-4838

Principal Investigator: Dr. John R. Stephenson

Other Investigators: Dr. Stuart A. Aaronson
Dr. Steven R. Tronick

Cooperating Units: Inside NIH
Dr. Joel S. Greenberger, VC, NCI
Dr. Tadao A. Aoki, VLL, NCI
Dr. Edward M. Scolnick, VLL, NCI

Outside NIH
Dr. Arthur A. Axelrad, University of Toronto, Canada
Dr. Roger E. Wilsnack, Huntingdon Research Laboratories, Baltimore, Maryland
Dr. Garth R. Anderson, Hazleton Laboratories, Vienna, Virginia

Man Years:

Total:	1.0
Professional:	1.0
Other:	0.0

Project Description

Objectives:

1. To isolate and characterize temperature sensitive mutants of murine leukemia viruses and to use these mutants in an attempt to elucidate the mechanism of murine leukemia virus replication and to examine the functional relationships between murine leukemia and sarcoma viruses.

2. To study the phenotypic expression of the sarcoma virus genome in MSV-transformed "nonproducer" mouse cells.

3. To determine the genetic factors regulating expression of endogenous RNA type C virus in normal mouse embryo cells.

Methods Employed:

1. Standard tissue culture procedures, including XC plaque assays for leukemia virus and focus formation assays for sarcoma virus.

2. Sensitive and specific radioimmunoassays for the viral structural proteins of different classes of mammalian type C viruses.

Major Findings:

1. Micro techniques for the isolation of temperature sensitive leukemia virus mutants have been developed. By these methods, it has been possible so far to isolate 27 temperature-sensitive leukemia virus mutants from clonal stocks of Kirsten and Rauscher leukemia viruses. Each mutant transmits to new cells with efficiency comparable to that of wild-type MuLV at the permissive temperature, but is at least 4-5 logs less efficient than wild-type at forming XC plaques at the nonpermissive temperature. The mutants all have very low rates of reversion to wild-type. These have been partially characterized and have been separated into three distinct classes based on the stage at which viral replication is blocked at the nonpermissive temperature. Studies will be carried out using the mutants to attempt to elucidate the mechanism of leukemia virus replication and to further examine the functional relationships between murine sarcoma and leukemia viruses.

2. Using similar procedures, three separate murine sarcoma virus nonproducer cell lines have been isolated which are temperature-sensitive for the maintenance of transformation. In each case, a viral rather than a cellular genetic mutation is the reason for the temperature-sensitive effect. Superinfection of one of the mutants with murine leukemia virus overcomes the temperature-sensitive change in the transformed state.

3. Morphologic revertants which contain avian or murine sarcoma viruses have been isolated from clonal lines of transformed mammalian cells. These lines are indistinguishable from nontransformed parent cell lines with respect to parameters such as saturation density and colony formation in depleted medium or on monolayers of contact-inhibited cells. The rate of glucose uptake had also reverted to normal. The malignant potential of one of the revertant lines was examined and found to be markedly reduced compared to that of the corresponding transformed cells. The differences in the susceptibilities of revertant cells to retransformation by the same or other oncogenic viruses suggest that different cellular genes may be involved in expression of transformation by various tumor viruses.

4. Sarcoma virus-transformed cells have been examined for the presence of a new virus-associated cell surface antigen by immunoelectron microscopy. By this procedure, a common antigen has been detected on the surface of nonproductively transformed and MuLV superinfected nonproducer cells of two different strains of murine sarcoma virus, Kirsten and Moloney. This antigen shows cross-reaction with murine sarcoma virus-transformed cell lines produced in two different mammalian species, rats and mice. Further, this antigen is distinct from previously described antigens on the surface of murine leukemia virus-induced cells, on the viral envelope, and on the surface of spontaneously or x-irradiation transformed cell lines.

5. Studies have been performed which show that chemicals can transiently activate endogenous type C viruses from embryo cells of the Balb/c mouse strain, but not from cells of another mouse strain, NIH Swiss. The number of genetic loci for inducibility of endogenous virus in Balb/c cells was investigated with cell lines derived from appropriate F_1, F_2, hybrid, and backcross generation embryos of these strains. A single genetic locus responsible for inducibility of virus in Balb/c cells was detected and tentatively designated Ind. A second locus, previously described in studies of mouse-cell susceptibility to exogenous virus infection, Fv-1, was found to be genetically nonlinked to Ind. This regulatory gene plays an important role in determining whether the induced viruses of Balb/c cells can persist after chemical activation.

Multiple genetic loci for induction of murine leukemia viruses are demonstrated in cells of the high leukemic incidence C58 mouse strain. The biologic properties of viruses at C58 inducibility loci are shown to markedly differ from those of viruses activated from mouse cells containing a locus for virus induction of the low leukemia incidence Balb/c strain. These findings are consistent with the hypothesis that the genes for virus induction in normal mouse embryo cells represent viral structural information.

Significance to Biomedical Research and the Program of the Institute:

Genetic studies of temperature-sensitive mutants of murine leukemia viruses should provide insight into mechanism of replication and transformation by viruses of the leukemia-sarcoma complex. An understanding of the genetic parameters regulating induction of endogenous virus of murine cells should eventually lead to methods of establishing whether human cells also contain endogenous type C viruses.

Proposed Course:

To examine mechanisms of RNA type C virus replication and to study cellular regulatory controls of expression of these viruses in normal cells of different species.

Honors and Awards:

None

Publications:

Stephenson, J.R., Reynolds, R.K., and Aaronson, S.A.: Isolation of temperature sensitive mutants of murine leukemia viurs. Virology 48: 749-756, 1972.

Stephenson, J.R., Scolnick, E.M., and Aaronson, S.A.: Genetic stability of the sarcoma viruses in murine and avian sarcoma virus transformed nonproducer cells. Int. J. Cancer 9: 577-583, 1972.

Stephenson, J.R. and Aaronson, S.A.: Genetic factors influencing C-type RNA virus induction. J. Exp. Med. 136: 175-184, 1972.

Stephenson, J.R. and Aaronson, S.A.: A genetic locus for inducibility of C-type virus in Balb/c cells: The effect of a nonlinked regulatory gene on detection of virus after chemical activation. Proc. Natl. Acad. Sci. USA 69: 2798-2801, 1972.

Stephenson, J.R., Reynolds, R.K., and Aaronson, S.A.: Characterization of morphological revertants of murine and avian sarcoma virus transformed cells. J. Virol. (in press).

Stephenson, J.R. and Aaronson, S.A.: Segregation of genetic loci for virus inducibility in high and low leukemia incidence strains of mice. Science. (in press).

Stephenson, J.R. and Aaronson, S.A.: Characterization of temperature sensitive mutants of murine leukemia virus. Virology. (in press).

Serial No. NCI-4857
1. Viral Leukemia and Lymphoma Branch
OASDVO, Division of Cancer
Cause and Prevention
2. Molecular Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Biochemical studies of the proteins of RNA tumor viruses

Previous Serial Number: Same

Principal Investigator: Dr. Steven R. Tronick

Other Investigators: Dr. Stuart A. Aaronson
Dr. John R. Stephenson

Cooperating Units: Inside NIH
Dr. Joel S. Greenberger, VC, NCI
Dr. Wade P. Parks, VC, NCI

Outside NIH
Dr. Garth R. Anderson, Hazleton Laboratories, Vienna, Virginia

Man Years:

Total:	1.0
Professional:	1.0
Other:	0.0

Project Description

Objectives:

1. To develop sensitive and specific assays for purified proteins of RNA tumor viruses.

2. To use these assays in studies on the mode of replication of RNA tumor viruses.

3. To characterize biochemically the defects in temperature-sensitive mutants of murine leukemia viruses.

4. To study the biochemical relationships between murine sarcoma and leukemia viruses.

Methods Employed:

Standard biochemical techniques for protein isolation and analysis; assays for enzymes of nucleic acid synthesis and degradation; radioimmunoassays for qualitative and quantitative characterization of tumor viruses and their proteins.

Major Findings:

A low molecular weight polypeptide (16,000 daltons) was isolated from Rauscher murine leukemia virus. A very sensitive and highly specific radioimmunoassay was developed for its quantitation. This polypeptide is virus-coded and antigenically distinct from another virion protein, the group-specific (gs) antigen. Different strains of MuLV contain antigens immunologically cross-reactive with the low molecular weight polypeptide. The presence of this polypeptide was detected in mouse and rat cells transformed by the S^+L^- sarcoma genome but not in the same cells transformed by the MSV nonproducer genome.

The assay for the 16,000 dalton polypeptide was used in studies of temperature-sensitive mutants of MuLV. The polypeptide was detected in some but not other ts mutant-infected cells at the permissive, but not the nonpermissive temperature.

Significance to Biomedical Research and the Program of the Institute:

Detailed characterization of tumor virus proteins is important for understanding the mode of replication of these viruses in mammalian cells and for understanding possibly the mechanism of the malignant transformation of cells by these viruses.

Proposed Course:

Other polypeptides will be isolated from various strains of MuLV and from other species of type C viruses. The assays for these polypeptides will be applied to studies such as those mentioned above, and will also be used to differentiate various mammalian type C endogenous viruses. Normal and transformed mammalian cells will be tested for the presence of these virus-coded polypeptides.

Honors and Awards:

None

Publications:

Tronick, S.R., Scolnick, E.M., and Parks, W.P.: Reversible inactivation of the deoxyribonucleic acid polymerase of Rauscher leukemia virus. J. Virol. 10: 885-888, 1972.

Serial No. NCI-4858

1. Viral Leukemia and Lymphoma Branch
   OASDVO, Division of Cancer
   Cause and Prevention
2. Genetics Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: A. Identification of human RNA tumor virus

B. Mechanism of transformation of RNA tumor viruses

Previous Serial Number: NCI-4837

Principal Investigator: Dr. Edward M. Scolnick

Other Investigators: Dr. Raoul Benveniste

Cooperating Units: Inside NIH
Dr. Wade Parks, VC, NCI

Outside NIH
Meloy Laboratories, Inc., Springfield, Virginia

Man Years:

Total:	1.0
Professional:	1.0
Other:	0.0

Project Description

Objectives:

A. We have been emphasizing less our attempts to identify a human type C virus since these are no longer candidate viruses and all results to date with other type C viruses have not provided any unequivocal evidence for type C virus in human cells. However, with the prior techniques we have developed, significant observations have been made in model systems in this direction.

1. Extensive investigations of mouse cells which contain virus but are not producing virus have been made. Most of these studies have been done with hybridization techniques using S_1 nuclease to detect hybridization. Significant levels of viral specific RNA have been detected in sarcoma transformed nonproducer rat or mouse cells. Surprisingly the RNA was found to have extensive homology with murine leukemia virus RNA.

To purify this RNA we have employed oligo-(dT)-cellulose chromatography. This technique allows us to purify the viral specific RNA based on its poly A content. Thus we can separate viral RNA from the ribosomal RNA of cells. This allows us to put much more RNA into hybridization reactions, and clarify saturation values. We are employing this technique to search in human tumors for viral specific RNA. By using purified mRNA we feel we can clarify low level reactions in these human cells.

2. To extend our earlier work on identification of viral reverse transcriptase, we developed a method to denature reversibly the mammalian viral reverse transcriptase. Guanidine hydrochloride agarose chromatography has been used. All mammalian viral polymerases have been found to have a molecular weight of 70,000 daltons. This is an additional useful criterion for identifying a putative human viral enzyme.

B. To study the mechanism of transformation by RNA tumor viruses, additional temperature sensitive mutants of RNA sarcoma viruses have been isolated. Several new mutants which transform cells at 32° have been isolated. These cells revert to normal at the nonpermissive temperature 39.5°C. Studies are in progress to identify the number of genes involved and what their products are.

In addition, a clonal isolate of a primate sarcoma virus has been obtained. This resembles the S+L- strain of murine sarcoma virus. It contains woolly gs antigen and woolly polymerase. Studies of cross species rescue have revealed that primate and murine helper viruses have equal ability to rescue primate or murine sarcoma viruses. The studies have also suggested that helper virus rescue involves merely an assembly of sarcoma components within helper structural proteins. Further details of the mechanism of rescue are in progress.

Methods Employed:

A. The methods involved in studies on the reverse transcriptase and gs antigens are column chromatography, ampholine isoelectric focusing, polyacrylamide gel electrophoresis, radioimmunoassays, complement fixation and immunodiffusion. Enzymatic assays, cesium sulfate centrifugation and hydroxyapatite chromatography have been used to study hybridization.

B. To study sarcoma mutants, standard tissue culture techniques are used. In addition, a method for replica plating cells is also used.

Major Findings:

A. By studying classes of viral RNA in nonproducer cells, clues as to what can be expected in human tumors may be found.

B. Several mutants of mammalian sarcoma virus have been isolated. They are being studied to try to determine the mechanism of transformation. Homologies between primate and murine sarcoma virus are being studied to compare transforming information.

Significance to Biomedical Research and the Program of the Institute:

A. This project is directly related to attempts to identify an RNA tumor virus as a causative agent in human cancer.

B. This project is to determine how such a virus causes cancer and, hopefully, thus to cure it.

Proposed Course:

A. This project will continue as detailed above.

B. This project will continue as detailed above.

Honors and Awards:

None

Publications:

Scolnick, E.M., Parks, W.P., and Todaro, G.J.: Reverse transcriptases as immunological markers for primate C-type viruses. Science 177: 1119-1121, 1972.

Scolnick, E.M., Stephenson, J.R., and Aaronson, S.A.: Isolation of temperature-sensitive mutants of murine sarcoma virus. J. Virol. 10: 653-657, 1972.

Tronick, S.R., Scolnick, E.M., and Parks, W.P.: Reversible inactivation of the deoxyribonucleic acid polymerase of Rauscher leukemia virus. J. Virol. 10: 885-888, 1972.

Benveniste, R. and Scolnick, E.M.: RNA in mammalian sarcoma transformed nonproducer cells homologous to murine leukemia virus RNA. Virology. (in press).

Scolnick, E.M. and Parks, W.P.: Identification of viral reverse transcriptases. Grossman, L. and Moldave, K. (Eds.); Methods in Enzymology. New York, Academic Press. (in press).

Scolnick, E.M., Aviv, H., Benveniste, R., and Parks, W.P.: Purification by oligo-(dT)-cellulose of viral specific RNA from sarcoma virus transformed mammalian nonproducer. J. Virol. (in press).

Serial No. NCI-4859

1. Viral Leukemia and Lymphoma Branch
   OASDVO, Division of Cancer
   Cause and Prevention
2. Genetics Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Purification and characterization of SV40 tumor antigen(s)

Previous Serial Number: None

Principal Investigator: Dr. Isaac C. Henderson

Other Investigators: Dr. David M. Livingston

Cooperating Units: Inside NIH
None

Outside NIH
Meloy Laboratories, Inc., Springfield, Virginia
Dr. Roger Wilsnack, Huntingdon Research Lab.,
Baltimore, Maryland

Man Years:

Total:	1.0
Professional:	1.0
Other:	0.0

Project Description

Objectives:

To isolate, purify and characterize SV40 T antigen and to develop a sensitive radioimmunoassay.

Methods Employed:

Standard methods of protein purification and identification, complement fixation assay, and tissue culture.

Major Findings:

Nuclear and crude cell extracts of both tumors and cells in culture which have been subjected to ammonium sulfate fractionation and serial column chromatography with DEAE, hydroxyapatite, phosphocellulose, and sephadex have yielded products purified 200-300 fold.

Cells labeled in vivo with a tritiated protein hydrolysate have proven to be a particularly useful source of T antigen because of the ease of following the labeled protein through serial purification steps in spite of its extreme lability.

However, with very high titer preparations of the antigen it has been possible to subject purified preparations to disc electrophoresis and recover significant complement fixation activity. It appears that little or no immuno-active T antigen enters a 7% gel; no more than 3-7 bands are seen on pH 8.7 and 4.5 gels. On sephadex G-100 the CF activity is found in the void volume. In light of published reports of T antigen molecular weights of less than 70,000 on immunologically inactive preparations, these data suggest there may be significant aggregation of the protein.

Highly purified hamster anti-T antibody has been successfully coupled to Sepharose, but thus far it has been impossible to obtain ideal conditions for binding and releasing significant amounts of T antigen from the solid immunoadsorbent.

Significance to Biomedical Research and the Program of the Institute:

Because of its small size, the limited number of proteins coded by SV40 DNA, and its known oncogenic potential, SV40 continues to be an excellent tool for studying the mechanism(s) of transformation. Since T antigen appears transiently in lytic infections and permanently in transformed cells both in culture and in tumors, even when no virus is present, and since it is one of only three or four non-coat proteins coded by SV40 DNA, it is very likely it plays a significant role in transformation or maintaining transformation in SV40 induced tumors. Its isolation may provide significant clues to the mechanism of oncogenesis or even provide a new means of treating cancer.

Proposed Course:

Complete isolation of T antigen and to prepare high titer rabbit anti-T to purified T antigen for radioimmunoassay and affinity chromatography.

Honors and Awards:

None

Publications:

None

Serial No. NCI-4860

1. Viral Leukemia and Lymphoma Branch
   OASDVO, Division of Cancer
   Cause and Prevention
2. Genetics Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Expression of the endogenous type C viral genome in normal and malignant cells

Previous Serial Number: None

Principal Investigator: Dr. Michael M. Lieber

Other Investigators: Dr. George J. Todaro
Dr. David M. Livingston
Dr. Raoul Benveniste

Cooperating Units: Inside NIH
None

Outside NIH
Meloy Laboratories, Inc., Springfield, Virginia

Man Years:

Total:	1.0
Professional:	1.0
Other	0.0

Project Description

Objectives:

To determine the extent of expression of the endogenous type C viral genome in normal and malignant mammalian cells; to define the cellular control mechanisms regulating type C virus expression and the associated changes in these systems with neoplastic transformation.

Methods Employed:

Normal and malignant animal cells in tissue culture are treated with various chemical and physical inducing agents to generate secretion of the endogenous type C virus. Electronmicroscopy, biochemical assays for the viral reverse transcriptase, gs antigens and viral RNA, and virologic assays in vitro using indicator cell lines are used to study the various parameters of endogenous virus production.

Major Findings:

The mouse cell line Balb/3T3 has been the object of recent studies. Spontaneously transformed clones of Balb/3T3 were isolated and cloned. One group of 4 clones were found to spontaneously and continuously release high titers of the endogenous type C virus. Five other transformed subclones were found to be virus-free, but treatment with 5-bromodeoxy-uridine (BrdU) induced production of the endogenous virus in large amounts. More detailed studies of the kinetics of the induction of endogenous type C virus were subsequently performed. Non-transformed Balb/3T3 clones never spontaneously released type C virus, and could be induced to release very small quantities only with BrdU; in contrast, cells transformed by mouse sarcoma virus, radiation, methylcholanthrene and also spontaneously were "superinducible", i.e., BrdU treatment caused virus production within 8 hours and resulted in secretion of very large quantities of virus with exponential kinetics.

Multiple attempts to induce the release of human endogenous type C viruses using BrdU from the many human tumor lines established in this laboratory have been unsuccessful to date.

Significance to Biomedical Research and the Program of the Institute:

These studies have established the relative failure of cellular control systems in neoplastic cells to keep the endogenous type C viral genome repressed. The phenomenon of type C virus release from transformed cells probably makes such cells more immunogenic, and may play an important role in host defenses against *in vivo* neoplastic transformation. In addition, they have provided significant knowledge about the cellular control mechanisms which keep genetic information repressed in mammalian cells.

Proposed Course:

We will investigate the spontaneous and induced release of endogenous type C viruses in synchronized cells and with various metabolic inhibitors to identify the controls of virus production. Additional studies are underway to demonstrate the protective effect of endogenous type C virus release during tumor cell challenge, and to investigate if the endogenous type C viruses are leukemogenic.

Finally, multiple efforts are underway to induce type C viruses from human tumor cells in tissue culture using a variety of chemical mutagenic agents.

Honors and Awards:

None

Publications:

Lieber, M.M. and Todaro, G.J.: Spontaneous and induced production of endogenous type C RNA virus from a clonal line of spontaneously transformed Balb/3T3. Int. J. Cancer. (in press).

Serial No. NCI-4861
1. Viral Leukemia and Lymphoma Branch
   OASDVO, Division of Cancer
   Cause and Prevention
2. Genetics Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The in vitro translation of the murine type C virus genome.

Previous Serial Number: None

Principal Investigator: Tikva K. Vogel

Other Investigators: Dr. E.M. Scolnick
Dr. W. Parks

Cooperating Units: Inside NIH
None

Outside NIH
Meloy Laboratories, Inc.
Rockville, Maryland

Man Years:

Total:	1.0
Professional:	1.0
Other:	0.0

Project Description

Objectives:

To translate in vitro 70S RNA from murine leukemia-sarcoma virus and to define the products thereof.

Methods Employed:

Krebs Ascites cells grown in mice are being used to synthesize viral proteins in vitro, with the eventual goal of identifying such proteins as viral by radioimmunoassay procedures. Oligo-(dt)-cellulose chromatography is being used to purify viral messenger RNA.

Major Findings:

None yet.

Significance to Biomedical Research and the Program of the Institute:

The preparation of labeled protein coded for by the murine sarcoma virus is of potential use in determining the molecular mechanism of transformation.

Proposed Course:

To follow leads in the program developed during the past year and to extend them with a goal of providing an understanding of human cancer.

Honors and Awards:

None

Publications:

None

Serial No. NCI-4862

1. Viral Leukemia and Lymphoma Branch
   OASDVO, Division of Cancer Cause and Prevention
2. Immunology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Introduction of humoral antibody to lymphocyte-mediated cytotoxicity in vitro against virus-induced and other tumors in order to clarify the relationships between cellular and humoral immunity

Previous Serial Number: None

Principal Investigator: Dr. Fujiro Sendo

Other Investigators:
Dr. Tadao Aoki
Dr. Ernest J. Plata
Dr. Luigi Chieco-Bianchi

Cooperating Units:

Inside NIH
Dr. Ronald B. Herberman, LCBGY, NCI
Dr. John Wunderlich, I, NCI

Outside NIH
Dr. Edward A. Boyse, Sloan-Kettering Institute for Cancer Research, New York, New York
Dr. Hidetoshi Sato, Sloan-Kettering Institute for Cancer Research, New York, New York

Man Years:

Total:	2.25
Professional:	1.00
Other:	1.25

Project Description

Objectives:

A. To determine specificities of surface antigens on virus-induced and other tumor cells by the introduction of antisera preabsorbed with various antigens to lymphocyte-mediated cytotoxicity tests.

B. To investigate, using the method described above, that surface antigens on irradiation-induced BALB/c leukemia BALB/RL1 cells may be specified by type C viruses released from these leukemia cells.

Methods Employed:

The ^{51}Cr releasing method is used for testing lymphocyte-mediated cytotoxicity (LMC) tests. To determine antigen specificity of tumor cells, LMC inhibition tests have been performed with either unlabeled various cells, antiserum-coated cells, or antisera preabsorbed with various cells. The type C viruses of BALB♂RL1 leukemia are harvested from the established suspension culture cell line.

Major Findings:

A. To obtain fundamental information, well known histocompatibility antigen systems in mice have been used for analysis of the antigen specificities by cell-mediated immunity. The results showed (a) that inhibition of LMC by absorbed antisera is a sensitive method to detect the specificity of cell surface antigens, and (b) that soluble antigens or antigen-antibody complexes cannot serve as a blocker of LMC at least in histocompatibility antigen systems using the ^{51}Cr releasing method. Based on these findings, murine virus-induced tumors are being investigated for i) antigen specificities, and ii) possible role of soluble antigens and/or antigen-antibody complexes in inhibition or acceleration of LMC in vitro and immunological enhancement in vivo.

B. BALB♂RL1 leukemia releases abundant type C viruses; the specificity of their envelope antigen is different from those of Gross and F.M.R. (Friend, Moloney, Rauscher) leukemia viruses (Aoki et al., in press). LMC against BALB♂RL1 cells was revealed by the spleen cells from (C57BL/6 ♀ x BALB/c ♂)F_1 hybrid mice immunized with the same leukemia cells. Furthermore, naturally occurring cytotoxic activities of normal spleen cells to BALB♂RL1 cells were observed in some strains of mice (C57BL/6, C58J and C3H/He), but not in others (BALB/c and AKR). LMC to BALB♂RL1 cells of normal spleen cells from (C57BL/6 ♀ x BALB/c ♂)F_1 hybrid mice of both sexes correlates with the transplantation resistance to this leukemia; namely, more than 50% of the female mice are resistant to transplantation of BALB♂RL1 and possess natural cell-mediated cytotoxic activity, but only 10% of the male population was positive. This finding suggests that naturally occurring cytotoxic activity of normal spleen cells to BALB♂RL1 may be related to the transplantation immunity. Moreover, it seems likely that surface antigens on BALB♂RL1 cells detected by LMC are induced by type C virus associated with this leukemia. Electron microscopy of the suspension culture cells of BALB♂RL1 revealed many type C viruses. We are investigating the possibility that other cell lines infected in vitro with this virus may acquire the surface antigens identical to those on the BALB♂RL1 cells.

Significance of Biomedical Research and the Program of the Institute:

Cell-mediated immunity to tumor cells is important when we try to control tumors by immunological methods, but the specificities of cell-mediated

immunity still remains obscure although extensive investigations have been performed by many researchers. It is worthwhile to determine the specificities of virus-induced and other tumors by using the methods described above. The study of the relationships between surface antigens and type C viruses of the tumor cells will contribute to the cancer virus program.

Proposed Course:

These activities will be maintained.

Honors and Awards:

None

Publications:

Sendo, F.: Enhancement of tumor antigenicity. In Kobayashi, H. and Tachibana, T. (Eds.): Tumor Immunology. Tokyo, Japan, Asakura, 1973 (in press).

Aoki, T., Sendo, F., and Kudo, T.: Immunology of virus-induced leukemias. In Japan Hematological Society (Ed.): Handbook of Hematology. Tokyo, Japan, Maruzen, 1973 (in press).

Serial No. NCI-4863
1. Viral Leukemia and Lymphoma Branch
OASDVO, Division of Cancer Cause and Prevention
2. Immunology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Immunological studies in murine onco-RNA-virus-induced and/or related tumors

Previous Serial Number: None

Principal Investigator: Dr. Luigi Chieco-Bianchi

Other Investigators: Dr. Tadao Aoki
Dr. George J. Todaro
Dr. Fujiro Sendo
Dr. Peter Fischinger

Cooperating Units: None

Man Years:

Total:	0.75
Professional:	0.50
Other:	0.25

Project Description

Objectives:

A. To study mechanisms of inefficient immune reactivity of the host to its own tumor.

B. To correlate humoral and cellular immune responses in vitro to the natural history of autochthonous tumor.

C. To characterize antigenically endogenous type C viruses.

Methods Employed:

A. Immune reactivity of mice neonatally infected with MuLV, BALB/c and C57BL mice injected at birth with MuLV (Moloney) will be immunized at adulthood with allogeneic cells from a transplantable leukemia originally induced by the same virus (MBL-2 cells in ascitic form). These mice will be studied for: (a) leukemia development, (b) virus neutralizing antibody production, (c) transplantation resistance, (d) cell mediated in vitro cytotoxicity, and (e) presence of blocking antibody. Virus neutralization

will be carried out in vitro using XC cell plaque assay and focus formation on S^+L^- cells.

^{51}Cr release and morphological lymphocyte microcytotoxicity tests will be used to evaluate both cell mediated immunity and blocking antibody.

B. BALB/c neonatally infected with MuLV-M will be injected at adulthood with murine sarcoma virus, Moloney strain (MSV-M). These mice will be observed for: (a) tumor induction, regression or progression, (b) production of cytotoxic and virus-neutralizing antibodies, (c) presence of cytotoxic lymphocytes and (d) production of blocking antibody.

Cell membrane directed cytotoxic antibody will be detected by ^{51}Cr release and dye exclusion tests. Assays for neutralization and cell mediated immunity will be performed as outlined in A.

C. Spontaneously transformed BALB/3T3 cells which are continuously releasing type C viruses will serve as producers of endogenous viruses. As controls, normal and spontaneously transformed BALB/3T3 cells which are not releasing viruses but are readily inducible to do it by treatment with IdU will be used. Antigenic characterization of the envelope of these viruses will be performed by neutralization tests with the standard PCl-typing serum as well as the G-typing rat serum, the (C57BL x BALB/c)F_1 anti-RL1, and C57BL anti-K36 sera. Infectivity will be tested on NIH/3T3 and BALB/3T3 cells. Inhibition of pseudotype formation on S^+L^- (FG 10) cells, and of reverse transcriptase will also be considered as parameters for neutralization of the virus activity by antisera.

Major Findings:

A. Previous work done has shown that mice infected at birth with MuLV-Gi (Graffi strain) fail to produce transplantation resistance and cytotoxic antibody against a syngeneic transplanted leukemia infected with MuLV-Gi. Moreover, mice neonatally infected with MuLV-Gi receiving at adulthood MSV-M, show high incidence of progressively growing tumors. These results seem to be partially related to a nonspecific immune depression caused by neonatal infection with MuLV.

B. Preliminary experiments performed have shown that endogenous type C viruses released from spontaneously transformed BALB/3T3 cells can be classified into at least two different populations: (a) type C viruses which carry an envelope antigen, xVEA, different from the typical MuLV envelope antigens, and (b) uncharacterized type C viruses which have neither xVEA nor the MuLV envelope antigens.

C. Because of the recent arrival of this researcher, results are not available at this time.

Significance to Biomedical Research and the Program of the Institute:

A, B, and C. The research approaches outlined here are specifically oriented to study: (a) the mechanisms underlying the inefficiency of immune response specifically directed against leukemia cells-associated antigens, (b) the degree of correlation, if any, between immune response measured in vitro and tumor behavior in vivo, and (c) the antigenic diversity of endogenous type C viruses.

Proposed Course:

These activities will be maintained.

Honors and Awards:

Fogarty International Fellowship

Publications:

None

Serial No. NCI-4864

1. Viral Leukemia and Lymphoma Branch
   OASDVO, Division of Cancer Cause and Prevention
2. Immunology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Immunological investigations of virus-induced tumors

Previous Serial Number: NCI-4844

Principal Investigator: Dr. Tadao Aoki

Other Investigators:
Dr. Luigi Chieco-Bianchi
Dr. Vincent W. Hollis, Jr.
Dr. Toshio Kudo
Dr. Fujiro Sendo
Dr. George Todaro

Cooperating Units:

Inside NIH
Dr. Stuart A. Aaronson, VC, NCI
Dr. Kenneth S.S. Chang, LCBGY, NCI
Dr. Peter Fischinger, VLLB, NCI
Dr. Claude Garon, DCBD, NIAID
Dr. Ronald B. Herberman, LCBGY, NCI
Dr. Robert J. Huebner, VC, NCI
Dr. Michael Potter, LCBGY, NCI
Dr. John R. Stephenson, VC, NCI
Dr. Kenneth K. Takemoto, LVD, NIAID

Outside NIH
Dr. Lloyd J. Old, Sloan-Kettering Institute for Cancer Research, New York, New York
Dr. Edward A. Boyse, Sloan-Kettering Institute for Cancer Research, New York, New York
Dr. Hidetoshi Sato, Sloan-Kettering Institute for Cancer Research, New York, New York
Dr. Hidesaburo Hanafusa, The Public Health Research Institute of the City of New York, Inc., New York, New York
Dr. Harry A. Wood, Boyce Thompson Institute for Plant Research, Yonkers, New York
Dr. Frank J. Dixon, Scripps Clinic and Research Foundation, La Jolla, California
Dr. Michael B.A. Oldstone, Scripps Clinic and Research Foundation, La Jolla, California

Serial No. NCI-4864

Dr. Roger E. Wilsnack, Huntingdon Research Center, Baltimore, Maryland
Dr. George Klein, Karolinska Institute School of Medicine, Stockholm, Sweden

Man Years:

Total:	3.0
Professional:	1.0
Other:	2.0

Project Description

Objectives:

A. To demonstrate the relationship between the cell surface "differentiation antigen" PC1 and xVEA$^+$ murine myeloma-associated type C viruses.

B. To examine possible viral etiology of murine myelomas.

C. To analyze antigenic properties of BALB/3T3 endogenous type C viruses.

D. Classification of Gross soluble antigens (GSA) and solubilization of surface antigens on Gross leukemia cells.

E. To demonstrate murine sarcoma virus-induced cell surface antigens.

F. To reveal the presence of cell surface antigen common to that on the type C virus envelope.

G. To clarify the diversity of murine type C viruses in terms of envelope antigens.

H. To study various immunological host responses to type C virus-associated antigens.

I. To study the relationships of fetal antigens to tumor specific antigens using F_1 hybrid mice.

J. Comparative study of visna, progressive pneumonia, and Rous sarcoma viruses by electron microscopy.

Methods Employed:

A. thru J. Many newly modified immunological methods, i.e. cytotoxicity tests, immunofluorescence tests, precipitation techniques, immunoelectron microscopy, and transplantation immunity in combination with absorption and/or inhibition of antiserum with various antigens, have been fully used in the animal system as a model for human tumor problems.

A major emphasis has been placed on naturally occurring antibodies and oncogenic viruses.

Major Findings:

A. thru C. Based on the finding of murine mineral oil-induced myeloma-associated virus (MuMAV) which carries a specific viral envelope antigen, xVEA, different from those on MuLV, it seems most likely that $xVEA^+$ MuMAV induces a cell surface "differentiation antigen" PC1. This hypothesis is substantiated by the following facts: (a) PC^+ BALB/c mice produce natural antibodies against xVEA and PC1 antigen; (b) mineral oil-induced primary BALB/c myelomas are always PC1 antigen-positive and produce $xVEA^+$ viruses, while a majority of mineral oil-induced primary and short-term transplanted myelomas in PC^- mouse strains become PC1 antigen-positive and produce either $xVEA^+$ alone or $xVEA^+$, uncharacterized type C viruses, and MuLV(Gross); and (c) some myelomas in PC^- mouse strains remain PC1 antigen-negative and produce no viruses.

It is of great interest that, when spontaneously transformed BALB/3T3 cells release complete type C viruses, these viruses can be classified into two different groups; (a) $xVEA^+$ viruses and (b) yet uncharacterized viruses. Furthermore, BALB/3T3 cells change from PC^- to PC^+ by transformation and virus-production.

D. GSA in the body fluids of AKR and C58 mice were classified according to the known specificity of G antigens in the murine Gross leukemia system. GSA showed the several specificities corresponding to G cell surface antigens, GCSAa, b, and c, and type-specific and group-specific viral envelope antigens, tsVEA and gsVEA, respectively. However, the plasma of C58 mice lacks GSAc. GSA corresponding to G_{IX} antigen was not detected in the body fluids at all.

In addition, G soluble antigens were obtained from Gross leukemia cells by repeated washing with saline and by autolytic sulfate precipiation. Sephadex G-150 chromatography indiccated heterogeneity in the molecular forms of the antigens. The specificity of these solubilized antigens contained both G cell surface antigens and viral envelope antigens.

E. Cells transformed by murine sarcoma virus (MSV) have been examined for the presence of new MSV-associated cell surface antigens by immunoelectron microscopy. A common antigen has been detected on the surface of nonproductively transformed cells infected by two different strains of MSV, Kirsten and Moloney. This antigen shows cross-reaction with the cell lines transformed by MSV that were produced in two different mammalian species, rats and mice. Further, this antigen is distinct from previously described antigens on the surfaces of cells infected by murine leukemia virus, on the viral envelope, and on the surfaces of spontaneously transformed cell lines or cell lines transformed by x-irradiation.

F. The serum of aged NZB mice reacts mainly with the viral envelope but not with the surface of murine virus-induced leukemias. However, recently

minute positive areas have been occasionally found on the cell surface of murine leukemias and myelomas by immunoelectron microscopy. These results suggest the formation of cell surface antigens common to those on the viral envelope. This has been supported by the results obtained in the avian sarcoma system; gs^+ and cellular helper factor$^+$ chick embryo fibroblasts absorbed out the activity against Rous sarcoma viral envelope antigens from the chicken anti-Bryan Rous sarcoma virus serum.

G. Various anti-murine type C virus sera reacted differently with the envelope of various murine tumor-associated type C viruses, suggesting the presence of a variety of type C viruses in terms of envelope antigen specificities.

H. While murine tumors usually grow progressively when their cells are transplanted into syngeneic inbred strains of mice, F_1 hybrid mice (one of the parents is syngeneic) instead of inbred mice are used and sometimes rejected as recipients of tumors. This has been found with irradiation-induced BALB/c leukemias, mineral oil-induced BALB/c myelomas, transplantable AKR spontaneous leukemias, etc. These resistant hybrid mice can also produce cell-mediated immunity as well as humoral antibody. It seems likely that tumor rejection by F_1 hybrid mice is related to type C virus production of tumors.

I. F_1 hybrid mice are capable of reacting much stronger with fetal antigens than inbred mice. Previous reports indicate the necessity of x-irradiation of embryo cells for the production of host reaction with fetal antigens, but this is not the case when F_1 hybrid mice are used. The so-called antiserum against fetal antigens prepared by immunization with embryo cells contains several types of antibodies against different antigens; fetal antigens and surface antigens common to those on the cell surface of normal thymocytes.

J. Visna virus and progressive pneumonia virus, agents which cause slow infections in sheep, possess several morphological properties quite similar to those of Rous sarcoma virus, i.e. spikes on their outer membrane, internal strandlike structures, and extracellular mature and budding viruses.

Significance to Biomedical Research and the Program of the Institute:

A. thru C. Some alloantigens or differentiation antigens on the cell surface may be induced by RNA type C virus. This may provide a key, changing to some extent previously established concepts concerning cell surface antigens which have been specified by the cellular genome. In this case, viral etiology of tumors will be more carefully analyzed in comparison with other normal cell-associated surface antigens (induced by viruses).

The possible viral etiology of murine myelomas has been established. The spontaneous phenotypic expression of endogenous type C viruses contributes to the oncogene theory, at least in the mouse system, for the possibility of pre-existing viral genome in a latent form has been clearly proven by

these results which further stimulate the investigation of viral etiology in other tumors of different species.

One population of previously uncharacterized endogenous type C viruses from BALB/3T3 cells suggests that broader virological investigation is needed.

D. The classification of Gross leukemia virus-associated surface antigens in soluble form produced by chemical solubilization as well as naturally produced will serve as the standard criteria of antigen specificities for the isolation of individual specific antigens. Consequently, these findings have made progress towards the establishment of vaccination and/or immunotherapy of virus-induced tumors as a model system for human tumor problems.

E. These results indicate a solution to the previously reported discrepancy in the presence of murine sarcoma virus genome-coded cell surface antigens; even nonproductively transformed sarcoma cells by murine sarcoma virus possess a common cell surface antigen clearly different from those induced by murine leukemia viruses and other factors. Although the amount of sarcoma antigens is very small, the determination of specific antigens will reveal the virological property of murine sarcoma virus and the immunological characteristics of sarcomas.

F. The presence of cell surface antigens common to those on the envelope of type C viruses is the revolutionary finding in the scientific field of virus-induced tumors. This may provide a clue to clarifying the repression mechanisms of oncogenic virus, and the analogous situation might be postulated in the human system.

G. and H. The fact that the individual as well as common antigens are present on the envelope of various type C viruses is a milestone in the vaccination of virus-induced tumors. In other words, if a common envelope antigen can be utilized for the neutralization of a variety of type C viruses the preparation of antiserum against this antigen would be a powerful means to prevent the cause of related tumors, whereas if the individual specific envelope antigen serves for their neutralization the multispecific antiserum will be required for vaccination.

I. Although fetal antigens are different from tumor specific antigens, if these two antigens accompany the same tumor cells, fetal antigens can be utilized for the immunotherapy of tumors in a simple manner. In addition, the finding that type C viruses exist in embryo tissues, especially in liver, suggests the possible induction of fetal antigens by these viruses. This postulate is also based on the broad spectrum of fetal antigen specificity in a variety of tumors.

J. The morphological similarity of slow infectious viruses to Rous sarcoma virus leads investigators of this field to pay attention to their other etiological properties once more.

Proposed Course:

The goals of these projects for the next fiscal year are:

A. thru C. To clarify the viral etiology of murine myelomas, to provide the direct evidence of the PCl antigen-production of $xVEA^+$ type C viruses, and to determine the characteristics of a new endogenous type C virus found in spontaneous transformed BALB/3T3 cells and mineral oil-induced murine myelomas.

D. To isolate different classes of Gross soluble antigens, in relation with blocking factors of cell-mediated immunity.

E. To analyze the specificity of other virus-induced nonproductive sarcoma cell surface antigens, e.g. monkey sarcoma virus-induced cell surface antigens.

F. To study the mechanism of induction of cell surface antigens common to those on the type C virus envelope in mammalian systems.

G. To reclassify type C viruses by analyzing envelope antigens.

H. To disclose the factor which induces the resistance to syngeneic transplanted tumors in F_1 hybrid mice.

I. To determine the specificity of fetal antigens and the role of type C viruses in the appearance of these antigens.

J. Further ultrastructural studies of oncogenic viruses will be performed.

For the investigation of all items mentioned above, new immunoelectron microscopy techniques should be developed that will improve upon present techniques. In addition, the results obtained in animal systems will be applied to the human system.

Honors and Awards:

Presented a paper entitled: "Type C virus-associated surface antigens" as a special lecture at Tohoku University, Sendai, JAPAN, September 18, 1972.

Presented a paper entitled: "Aging and cancer—immunological aspects" at the Symposium on "Aging and Cancer" in the 31st Annual Meeting, Proceedings of the Japanese Cancer Association, in Nagoya, JAPAN, September 27, 1972.

Presented a paper entitled: "Analysis of type C virus associated surface antigens by immunoelectron microscopy," at Oak Ridge National Laboratory in Oak Ridge, Tennessee, December 6, 1972.

Presented a paper entitled: "Immunological analysis of murine myelomas and their associated type C viruses" at the Work Shop of Myeloma, at NIH, Bethesda, Maryland, February 8, 1973.

Presented a paper entitled: "Cell surface antigens of normal and neoplastic lymphohematopoietic cells" at the 26th Annual Symposium on Fundamental Cancer Research, "Immunological Aspects of Neoplasia" in Houston,Texas, March 7, 1973.

Publications:

Aoki, T. and Johnson, P.A.: Suppression of Gross leukemia cell-surface antigens: A king of antigenic modulation. J. Natl. Cancer Inst. 49:183-192, 1972.

Herberman, R. and Aoki, T.: Immune and natural antibodies to syngeneic murine plasma cell tumors. J. Exp. Med. 136:94-111, 1972.

Author: Aoki, T. and Hashimoto, Y.: Tumor Immunology. Tokyo, Shinjiku-Shobo, 1972, 137 pp.

Aoki, T., Herberman, R.B., Johnson, P.A., Liu, M., and Sturm, M.M.: Wild-type Gross leukemia virus: Classification of soluble antigens (GSA). J. Virol. 10:1208-1219, 1972.

Herberman, R.B., Aoki, T., and Nunn, M.E.: Solubilization of G(Gross) antigens on the surface of G leukemia cells. J. Natl. Cancer Inst. 50:481-490, 1973.

Aoki, T., Stephenson, R.J., and Aaronson, S.A.: Demonstration of a cell surface antigen associated with murine sarcoma virus by immunoelectron microscopy (Kirsten and Moloney strains/MuLV/viral envelope antigens). Proc. Natl. Acad. Sci. 70:742-746, 1973.

Aoki, T.: An analysis of antigens on the surface of murine leukemia. In Dutcher, R.M. and Chieco-Bianchi, L. (Eds.): Unifying Concepts of Leukemia. Bibl. Haemat. 39:307-315, 1973.

Takemoto, K.K., Aoki, T., Garon, C., and Sturm, M.M.: Comparative studies on visna, progressive pneumonia and Rous sarcoma viruses by electron microscopy. J. Natl. Cancer Inst. 50:534-547, 1973.

Plata, E.J., Aoki, T., Robertson, D.D., Chu, E.W., and Gerwin, B.I.: An established cultured cell line from human breast carcinoma. J. Natl. Cancer Inst. (in press).

Aoki, T.: Cell surface antigens of normal and neoplastic lymphohematopoietic cells. Proceedings of the Twenty-sixth Annual Symposium on Fundamental Cancer Research, In Hersh, E. (Ed.): Immunological Aspects of Neoplasia. (In press).

Aoki, T. and Todaro, G.J.: Antigenic properties of endogenous type C viruses from spontaneously transformed clones of BALB/3T3. Proc. Natl. Acad. Sci. (in press).

Aoki, T., Sendo, F., and Kudo, T.: Immunology of virus-induced leukemias. In Japan Hematological Society (Ed.): Handbook of Hematology. Tokyo, Japan, Maruzen (in press).

Kudo, T. and Aoki, T.: Specific antigens on the surface of tumor cells. In Kobayashi, H. and Tachibana, T. (Eds.): Tumor Immunology. Tokyo, Japan, Asakura (in press).

Aoki, T. and Johnson, P.A.: Aging and oncogenesis: Immunological aspects. Igaku-no-Ayumi (in press).

Serial No. NCI-4865

1. Viral Leukemia and Lymphoma Branch
   OASDVO, Division of Cancer Cause
   and Prevention
2. Immunology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Immunotherapy of murine leukemia cells and analysis of fetal antigens

Previous Serial Number: None

Principal Investigator: Dr. Toshio Kudo

Other Investigators:
Dr. Tadao Aoki
Dr. Vincent W. Hollis, Jr.
Dr. Ernest J. Plata
Dr. Fujiro Sendo
Dr. Luigi Chieco-Bianchi

Cooperating Units:
Inside NIH
Dr. Robert J. Huebner, LCBGY, NCI
Dr. Kenneth S.S. Chang, LCBGY, NCI

Man Years:

Total:	2.25
Professional:	1.0
Others:	1.25

Project Description

Objectives:

A. To develop immunotherapy using formalin-fixed syngeneic malignant cells in the virus-induced leukemia systems.

B. To detect tumor-specific transplantation antigens (TSTA) by transplantation of syngeneic virus-induced leukemias into F_1 hybrid mice and by analysis of antigen-specificities with humoral antibody and cell-mediated immunity.

C. To analyze fetal antigens by utilizing F_1 hybrid mice and young embryos (less than 10 days) in comparison with malignant cell-specific antigens.

Methods Employed:

A. and B. Formalin fixation method: Leukemia cells were fixed by 0.1%

formalin solution (formalin was diluted with 0.15 M phosphate buffer solution, pH 7.2, to 1:1,000) for 1 hour to 3 weeks at 4°C with periodical shaking.

Immunization with various numbers of fixed or untreated cells was performed in the syngeneic or F_1 hybrid system and the hyperimmunized mice were challenged subcutaneously with untreated viable leukemia cells.

C. Hyperimmune serum was obtained by immunizing F_1 hybrid mice with either x-ray irradiated embryo cells or untreated embryo cells from parent inbred strains.

For the examination of antigen specificity, cytotoxicity tests, immunofluorescence microscopy are used in combination with various absorption techniques.

Major Findings:

Since all projects mentioned above were initiated in October, 1972, the findings obtained are limited.

A. In the first place, various concentrations of formalin solution were used to find out the best one for suitable fixation of leukemia cells and for preservation of antigenicity. When E♂G2 cells were treated with either 1% or 10% formalin solution for 1 hour, their antigenicity was almost lost. Since 0.1% formalin solution was the best for preservation of antigenicity, this concentration was chosen for this experiment.

In the H-2^b system, antigenicity of E♂G2 cells treated with 0.1% formalin solution for three weeks proved to be preserved.

The viability of the leukemia cells was examined by both trypan blue staining and inoculation to syngeneic mice. Discrepancies between trypan blue staining and tumor growth were observed; i.e. only 38% of E♂G2 cells treated with 0.1% formalin solution for 2 hours were stained but did not grow in C57BL/6 mice.

B. When 15 x 10^6 K36 cells, transplanted AKR spontaneous leukemia, were inoculated subcutaneously into three (C57BL/6 x AKR)F_1 hybrid mice, two mice died of tumor growth 12 days after inoculation. One mouse, whose transplanted tumor was completely regressed, was challenged successively with 10 x 10^6, 40 x 10^6, and 50 x 10^6 viable K36 cells. The mouse challenged showed no sign of tumor growth. When 20 x 10^6 K36 cells were transplanted subcutaneously into six (C57BL/6 x AKR)F_1 hybrid mice, however, three out of six mice yielded to progressively growing tumors while the other tumors regressed completely.

These findings indicate the resistance of (C57BL/6 x AKR)F_1 mice to K36 cells. This resistance may be derived from the following two factors;

a) F_1 hybrid mice usually respond stronger to antigens than inbred mice, and b) wild-type Gross leukemia K36 produced at least two antigenically different type C viruses (Aoki et al., unpublished observations) which may induce stronger antigenicity.

C. According to previous reports, irradiation of embryonic cells is necessary to unmask fetal antigens for immunization. When F_1 hybrid mice are used for immunization instead of inbred mice, however, irradiation is not required to obtain the high titer antiserum. This antiserum positively reacted with C57BL/6 leukemia EL4 cells by cytotoxicity tests. EL4 cells possess various antigens on the cell surface; i.e. "X," "E," "L," and fetal antigens and antigen(s) common to those on the normal thymocyte. Since this antiserum reacted with either embryonic cells or normal thymocytes of syngeneic mice but not with normal spleen cells of syngeneic mice, it has been proven that this antiserum contains specific antibody against fetal antigens(s).

Significance to Biomedical Research and the Program of the Institute:

A. and B. The advantage of the formalin fixation method is the ability to stabilize both cells and viral antigens. This method may provide the way to immunotherapy and prophylaxis of cancer.

C. Analysis of fetal antigens utilizing F_1 hybrid mice may provide further information on the relationship between fetal antigens and tumor specific antigens.

Proposed Course:

These activities will be maintained.

Honors and Awards:

None

Publications:

Kudo, T. and Aoki, T.: Specific antigens on the surface of tumor cells. In Kobayashi, H. and Tachibana, T. (Eds.): Tumor Immunology. Tokyo, Japan, Asakura, 1973 (in press).

Aoki, T., Sendo, F., and Kudo, T.: Immunology of virus-induced leukemias. In Japan Hematological Society (Ed.): Handbook of Hematology. Tokyo, Japan, Maruzen, 1973 (in press).

Serial No. NCI - 4891

1. Viral Leukemia and Lymphoma Branch
   OASDVO, Division of Cancer
   Cause and Prevention
2. Viral Pathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Studies on the biology of DNA and RNA oncogenic viruses

Previous Serial Numbers: Same

Principal Investigator: Dr. Herbert K. Oie

Other Investigators: Dr. Yoji Ikawa
Dr. Dharam V. Ablashi
Dr. Adi F. Gazdar

Cooperating Units: Inside NIH
Dr. Samuel Baron, LBV, NIAID
Dr. Gary Pearson, VBB, NCI
Dr. Ursula Heine, VSS, NCI

Outside NIH
None

Man Years:

Total:	1.0
Professional:	1.0
Other:	0.0

Project Description

Objectives:

1. To investigate *in vitro* the transforming potential and other biological properties of *Herpesvirus saimiri*.

2. To determine the biologic properties of viruses isolated from rat mammary tumors.

3. To determine some of the changes in cellular functions resulting from exposure to oncogenic viruses and other carcinogens, with primary emphasis on the interferon systems.

Methods Employed:

1. Virus and viral antigens were assayed by the complement fixation tests, neutralization test, immunofluorescence, plaque assay, and electron microscopy.

2. Cells were cultured by routine tissue culture methods. The tumorigenicity of cells was tested by animal inoculation. Virus production was monitored by the complement fixation test for rat gs antigen and reverse transcriptase assays.

3. Interferon was assayed by a hemagglutinin yield reduction method.

Major Findings:

1. A continuous cell line from a spontaneously transformed rhesus monkey embryo cell culture has been established. The cells appear epithelioid, are not contact inhibited, have a doubling time of about 22-24 hours, and do not produce any endogenous virus. The cells grow in soft agar and at temperatures ranging from 33°C to 40.5°C. The following viruses have been shown to grow in this cell line: vesicular stomatitis virus, Sindbis virus, herpes simplex virus, Herpesvirus saimiri, a human cell-adapted strain of Rauscher leukemia virus, Woolly monkey virus and the Mason-Pfizer monkey mammary tumor virus. Infection of the spontaneously transformed rhesus embryo cells with Herpesvirus saimiri has resulted in the establishment of a persistently infected cell culture. This culture has undergone more than 10 passages during the past 5 months and is still producing virus. Changes in medium and/or incubation temperature can be manipulated to favor virus replication or cell growth. This culture is presently being used as a source of infectious virus and viral antigens for virologic and immunologic studies.

2. Spindle-shaped cells have been isolated in culture from a rat mammary tumor. The original tumor has been continuously maintained in vivo. Cells recently cultured from three mammary tumors from this in vivo tumor line grew as epithelioid cells. These epithelioid cells as well as several clones derived from them produced carcinomas in newborn Sprague-Dawley rats. All produce virus containing rat gs antigen and reverse transcriptase activity.

3. A clonal line of S+L- 3T3FL cells has been shown to produce unusually high yields of interferon. The parent 3T3FL line and another clone of S+L- cells proved to be poor interferon producers. Efforts are underway to determine the necessity for

the presence of the sarcoma genome in the cells for high interferon production by isolation of "flat" cells.

Significance to Biomedical Research and the Program of the Institute:

Neoplastic diseases in man resembling Herpesvirus saimiri induced leukemia and lymphoma and the rat mammary tumor, to name a few, do exist. Comprehensive studies of such oncogenic viruses and tumor cells in vitro could result in the establishment of useful model systems. Investigations with these model systems could provide valuable information toward the understanding of human cancer. Also, these model systems could be useful for testing of anti-cancer drugs.

Honors and Awards:

None

Proposed Course:

1. The biologic properties of the Herpesvirus saimiri produced in the spontaneously transformed rhesus cells will be studied.

2. The virus produced in the epithelioid cells isolated from rat mammary tumor will be further characterized, especially with respect to its oncogenic potential.

3. The study of the effect(s) on the interferon system of cell transformation by viruses will be extended to cells transformed spontaneously and cells transformed by radiation and chemical carcinogens.

Publications:

Oie, H. K., Buckler, C. E., Uhlendorf, C. P., Hill, D. A., and Baron, S.: Improved assays for a variety of interferons. Proc. Soc. Exp. Biol. Med. 140: 1178-1181, 1972.

Oie, H. K., Gazdar, A. F., Buckler, C. E., and Baron, S.: High interferon producing line of transformed murine cells. J. Gen. Virol. 17: 107-109, 1972.

SUMMARY REPORT

VIRAL BIOLOGY BRANCH
July 1, 1972 - June 30, 1973

The Viral Biology Branch conducts research on virus and host factors related to carcinogenesis and the development and evaluation for cancer control in experimental systems. Investigations are conducted to detect and identify the nature of virus activity in tumor tissue, study the effect on tumorigenesis of interaction between viruses co-infecting the host, determine the biological behavior of neoplastic cells, characterize virus and tumor specific antigens, and examine biochemical events related to viral infection and cell transformation. Ultrastructural studies permit detection and morphological characterization of viruses associated with disease processes and their effect upon the internal organization of the cell. Selected experimental animals and cell cultures are used to evaluate combined chemotherapeutic and immunotherapeutic measures for effectiveness in control of tumor growth.

The Section of Cell Biology seeks to acquire knowledge of malignant cell behavior and of the nature of the neoantigens which appear on tumor cell surface membranes. The immune response of the host to tumor cell surface antigens is studied, and methods are developed to increase immunity developed against these antigens to provide improved measures for immunotherapeutic control. The Section of Experimental Pathology has given emphasis to the investigation of neoplasms associated with herpes-type virus activity. A definition of the causal relationship of herpesviruses to specific neoplasms is sought by the study of host immune responses to virus-induced antigens at various stages during the pre- and post neoplastic phases of the disease. The humoral factors affecting the course of a neoplastic disease process in animal hosts have been given particular attention. The Section for Human Tumor Studies combines viral, immunological and biochemical approaches to detect, isolate and characterize viruses associated with human and some animal tumors. Protein products of virus gene expression, in vitro protein synthesis with viral messenger, and the relationship of these proteins to cellular transformation are under investigation. The Section of Virus and Disease Modification is concerned with two areas of research related to virally-induced neoplasia. One aspect involves the effect of co-infections by oncogenic and non-oncogenic viruses on the manifestation of neoplastic disease in experimental animals. The other primary course of study is directed to the development and evaluation of combined chemotherapeutic and immunotherapeutic measures for control of tumor cell growth in animal hosts. The Section of Electron Microscopy devotes its efforts to the detection of viruses in biological material, their morphology, and the localization of viral antigens within host cells. The ultrastructural changes occurring in virus-infected cells are determined in relation to the reproductive cycle of the agent. An important contribution is made by this Section to the overall requirements of other intramural investigators in Viral Oncology for electron microscopic examination of biological materials and photographic services.

The Office of the Chief coordinates research by the various Sections with due recognition of the scientific freedom of the individual investigators. The office is responsible for establishing collaborative efforts between investigators within the Branch and in other areas of NIH or elsewhere. The Chief serves as Chairman of the Developmental Research Segment of the Special Virus Cancer Program. In this capacity he is responsible for the management of the extramural research contracts within the Segment's program.

Following published reports that endogenous animal RNA tumor viruses could be activated by treatment of cultured cells with halogenated uridine, the technique was applied to normal bovine cell cultures carried in our laboratories. The treatment resulted in the release of virus particles possessing RNA-dependent DNA polymerase (RDDP) activity. The technique was also successfully applied to a human tumor cell culture, and the agent, released in small amounts, is presently undergoing preliminary characterization, both intramurally and within the research contract program. The human cell derived virus was shown to have a C-type morphology, a density of 1.16 g per ml in a sucrose gradient, and RDDP activity. Upon infection of whole human embryo or human bone marrow cultures, proliferating foci of morphologically altered cells were observed, but no significant amounts of virus was shed into culture fluids. Superinfection by this agent of some cell lines chronically infected with C-type RNA tumor virus inhibited production of the latter. Cytopathogeneic effects were observed in other chronically infected cells following superinfection. The inhibitory activity may make it possible to acquire interesting data on virus gene expression.

In collaborative studies with other laboratories, an attempt was made to show a correlation between the presence of virus-like particulates in human milk, the RDDP activity associated with fractions separated from the milk, and the breast cancer history of the milk donor. RDDP activity was found to be no higher in preparations from milk of donors with a family history of breast cancer, or any other cancer, than in those from donors without a history of cancer. Further, no correlation was determined between the presence or absence of particulates in milk and RDDP activity.

The Epstein-Barr virus (EBV) has a strong association with Burkitt's lymphoma, but the lack of an experimental animal responsive to infection has impeded progress in defining its role in the cause of this neoplasm. Collaborative studies with other investigators outside the Branch demonstrated characteristics exhibited by Herpesvirus saimiri (HVS) which were quite similar to those of EBV. HVS infections in marmosets and owl monkeys terminate in fatal lymphoma or leukemia. "Early" antigens were found to be produced upon infection of Vero cells by HVS, mimicking the observations made on human cell infections by EBV. Whereas the squirrel monkey, the natural host of the virus, shows only a transient antibody response to "early" antigen and no pathology following primary infection by HVS, the owl monkey and marmoset develop antibodies to this antigen, but only at the time when there is evidence of abnormal lymphoproliferation. This is a striking parallel to the relationship between antibodies to "early" EBV-induced antigen and the course of Burkitt's lymphoma in humans.

The motility of cells in vitro was studied to gain knowledge of the phenotypic attributes of cells that correlate with the malignant state. The inhibition of cellular mobility by population density in vitro of 3T3 mouse fibroblasts was compared with the inhibition determined for viral transformants of 3T3 cells, spontaneous transformants, and chemically-induced fibrosarcoma cells. The degree of diminution of motility observed did not correlate well with the tumorigenicity exhibited by these cell strains. Procaine hydrochloride in pharmacoligic doses reversibly inhibited cell mobility and division, suggesting that ionic permeability of the cell membrane had some influence on cell mobility.

The examination of molecular events at the translational level which are associated with viral replication was undertaken as an approach to the definition of products of virogene expression related to cellular transformation. More than forty polypeptides ranging in molecular weight from several hundred thousand to several thousand daltons may be recognized in preparations of highly purified C-type RNA viruses. Relative to the few major components, the majority are present in minute quantities. A method has been developed so that these minor components, labeled in vitro, may be purified by conventional techniques. A subcellular protein synthesizing system was isolated from both Rauscher leukemia virus infected cell cultures and fresh rat liver. Both systems are capable of de novo protein synthesis in response to exogenous messenger RNA. Preliminary results with AMV RNA directed proteins in the rat liver system indicated that some virus-specific zones resolved by gradient polyacrylamide-SDS gel electrophoresis were present when compared to AMV capsid proteins.

The majority of animal and human tumor cells possess surface membrane antigens which are capable of inducing a weak immunological rejection reaction in the host. Attempts to obtain these tumor transplantation antigens in a cell-free immunogenic form were unsuccessful. However, if tumor cells were first infected with influenza virus, antigenicity was retained by cell-free preparations, which were shown to confer protection of the immunized host against challenge in tumor transplant rejection assays. Tumor specific cell surface antigen was solubilized by KCl extraction and shown to inhibit the binding of specific antibody to the antigen in tumor cells. Partial purification of the soluble antigen was achieved. In the course of these studies, an unusual monosaccharide, tentatively identified as a dideoxyhexosamine, was detected in six different methylcholanthrene-induced fibrosarcomas. Investigations are being extended to resolve and characterize multiple antigens present on cell surface membranes of selected tumors and to determine the mode and kinetics of their biosynthesis.

The appearance of antigens on the surfaces of cells in the course of infection and replication of woolly monkey sarcoma virus was followed by immunoelectron microscopy and immunofluorescence microscopy. Virus specific antigen appeared as small discrete points on the cell surface during the first 24 hours following infection and prior to the production of virus particles. The areas on cell surfaces occupied by viral antigen increased with time, and intracellular viral antigen could be readily detected 72

hours after infection, when virions were being produced. After the seventh day post-infection, extracellular virus could no longer be readily detected, and virus antigens could be demonstrated in only 20 percent of the cell population. However, replenishment of the culture with fresh medium resulted in the appearance of increased numbers of cells with virus antigen within six hours. These observations were confirmed by the results obtained with parallel cultures in which the cytolytic activity of an antiserum reactive with virus-induced cell antigens was used to follow viral expression in infected cells. The observations show the requirement of active cell metabolism for woolly virus production which contrasts with experience with herpesviruses associated with oncogenic processes wherein virus expression is increased in metabolically-deficient cells.

Humoral factors influencing the outgrowth of murine sarcoma virus-induced tumors were examined in the sera taken from mice with progressively growing tumors, in sera taken after tumor regression, and in sera taken from mice immunized with allogeneic cells of tumors induced by the virus. Only the serum from mice with progressively growing tumors had low titers for tumor cell membrane antigen and for virus neutralization. Preinoculation of mice with this serum enhanced the growth of a tumor cell challenge. Although none of the sera were cytotoxic for sarcoma virus-induced tumor cells, serum from regressor and from immunized mice were effective in inhibiting outgrowth of a tumor cell challenge. The degree of effectiveness in preventing tumor outgrowth was directly related to the serum titers, indicating the value of this measure of serum potency in tumor inhibition.

Over 36 compounds were tested for their capability to inhibit the reproduction of murine leukemia and sarcoma viruses in vitro. Only 16 showed reproducible activity. Five of the most active demonstrated inhibitory activity against these viruses in vivo. Among the chemicals tested for non-specific stimulation of the activity of the reticuloendothelial system, imidazole thiazole, pyran copolymer and Tiloron proved very effective in increasing the survival of leukemic mice in chemotherapeutically induced remission. Presumably, their activity is associated with the capability to stimulate a non-specific cellular immune response. Bis-chloronitrosourea compounds demonstrated inhibitory capability when tested against the transplantable Lewis lung tumor, a neoplasm which is refractory to many drugs tested.

In a collaborative experiment with investigators under a research contract for the study of the activation of endogenous mouse leukemia viruses in the graft versus host reaction, the administration of high-titered interferon was found to completely inhibit virus activation. In this respect, interferon may prove to be an effective agent in blocking events leading from immune responses against histocompatability antigens to virus activation and subsequent oncogenesis.

The effectiveness of immunostimulation in prolonging remission following chemotherapy was explored with virally-induced lymphomas of the mouse. Long term survival was reproducibly achieved through treatment of mice with the Phipps strain of BCG after the tumor cell burden was reduced by drugs.

Corynebacterium granulosum inoculated in different doses had similar activity. Treatment by immunostimulators had no effect on the control of the spontaneous transplantable Nova rat leukemia, which appears to be free of virus particles. Immunological investigations failed to demonstrate tumor-associated antigens on the Nova tumor cells, suggesting that the immunogenicity of tumor cell surface antigens is important to effective response to immunostimulation.

Other Activities of the Branch:

During this reporting period, 11 papers covering various aspects of viral oncology were published by senior investigators of the Branch and 14 papers are in press.

Members of the Branch presented lectures by invitation to research groups in this country and abroad and discussed research findings at various scientific meetings. The Branch also provided training in a variety of experimental procedures to outside visitors. Senior members of the Staff served as project officers on research contracts and participated in site visits to different laboratories in the research contract program of the Institute.

In serving the intramural investigators within the Viral Oncology Area, the Electron Microscopy Section processed 718 samples for ultrastructural study. In addition, the Section's Photographic unit assisted investigators in different laboratories by photographing specimens and by processing electron photomicrographs for study and inclusion in manuscripts.

Serial No. NCI-4874
1. Viral Biology Branch
OASDVO, Division of Cancer Cause and Prevention
2. Electron Microscopy Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Ultrastructural Studies on Tissue and Virus in Relation to Neoplastic Diseases

Previous Serial Number: Same

Principal Investigator: Dr. Harish C. Chopra

Other Investigators: Dr. Angelo P. Andrese (NCI-4897)
Dr. Walter D. Holder (NCI-4898)

Cooperating Units:

Inside NIH

Dr. D. Ablashi, VLLB, NCI (NCI-4834)
Dr. A. Gazdar, VLLB, NCI (NCI-4824)
Dr. B. Gerwin, VLLB, NCI (NCI-4846)
Dr. Y. Ikawa, VLLB, NCI (NCI-4847)
Dr. G. Pearson, VBB, NCI (NCI-4880)
Dr. P. Ebert, VBB, NCI (NCI-4876)

Outside NIH

Dr. M. Brennan, Michigan Cancer Foundation, Detroit, Michigan
Dr. M. Rich, Michigan Cancer Foundation, Detroit, Michigan
Dr. M. Mason, Mason Research Institute, Worcester, Massachusetts
Dr. A. Bogden, Mason Research Institute, Worcester, Massachusetts
Dr. D. Fine, Bionetics Research Laboratories, Bethesda, Maryland
Dr. R. Pienta, Bionetics Research Laboratories, Bethesda, Maryland
Dr. H. Rabin, Bionetics Research Laboratories, Bethesda, Maryland

Man Years:

Total:	4.2
Professional:	1.0
Other:	3.2

Project Description

Objectives:

1. Detect and characterize virus-like particles in the milk of women with family history of cancer.

2. Attempt isolation of viruses from dog mammary carcinomas.

3. Study mouse mammary tumor virus in vitro.

4. Continue studies of M-PMV and R-35 MTV infected animals.

5. Provide electron microscopic and photographic support to other NCI investigators.

Methods Employed:

Human milk specimens were processed to purify and concentrate milk particles by density gradient fractionation. Human mammary carcinoma biopsy materials and cultured cells were prepared by thin sectioning and examined by electron microscopy for the presence of virus and for cellular ultrastructure. The cultured mammary tumor cells were treated with various carcinogens in attempts to activate virus production. Electron micrographs were interpreted and evaluated.

Major Findings:

1. Electron microscopic studies were continued in a search for virus or virus-like particles in the purified and concentrated fractions of milk obtained from women with or without a family history of cancer. The milk samples from 460 women, along with their family history of cancer were received from the Michigan Cancer Foundation, Detroit and were processed by density gradient centrifugation. The banding at densities of 1.16 to 1.18 g/ml were examined by the negative staining technique of electron microscopy. These investigations revealed that only 50 of 460 milk specimens contained particles morphologically resembling the Mason-Pfizer monkey virus (M-PMV) isolated from a breast tumor of a rhesus monkey. No type B particles resembling the mouse mammary tumor virus were observed in these samples. There was no correlation between the presence of the particles in human milk and the family history of cancer among the milk donors. An attempt was also made to correlate the presence of milk particles with the reverse transcriptase activity detected in these milk specimens; no consistent correlation could be established. During this reporting period further studies were undertaken to demonstrate the viral nature of these particles from human milk.

In collaboration with Dr. Marvin Rich of the Michigan Cancer Foundation, the infectivity of the milk particles is being assayed on a number of human cell cultures. The milk specimens which were positive for the presence of particles by electron microscopy are being inoculated into the cultures. As yet no significant results demonstrating the viral nature of the particles from milk have been obtained.

2. Investigations to detect and isolate a viral agent from dog mammary tumors were continued during the past year. In addition to previously established cell cultures of dog mammary carcinomas, two new cell cultures were initiated and are being explored for the presence of virions. These cultures are being regularly monitored by electron microscopy and as yet none have been found to contain virus particles. Cultures treated with IUdR and BUdR have failed to show evidence of virus production. The original biopsies of the dog tumors also appeared to be free of virus particles.

3. During this reporting period, investigations were initiated to attempt to propagate mouse mammary tumor virus in tissues from different strains of mice. Also the morphological ultrastructure of mouse MTV is being analyzed by cytochemical techniques. Cell cultures from C_3H and Balb/C mice which are propagating mouse mammary tumor virus are being maintained. Two of these cell cultures originated from C_3H mammary tumors are now established cell lines, having been maintained in our laboratory for four months. At this time the cultures are slow virus producers, and an attempt is being made to reactivate the virus by chemical and hormonal treatment.

4. We have continued investigations on the cell transforming ability of (M-PMV) virus, and its oncogenic potential in vivo. Preliminary in vitro studies demonstrating transformation of rhesus foreskin cells following infection by M-PMV were published. Tumorigenic potential of these transformed cells is being investigated in the newborn rhesus monkeys in which palpable masses have been induced at the site of inoculation of the transformed cells. The oncogenic potential of cell-free M-PMV in rhesus monkeys inoculated neonatally is being investigated in collaboration with investigators at the Mason Research Institute. These studies are aimed at definition of the role of hormonal imbalance as a cofactor with M-PMV infection in the induction of mammary neoplasia in the rhesus monkey. Electron microscopy demonstrated that the enlarged lymph nodes (lymphadenopathy) from seven of nine newborn female rhesus monkeys inoculated subcutaneously and intraperitoneally with cell-free virus contained characteristic M-PMV virus particles; lymph nodes from an un-inoculated rhesus monkey showed no virus. Further investigations to determine viremia in the inoculated animals, and in vivo oncogenic potential of M-PMV are in progress.

5. The R-35 virus, isolated from a rat mammary carcinoma, is being investigated for its etiological role in rat mammary cancer. Previously, we reported that this virus transformed cultured normal rat mammary tissue. However, it was observed that the transformation of rat mammary gland cell cultures was inefficient and inconsistent. In collaboration with the Mason Research Institute, experiments were initiated to determine whether R-35 virus is oncogenic in its natural host, the Sprague-Dawley rat. Dr. Bogden submitted for electron microscopy study a total of 15 malignant and 18 benign tumors from the virus inoculated rat population, and 2 malignant and 16 benign tumors that occurred in the control population. Although it is quite significant that the malignant tumors appeared in the virus inoculated population earlier (4-5 months of age) than in the Control group (9-13 months), none of the tumors contained R-35 virus particles. Further collaborative studies will be pursued during the next year.

6. The Electron Microscopy Section has provided electron microscopic and photographic support to various investigators within the Viral Oncology Area. During the past year, routine as well as collaborative electron microscopic support was given to several NCI research projects. The service support personnel processed 718 samples for electron microscopic study. Several collaborative research projects, reported previously, were completed this year and the reports have either been published or submitted for publication.

Significance to Biomedical Research and the Program of the Institute:

Ultrastructural studies on tissues and viruses in relation to neoplastic diseases have contributed significantly to the virus-cancer program, especially for virus detection in human and subhuman primates. Further, collaborative investigations with other NCI investigators have provided necessary support on the ultrastructural aspects of various research projects.

Proposed Course:

The current research activities will be maintained.

Other Activities:

Served as a member of the Working Group for the Breast Cancer Segment, SVCP, and as assistant project officer on contract number NIH-70-2204 with the Mason Research Institute. Trained investigators from other laboratories in procedures for quantitation of virus by electron microscopy. Provided support to other NCI investigators by interpreting ultrastructural details in electron micrographs.

Publications:

Chopra, H. C., Fine, D., Pienta, R. and Woodside, N.: Studies on oncogenic properties of a virus isolated from the monkey breast tumor. Medical Primatology, 3, 101-106, (Karger, Basel, 1972).

Chopra, H. C., Woodside, N., Kvedar, J., Albert, S. and Brennan, M. J.: Electron microscopic search for oncorna-type particles in human milk. J. Inst. Nat. Res. Med., France (Symposium), 321-333, 1972.

Fine, D. L., Pienta, R. J., Valerio, M. G. and Chopra, H. C.: Current studies on a virus isolated from a breast carcinoma of rhesus monkey. J. Inst. Nat. Res. Med., France (Symposium), 197-212, 1972.

Hooks, J., Gibbs, C. J., Chopra, H. C., Lewis, M., and Gajdusek, D. C.: Spontaneous transformation of human brain cells grown in vitro and description of associated virus particles. Science, 176, 1420, 1972.

Fine, D. S., Kingsbury, E. W., Valerio, M. G., Kubicek, M. T., Landon, J. C. and Chopra, H. C.: Nature, New Biology, 238, 191-197, 1972.

Pienta, R. J., Fine, D. L., Hurt, T., Smith, C. K., Landon, J. C. and Chopra, H. C.: Transformation of rhesus foreskin cells by Mason-Pfizer monkey virus. J. Nat. Cancer Inst. 48, 1913-1917, 1972.

Bergs, V. V., Pearson, G., Chopra, H. C. and Turner, W.: Spontaneous appearance of cytopathology and rat C-type virus (WF-1) in a rat embryo cell line. Int. J. Cancer 10: 165-173, 1972.

Presentations:

Dr. Chopra presented a paper entitled: Studies on Oncogenic Properties of a Virus Isolated from the Monkey Breast Tumor at the Third Conference on Experimental Medicine and Surgery in Primates, Lyon, France.

Dr. Chopra presented a paper entitled: Current Studies on a Virus Isolated from a Breast Carcinoma of Rhesus Monkey at the 7th Meeting on Breast Cancer in Animals and Man, Grenoble, France.

Dr. Chopra presented a paper entitled: Electron Microscopic Search for Oncorna-type Particles in Human Milk at the 7th Meeting on Breast Cancer in Animals and Man, Grenoble, France.

Serial No. NCI-4875

1. Viral Biology Branch
   OASDVO, Division of Cancer Cause & Prevention
2. Virus & Disease Modification Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Control of Oncogenic Viruses and Induced Diseases Mediated by External Factors

Previous Serial Number: Same

Principal Investigator: Dr. Michael A. Chirigos

Other Investigators: Dr. Wilna A. Woods (NCI-4877)
Dr. John W. Pearson (NCI-4878)
Dr. Takis S. Papas (NCI-4899)

Cooperating Units: Inside NIH:
Dr. S. Chaparas, LBP, DBS
Dr. N. Sher, LBP, DBS
Dr. H. Wood, DDB, DCT
Dr. K. Perk, VBB, DCCP

Outside NIH:
Dr. G. Spahn, Microbiological Associates, Inc., Bethesda, Maryland
Dr. L. S. Rapstein, Microbiological Associates, Inc., Bethesda, Maryland
Dr. C. Bowles, Hazleton Laboratories, Falls Church, Virginia
Dr. E. Furusawa, University of Hawaii, Medical School, Honolulu, Hawaii
Dr. M. Hirsch, Massachusetts General Hospital, Boston, Massachusetts
Dr. P. Black, Massachusetts General Hospital, Boston, Massachusetts
Dr. W. Jergelsky, NIEHS
Dr. R. Simpson, Rutgers University, New Brunswick, New Jersey

Man Years:

Total:	3.0
Professional:	1.0
Other:	2.0

Project Description

Objectives:

The overall aim of this project is to determine the most effective means of preventing and/or controlling leukemias, sarcomas and carcinomas. Several therapeutic approaches are examined in animal model systems to assess the most feasible approach that can be employed to exert maximum antitumor activity with the least deleterious effect on normal host factors. Host factors which may be affected by therapy are immuno-competence, organ and/or cell toxicity, kidney or liver damage.

Methods Employed:

A. Virus and Tumor Animal Systems.
The following are employed for in vitro and in vivo studies.
1. The Rauscher, Friend, Moloney, Graffi and Gross leukemogenic viruses, and the Moloney, Harvey and PV sarcomas.
2. AKR mice with spontaneous lymphoma.
3. Rat Gross, Nova and Dunning leukemias.
4. Lewis lung carcinoma.
5. DMBA & 3 MC induced fibrosarcomas.
B. Humoral and cellular antibodies as measured by standard procedures or new methods developed by Section staff.
C. In vitro and in vivo assays for measuring viral replication. (X-C syncytia formation for MuLV and Focus assay for MuSV).

Major Findings:

1. Inhibitors: Employing the XC syncytia formation assay for murine leukemia viruses and the focus assay for murine sarcoma viruses over 36 compounds were tested for viral inhibitory activity. Only 16 showed reproducible activity in vitro. The most active agents were: Streptonigrin; 3 Rifamycin derivatives; Distamycin; Compound 182/30; 2 extracts of plant alkaloids; Amphotericin B; Streptovaricin A, B and Complex; and BNS 217.

Of the most active agents, demonstrating in vitro activity, 5 have been tested in vivo and show good antiviral activity against Rauscher leukemia virus and Murine sarcoma virus. A few of these agents have been tested for reverse transcriptase inhibitory activity and four were very potent inhibitors.

2. Immuno-stimulators: Several chemicals have been tested for nonspecific RES stimulatory activity. Three of these chemicals, when used in concert with chemotherapy, have been found to be very effective in significantly extending the survival time of leukemic mice (Imidazole thiazole, Pyran copolymer and Tiloron). These drugs when used alone have little to very limited antitumor activity. However, when applied to leukemic animals in chemotherapeutically induced remission they provoke an immuno-stimulatory activity which results in a substantial number of cures. Since these drugs

cause an earlier rejection of skin grafts in mice, it is felt that they act by stimulating a nonspecific cellular immune response.

3. Lewis Lung tumor: A syngeneic transplantable mouse lung tumor model has been established. Quantitative responses are possible through the lung lesion assay method we have developed. This tumor model is being presently used to assess the ability to prevent and/or control metastatic lesions from developing in the lung. The tumor has been found to be refractory to many of the drugs we have tested. However, the bis-chloronitrosourea compounds have been found to be very effective against both the primary tumor and the metastatic lung lesions. Preliminary results indicate that immunostimulators used in conjunction with drugs act synergistically.

4. DMBA & 3MC induced fibrosarcomas. Several DMBA & 3MC carcinogen-induced tumors have been established in BALB/c and C_{57}Bl/b adult mice. The tumors have been histologically identified as fibrosarcomas. These solid tumors will serve as a model for studies on chemotherapy and immunotherapy. Current studies show that these tumors respond to Cytoxan therapy.

5. Inhibition of leukemia virus activation in Graft versus Host Disease by Interferon therapy. Immune dyscrasias in the mouse have been found to be associated with activation of leukemia viruses. In the graft versus host (GVH) reaction, virus activation becomes apparent soon after injection of hybrid F_1 animals with parental cells, and the activated virus can be shown to be causally related to the malignant lymphomas that subsequently develop in the F_1 animals. In collaboration with Dr. Paul Black it was shown that treating F_1 mice undergoing a GVH disease with interferon completely protected them against virus activation. Interferon treatment of GVH-animals not only altered virus replication in these animals, but also modified the nature of the GVH-reaction itself. The results indicate that interferon shows promise of effectiveness in blocking the chain of events leading from immune responses against histocompatability antigens to virus activation and subsequent oncogenesis.

6. Combined Chemotherapy and Immunostimulation of Canine Spontaneous Lymphosarcoma. In collaboration with Dr. C. Bowles of Hazleton Laboratories, Inc., Contract NCI 69-2079, we presently have under treatment 4 dogs with stage 2 and 3 lymphosarcoma. Combined cytoxan, Vincristine and Prednisone induced a remission of the lymphadenopathy. These animals have been injected with the Brazilian strain of BCG in the lymphnode areas. Current observations indicate that the lymphadenopathy, and hematological evidences of remission in the BCG treated animals is being maintained.

Significance to Biomedical Research and the Program of the Institute:

The multi-disciplinary approach represented in this project is almost entirely directed towards the prevention, treatment and control of cancer. It is necessary to work with animals systems to gain basic information which is ultimately applied to the control of human neoplasia. The greatest strides made in controlling cancer are with drug therapy. However,

chemotherapy has several serious limitations. Studies concerned with immunotherapy and immunostimulants, described herein, show these to be effective control measures particularly in combination with drug therapy. Non-drug therapeutic measures are presently at hand, and if better understood and judiciously applied, can serve to improve measures for control of human cancer.

Proposed Course:

Each of the areas described under objectives will be pursued. More emphasis will be placed on quantitative and qualitative studies in three specific areas, i.e., Inhibitors, Immune-Stimulators, and Oncornavirus Activation and/or Retardation. Biochemical parameters will be investigated with the active inhibitors to assess mechanisms(s) of action. In vitro assays will be developed and employed to assess whether the immunostimulatory activity demonstrated by drugs is of humoral and/or cellular type. Chemical immunostimulatory studies will be extended to carcinogen-induced tumors as well as to leukemias to determine effectiveness in these tumors.

Further studies will be pursued on the activation of covert infections by RNA tumor viruses and inhibition of the process. Results of interferon treatment causing an inhibition of the virus activation which occurs during a GVH reaction shows interferon to hold promise of being a possible effective blocker of the chain of events leading from immune responses against histocompatibility antigens to virus activation and subsequent oncogenesis. In this regard, interferon may well prove to be an important prophylactic agent against cancers in human transplant recipients and in patients with chronic immunological disorders.

Other Activities:

Serves as Associate Chief of the Viral Biology Branch and Vice-Chairman of the Developmental Research Segment of the SVCP. Serves as a Work Group Member of the Biohazards Control and Containment Segment of the SVCP, and Project Officer or Assistant Project Officer on 10 contracts in the Special Virus Cancer Program.

Honors and Awards:

Dr. Chirigos received an Honorary Doctor of Science Degree from Western Maryland College during the 1972 Commencement exercises at the College. Dr. Chirigos, by invitation, presented lectures at the Division of Medical Oncology, and Department of Medicine, University of Virginia, and at the Conference on Virus Tumorigenesis and Immunogenesis held at the Pennsylvania State University, and spoke in regard to the most recent developments in therapy applied to Cancer and Viral Oncogenesis. He also particpated in the International Conference on BCG in Therapy of Cancer held at the NCI in Bethesda, Maryland and in the Conference on Immunology of Carcinogenesis held in Gatlinburg, Tennessee.

Publications:

Pearson, J. W., Pearson, G. R., Gibson, W. T., Chermann, J. C., and Chirigos, M. A.: Chemoimmunostimulation Therapy Against a Murine Leukemia. Cancer Research 32: 904-907, 1972.

Chirigos, M. A., Pearson, J. W., Spahn G., and Rutman, R.: Current Studies on Oncorna Virus Therapy. Bibliotheca Haematologica, Vol. 39, Vth International Symposium on Comparative Leukemia Research (In Press), 1972.

Chirigos, M. A., Pearson, J. W., Woods, W. A., and Spahn, G.: Immunotherapy and Chemotherapy in Murine Leukemia. Tumor Viruses and Immunity Conference. Academic Press (In Press), 1973.

Woods, W. A., Massicot, J., Webb, J., and Chirigos, M. A.: Inhibitory Effect of Streptonigrin on a Murine Sarcoma Virus-Induced Tumor Cell Line (MSC) and Selection of Drug Resistant Clones. In Vitro (In Press), 1973.

Pearson, J. W., Chaparas, S. D., and Chirigos, M. A.: Effect of Dose and Route of BCG Inoculation in Chemoimmunostimulation Therapy of a Mouse Leukemia. Cancer Research (In Press), 1973.

Serial No. NCI-4876

1. Viral Biology Branch
   OASDVO, Division of Cancer
   Cause and Prevention
2. Human Tumor Studies Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Biochemical Alterations Occurring in Host Tissue as a Result of Oncogenic Virus Infection and Tumor Induction

Previous Serial Number: Same

Principal Investigator: Dr. Paul S. Ebert

Other Investigators: None

Cooperating Units: Inside NIH:
Dr. M. A. Chirigos, VBB, NCI (NCI-4875)
Dr. G. F. Vande Woude, VBB, NCI-4893)
Dr. W. G. Robey, VBB, NCI (NCI-4895)
Dr. R. Ascione, VBB, NCI (NCI-4902)
Dr. G. Pearson, VBB, NCI (NCI-4880)
Dr. H. Chopra, VBB, NCI (NCI-4874)
Dr. D. P. Tschudy, Metabolism Branch, NCI
Dr. D. N. Buell, GL&C, NCI
Dr. Y. Ikawa, VLLB, NCI, Visiting Scientist
Dr. E. Weinbach, LPD, NAID

Outside NIH:
Dr. B. Gerwin, Bionetics
Dr. D. Gillespie, Bionetics

Man Years:

Total:	2.0
Professional:	1.0
Other:	1.0

Project Description

Objectives:

1. To investigate the possible viral etiology of breast cancer by examining the RNA-directed DNA polymerase in human milk.

2. To investigate the role of heme biosynthetic activity in RNA tumor virus production.

3. To investigate the process of differentiation by following the early enzymatic changes in primitive cells which differentiate to red cells in vitro when incubated with Dimethylsulfoxide (DMSO).

Methods Employed:

Conventional biochemical procedures were utilized to observe the alterations occurring in normal and virus-infected cells in culture. Virus particles and viral RNA were concentrated and purified by centrifugation and standard density gradient techniques. Reverse transcriptase was measured by techniques described by Todaro and Spiegelmen. Briefly, virus particles were disrupted by detergent treatment, incubated with ^{3}H-dTTP and rA·dT (template-directed polymerase reaction) or with 3 non-radioactive deoxyribonucleotides and ^{3}H-dTTP (endogenous reaction) and the acid-precipitable product of the reaction was collected on Millipore filters and counted in a scintillation counter.

Major Findings:

1. Reverse transcriptase activity in human milk: Attempts were made to correlate reverse transcriptase activity and the presence of particles by electron microscopy in human milk with the breast cancer history of the donor. The presence of reverse transcriptase by 3 methods (phosphocellulose chromatography, endogenous reaction, simultaneous detection) was not significantly higher in milk donors with a positive family history of breast cancer or of any other cancer than in donors with normal histories. Moreover, there was no correlation between the detection of a poly (dT) synthetase with either the endogenous reaction or simultaneous detection procedures, or with the detection of virus-like particles by electron microscopy. Thus, in contrast to a report from another laboratory, no correlation was determined between virus-like enzyme activity and particles observed with human milk with a positive family cancer history of the donor.

2. Heme biosynthesis and reverse transcriptase activity in rat cell lines producing C-type virus: The heme biosynthetic and reverse transcriptase activities were studied in the WF-1, RMTL-8 and R-35 rat fibroblastic cell lines which shed C-type particles. The synthesis of heme in the WF-1 tumor cell line was 4 times the level in both cell homogenates and mitochondria of the WF-1 cell line as compared to normal fibroblasts. Moreover, the levels of virus production in the normal line and 4 virus producing lines, as determined by electron microscopy and immunofluorescence, was found to be proportional to the amount of ALA synthetase activity. All of the virus-producing lines contained DNA polymerase activity using the synthetic rA·dT-template, and showed a 60-70S RNA component which served as a template for DNA synthesis.

3. Heme biosynthesis in a cell line which differentiates to red cells in vitro: A Friend leukemia virus-transformed cell line T3C12 can be induced to undergo changes which are analagous to those involved in the differentiation of erythrocytic precursors. The line develops erythrocyte membrane-specific antigen, accumulates heme and hemoglobin, and undergoes morphologic changes similar to normoblastic maturation. In the presence of DMSO, hemoglobin production, as detected by benzidine-staining is not maximal until the 6th day after DMSO administration, but increased ALA synthetase activity can be detected by 28 hours. Enzyme activity is elevated about ten-fold in the presence of 1.8% DMSO for about 4 days and then decreases to the level of non-DMSO-treated cells. There is an optimal level of DMSO at which ALA synthetase induction is maximal at 4 times the control level. Allylisopropylacetamide, an in vivo inducer of ALA synthetase in the liver increases enzyme activity in the control cells by 60% and 24% over the level of activity in DMSO-stimulated cells, suggesting that agents other than DMSO can induce ALA synthetase in this cell line.

Significance to Biomedical Research and the Program of the Institute:

Investigations on the reverse transcriptase in human milk, in conjunction with electron microscopic studies, could provide further evidence to substantiate the viral etiology of human breast cancer.

Studies on ALA synthetase and heme biosynthesis activity in virus-producing tissue culture cells can lead to a better understanding why virus-producing cells have an increased requirement for heme and heme-containing compounds. Investigations of early enzymatic changes during the differentiation of Friend leukemia virus-transformed cells to red cells could lead to a better understanding of the role of virus in the control of differentiation.

Proposed Course:

Two cells which differentiate to red cells in vitro will be further investigated to determine what factors promote or control differentiation. Different inducers of ALA synthetase will be tested to determine what compounds other than DMSO can induce red cell differentiation.

Attempts will be made to determine why tumor virus production stimulates heme production and what heme products or heme containing enzymes are required by the virus-producing cell.

Honors and Awards:

Dr. Ebert, by invitation, participated in the Mammary Tumor Virus Molecular Biology Conference at Cherry Hill, N.J., on December 11, 1972.

Publications:

1. Gerwin, G. I., P. S. Ebert, H. D. Chopra, S. G. Smith, J. P. Kvedar, S. Albert, M. J. Brennan. DNA Polymerase Activities of Human Milk. Science (in press).

Serial No. NCI-4877

1. Viral Biology Branch
   OASDVO, Division of Cancer
   Cause & Prevention
2. Virus & Disease Modification
   Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Modification of normal and neoplastic cells by oncogenic and non-oncogenic viruses.

Previous Serial No.: Same

Principal Investigator: Dr. Wilna A. Woods

Other Investigators: Dr. M. A. Chirigos (NCI-4875)
Dr. J. W. Pearson (NCI-4878)
Dr. D. B. Schwartz (NCI-4879)
Dr. T. S. Papas (NCI-4899)

Cooperating Units: Inside NIH:
Dr. S. Chaparas, FDA

Outside NIH:
Dr. C. A. Bowles, Hazleton Laboratories,
Falls Church, Virginia
Dr. W. Kerber, Hazleton Laboratories,
Falls Church, Virginia
Dr. W. Jergelsky, NIEHS
Dr. G. Spahn, Microbiological Associates, Inc.
Bethesda, Maryland
Dr. R. Simpson, Rutgers University
New Brunswick, New Jersey

Man Years:

Total:	2.0
Professional:	1.0
Other:	1.0

Project Description

Objectives:

1. To investigate intracellular interactions between oncogenic and non-oncogenic viruses *in vitro*.
2. To investigate alteration of antigenic composition of tumor cell

membranes by infection with non-oncogenic viruses.
3. To investigate use of tumor cells with altered membrane antigenic profiles in immunotherapy and prophylactic vaccination.
4. To investigate the effect of chemotherapeutic agents on RNA tumor virus replication in vitro.

Methods Employed:

A. In vitro systems:
1. Cell lines of murine, canine, opossum, and human origin have been established and characterized. These lines can now be modified experimentally for basic immunologic and virologic studies.
2. ^{3}H-uridine incorporation into particles banding at 1.16 g/ml in a sucrose density gradient centrifugation is being used to identify presence of RNA in viral particles and demonstrate the presence of viral particles in cells.
3. Radio-isotope procedures for detecting and characterizing RNA, DNA and protein synthesis are now established on a routine basis.
4. Routine procedures used in this laboratory include virus titrations (focus formation by MSV-M, X-C plaque formation, plaque formation by lytic virus, fluorescent focus assay for MuLV) and antigenic determinations (membrane fluorescence, cytotoxic antibody, fluorescent antibody staining of fixed cells, CF, hemagglutination inhibition, and neutralizing antibody titrations).
B. In vivo systems:
1. AKR and D_2AK mice are used for assay of AKR-A tumorigenicity and effectiveness of immunotherapy procedures.
2. CDF_1 mice are used for assay of the RCS tumor cell line grown in vitro and for immunotherapy experiments on this tumor.
3. BALB/c mice are used in MSV-M studies.
4. Dogs bearing spontaneous tumors in holding at Hazleton Laboratories are being studied in immuno- and chemotherapy experiments.

Major Findings:

1. In vitro inhibitor studies on MSV-M virus and tumor cells have been pursued and data from preliminary experiments submitted for publication. Briefly, the drug streptonigrin has been found to block viral synthesis when added to culture medium during the first few hours after infection and to inhibit colony formation by MSC cells (MSV-M induced tumor cell line). The replication of virus by these chronically infected cells is also decreased by 2 or more logs.
2. Streptonigrin resistant clones of MSC cells and AKRA cells have been established. Work is in progress to determine the tumorigenic characteristics of these cells and the properties of the viruses being produced by them.
3. In collaboration with Dr. D. B. Schwartz, in vitro cytotoxicity studies were pursued to determine the immunological status of BALB/c mice bearing MSV-M induced tumors and treated with BCG. No differences were noted between treated and untreated animals.

4. Viruses and cell lines have been cloned and characterized for studies on the interaction between oncogenic and non-oncogenic viruses. These studies are being carried out in collaboration with Dr. R. Simpson of Rutgers University.
5. ^{3}H-uridine labeling of normal NIH Swiss mouse embryo cells revealed a consistant peak of incorporation into particles banding at a density of 1.16 g/ml in a sucrose density gradient. The properties of these particles are under investigation.
6. We have confirmed the presence of virus-like particles in a mastosarcoma cell line. The particles band at a density of 1.16 g/ml in a sucrose density gradient and incorporate ^{3}H-uridine.
7. In cooperation with Dr. William Jurgelsky of NIEHS, several tumors induced in opossums with chemical carcinogens have been established in vitro. Two tumors of developing tooth buds and 2 nephroblastomas are in culture. The cultured cells were inoculated into opossums of various ages for tumorigenicity studies, and have been submitted for electron microscopic analysis.
8. Various inhibitors of viral reverse transcriptase activity are being tested for their effect on viral replication and on tumor cell colony formation.
9. Dogs bearing spontaneous lymphosarcomas are being treated by combination chemo-immunotherapy at Hazleton Laboratories. The effect of BCG on the immune system is being monitored in vitro using a variety of antigens and mixed lymphocyte reactions.
10. The C-type particles observed by electron microscopy in PK-15 (pig kidney) cells were demonstrated to have biochemical and biophysical properties associated with the oncorna virus group: density of 1.16 g/ml in a sucrose gradient, 70S RNA, and the RNA dependent DNA polymerase. The group specific interspecies antigen, gs-3, was not present. Evidence of a latent infection with a porcine parvovirus was also obtained.

Significance to Biomedical Research and the Program of the Institute:

The study of the interaction between oncogenic virus and non-oncogenic virus may be of importance in determining the "trigger" mechanism for tumor induction in animals (including man) carrying an oncogenic virus genome from conception.

The study of the mechanisms of action of potentially therapeutic drugs on both cell and virus replication has value for screening such agents in vitro before application in vivo.

The study of ^{3}H-uridine incorporation into high molecular weight RNA of malignant cells is of importance for the detection of cells bearing defective viral genomes.

Proposed Course:

1. The significance of ^{3}H-uridine incorporation into high molecular weight RNA of malignant cells of man and other animals will be studied.

2. Studies on the effectiveness of streptonigrin for prevention and interruption of viral and cellular replication will be pursued.
3. Virus-virus interactions involving Gross leukemia virus and Germiston arbovirus, as well as, MSV and Guaroa arbovirus will be investigated.
4. The effect of BCG and other immune stimulators on the host immune defenses against tumor cells will be investigated using in vivo and in vitro monitoring techniques.

Honors and Awards:

None

Publications:

Woods, W. A., Massicot, J., Webb, J., and M. A. Chirigos: Inhibitory Effect of Streptonigrin on a Murine Sarcoma Virus-Induced Tumor Cell Line (MSC) and Selection of Drug Resistant Clones. In Vitro (In Press), 1973.

Chirigos, M. A., Pearson, J. W. Woods, W. A., and Spahn, G.: Immunotherapy and Chemotherapy in Murine Leukemia. Tumor Viruses and Immunity Conference. Academic Press (In Press), 1973.

Woods, W. A., Turner, W., and Chirigos, M. A.: Coinfection of Mouse Spleen Cells with Murine Sarcoma Virus and Guaroa Virus. Applied Microbiology, December, 1972. (In Press)

Bowles, C. A., Kerber, W. T., Rangan, S. R. S., Kwapien, R., Woods, W. A., and Jensen, E. M.: Characterization of a transplantable canine immature mast cell tumor. Cancer Research 32: 1432-1441, 1972.

Bowles, C. A., Hagen, W. Ditmore, J., Kerber, W. T., Woods, W. A., and Jensen, E. M.: Immunofluorescent studies of cultured canine tumor cells. Int. J. Cancer, 10: 28-35, 1972.

Serial No. NCI-4878

1. Viral Biology Branch
   OASDVO, Division of Cancer
   Cause & Prevention
2. Virus & Disease Modification
   Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Modification of Oncogenic Viruses and Disease Induction by Immunological, Biological, Chemical and Physical Methods

Previous Serial Number: Same

Principal Investigator: Dr. John W. Pearson

Other Investigators: Dr. M. A. Chirigos (NCI-4875)
Dr. W. A. Woods (NCI-4877)
Dr. T. A. Papas (NCI-4899)

Cooperating Units: Inside NIH:
Dr. S. Chaparas, FDA
Dr. N. Sher, FDA

Outside NIH:
Dr. G. Spahn, Microbiological Associates, Inc., Bethesda, Maryland

Man Years:

Total:	4.0
Professional:	1.0
Other:	3.0

Project Description

Objectives:

The overall aim of this project is to effectively control virus-induced, spontaneous and transplantable leukemias and lymphomas by several therapeutic approaches. The therapeutic approaches encompass individual or combination drug therapy, immunotherapeutic methods, vaccines, and the application of interferon. Any therapy leading to effective control will be submitted for preclinical testing, evaluation and eventual clinical trial.

Methods Employed:

Mouse Virus and Tumor Animal Systems:
1. The Friend, Rauscher, Moloney leukemogenic viruses, the Moloney sarcoma

virus, and the plasma passage virus varient (MSV-PV) are employed for in vivo studies.
2. Transplantable tumors used are: a Moloney lymphoid leukemia ascites line (LSTRA and MCAS-10), a transplantable tumor line induced by Graffi leukemia virus, a Gross virus transplantable tumor line and a transplantable Lewis Lung carcinoma line.
3. AKR mice, which have a high incidence of spontaneous leukemia are used for in vivo tests.
4. Rat transplantable tumors are presently under investigation and are being carried not only in vivo but also in vitro. These include: (a) a Nova lymphoid leukemia ascites line; (b) a transplantable ascites Gross leukemia line and (c) a Dunning lymphosarcoma transplantable line.

Major Findings:

Chemo-Immunostimulation Therapy: Investigations have been underway utilizing a murine lymphoid leukemia (LSTRA) in which 2 immunostimulators have been used as adjuncts to effective drug therapy in order to achieve complete and long lasting remissions in diseased animals. Subcutaneous inoculation of 1 X 10^4 LSTRA cells results in detectable systemic leukemia 7 days after tumor cell inoculation followed by the development of splenomegaly and lymphadenopathy culminating in death between the 12th and 15th day of post-inoculation. Treatment of leukemic mice (Day 7) with 30 mg/kg of BCNU resulted in a remission period of approximately 9 to 10 days during which, prior to relapse, no systemic disease could be detected. The effect of BCG (Phipps strain) with regards to time, dose and route of administration was studied during the period of drug induced remission. Effective therapy was attained with combined drug and BCG treatment in obtaining a high percentage of long term survivors apparently "cured" of leukemia. This response has been consistently reproduced with 50 to 100% long term survivors as compared to 20-25% survivors obtained in groups treated with drug only. The protective effect afforded by BCG appeared to be independent of a wide range of doses. Injection of doses containing 8 X 10^7 organisms resulting in a significant number of long term survivors. No leukemic cells were observed in any of the tissues histologically examined from the "cured" animals. Likwise, Corynebacterium granulosum (CG) when administered at different doses and regimens following drug therapy against LSTRA resulted in a significant number of long term survivors. The effect of CG appeared to be independent of dose and regimen; 50, 100, 200 or 400 µg's of CG given singly or multiply resulted in 60 to 75% long term survivors as compared to 20 to 25% survivors in the drug treated group alone. Investigations are in progress utilizing chemoimmunostimulation therapy against other virus-induced murine leukemia and tumor systems as well as to determine the mechanism(s) of action of both BCG and CG.

Combination drug therapy studies against the spontaneous leukemia occurring in AKR mice have been in progress. A high percentage of AKR mice came down with leukemia at 7 to 8 months of age.

Hematological parameters as well as spleen and/or node enlargement were utilized to diagnose the disease. Of the various regimens of drug therapy tested for the induction of remission, treatment with 30 mg/kg of prednisone and 0.50 mg/kg of vincristine once a week for 2 weeks appeared to be best. A remission period of approximately 10 to 14 days was obtained with no apparent toxicity. As leukemic mice become available, the efficacy of nonspecific immune stimulators such as BCG, CG, pyran copolymer and other defined chemicals will be tested to determine whether the remission period is increased or whether long term survival with freedom from the disease will follow drug therapy.

A spontaneous Nova rat leukemia (NRL) has been utilized as a model system for testing the efficacy of chemoimmunostimulation therapy. Histologically, the disease is a lymphoblastic leukemia with virtually all organs densely infiltrated by leukemic cells. Electron microscopic observation of diseased tissues has failed to show any "C" type virus particles. Subcutaneous inoculation of approximately 1.0 X 10^5 NRL tumor cells results in the death of all animals within 19 to 25 days. Treatment of tumor bearing rats (12-15 mm's) with 60 mg/kg of cytoxan produced a complete tumor remission period for approximately 11 days before recurrence of the tumor and subsequent death of all animals. The administration of BCG or CG at different time intervals during the period of tumor remission failed to be any more effective than groups of animals treated with drug alone. Preliminary immunological investigations have failed to identify the presence of a tumor-associated antigen on the Nova tumor cells. Based on these findings involving the NRL, it is our belief that in order for immunostimulation therapy to be advantageous to the host there must first exist an immune response against antigen(s) on the tumor. Studies are now in progress to attempt to augment the host immune response to the Nova tumor if indeed a foreign antigen does exist.

A transplantable virus-associated Gross virus-induced leukemia has been maintained in WF/u rats. This leukemia kills animals in approximately 12 to 15 days with all diseased rats exhibiting splenomegaly as well as lymphadenopathy. At present, we have been unable to effectively control this disease with drug therapy. However, a regimen of therapy using prednisone, cytoxan and vincristine over a period of a week gives a remission period of 5 to 6 days with no apparent toxicity. Studies are currently underway to increase the period of remission. Once a satisfactory remission period has been obtained a variety of nonspecific immune stimulators will be tested in hopes of extending the period of remission following drug therapy.

Significance to Biomedical Research and the Program of the Institute:

These studies are directed toward the prevention, treatment and control of virus-induced or spontaneous leukemias, lymphomas, and sarcomas in animal systems. Comparisons made by the use of drugs, alone and in combination with immunostimulators, immunotherapy and vaccines may elucidate factors improving the effectiveness of therapeutic measures.

Proposed Course:

Research in each of the areas described under objectives will continue. More emphasis will be placed in maintaining prolonged periods of remission following chemotherapy. The use of immunostimulators alone and in combination with syngeneic and allogeneic vaccines will be investigated in both mouse and rat leukemia and sarcomas following drug therapy. Similar approaches will be attempted against the spontaneous AKR leukemia. It is hoped that information gained from these investigations may be applied toward the control of human cancer.

Honors and Awards:

None

Publications:

Pearson, J. W., Pearson, G. R., Gibson, W. T., Chermann, J. C., and Chirigos, M.A.: Chemo-Immunostimulation Therapy Against a Murine Leukemia Cancer Research 32: 904-907, 1972.

Pearson, J. W., Chaparas, S. D., and M. A. Chirigos: Effect of Dose and Route of BCG Inoculation in Chemoimmunstimulation Therapy of a Mouse Leukemia. Cancer Research (In Press), 1973.

Pearson, G.R., Redmon, L. W., and Pearson, J. W.: Sero-chemotherapy Against a Moloney Virus Induced Leukemia. Cancer Research (In Press), 1973.

Chirigos, M. A., Pearson, J. W., Woods, W. A., and Spahn, G.: Immunotherapy and Chemotherapy in Murine Leukemia. Tumor Viruses and Immunity Conference. Academic Press (In Press), 1972.

Chirigos, M. A., Pearson, J. W., Spahn, G., and Rutman, R.: Current Studies on Oncorna Virus Therapy. Bibliotheca Haematologica, Vol. 39, Vth International Symposium on Comparative Leukemia Research (In Press), 1972.

Serial No. NCI-4880

1. Viral Biology Branch
   OASDVO, Division of Cancer
   Cause and Prevention
2. Experimental Pathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Immunological and Virological Studies on Human and Animal Tumors

Previous Serial Number: Same

Principal Investigator: Dr. Gary R. Pearson

Other Investigators: Inside NIH:
Dr. Dharam Ablashi, Viral Leukemia and Lymphoma Branch, NCI (NCI-4834)
Dr. John Pearson, Viral Biology Branch, NCI (NCI-4878)
Dr. Charles Boone, Viral Biology Branch, NCI (NCI-4883)
Dr. Paul Ebert, Viral Biology Branch, NCI (NCI-4876)
Dr. Alan Rabson, Laboratory of Pathology, NCI

Outside NIH:
Dr. Harvey Rabin, Litton Bionetics Research Laboratories, Kensington, Maryland
Dr. George Klein, Karolinska Institute, Stockholm, Sweden
Dr. Friedrich Deinhardt, Rush-Presbyterian-St. Luke's Medical Center, Chicago, Illinois

Man Years:

Total:	3.5
Professional:	1.0
Other:	2.5

Project Description

Objectives:

1. To investigate the use of well-characterized immune serum in transferring immunity to virus-induced tumor transplants and to attempt to identify the active elements in serum capable of conferring resistance.

2. To investigate the immune responses of non-human primates to Herpesviruses saimiri infection as an immunological model for Epstein-Barr virus infection in man.

3. To collaborate with other scientists at NCI on problems of mutual interest.

Methods Employed:

1. Mice were immunized with MSV or allogeneic tumor cells producing virus. At appropriate times following the immunizations, the mice were bled and the sera pooled into different groups depending on the method of immunization. The sera were heat-inactivated and then tested for antibodies directed against MSV-associated antigens by membrane immunofluorescence, neutralization of the XC reaction and cytotoxicity. Recipient mice were then inoculated intraperitoneally with 0.2 ml of a test serum. The sera were tested alone against a transplantable MSV-induced sarcoma and a MLV-induced lymphoma (LSTRA) or in combination with drug-therapy when tested against established disease. The mice were monitored twice weekly for the presence of tumor, size and death.

2. Sera were collected from marmosets, owl monkeys and squirrel monkeys at different times following inoculation with HVS. The sera were heat inactivated and then tested for antibodies directed against HVS-induced cell membrane antigens (MA), HVS-induced late antigens (LA) and HVS-associated early antigens (EA) by immunofluorescence assays. Target cells for detecting MA were viable Vero, owl monkey kidney or lymphoid cells infected with HVS. Acetone-fixed smears of HVS infected Vero or owl monkey kidney cells were used as targets for demonstrating antibodies to LA. Acetone-fixed smears of HVS-infected Vero cells grown in the presence of 20 ug/ml cytosine arabinoside served as the source of antigen for EA antibody determinations. All immunofluorescence assays were performed by the indirect method using fluorescein isothiocyanate-conjugated goat anti-human gamma globulin.

Major Findings:

1. Studies were continued investigating the effectiveness of immune sera in preventing the outgrowth of MSV and MLV-induced tumor transplants and in the treatment of mice with systemic MLV-induced leukemia either alone or in combination with chemotherapy. The serum donors were (1) BALB/c mice bearing progressively growing MSV-induced tumors (referred to as progressor serum). (2) BALB/c mice following regression of MSV-induced tumors (regressor serum); and (3) CDF_1 mice immunized with allogeneic tumor cells (C57BL origin) induced by MSV (allogeneic serum). The regressor and allogeneic sera contained high antibody titers as determined by the membrane immunofluorescence (MF) and neutralization assays with pools of allogeneic serum consistently containing the highest titers by both assays. The progressor serum contained low titers as determined in these two tests. None of the sera contained antibody cytotoxic against three different

Moloney virus-induced tumor cell lines. The results demonstrated that the effectiveness of these sera in preventing the outgrowth of MSV or MLV-induced tumor transplants was directly related to the antibody concentration in the sera as determined by MF and neutralization. The allogeneic serum preparation was the most effective of the three preparations. Preinoculation of progressor serum before cell challenge resulted in tumor growth enhancement. The allogeneic serum was also effective when inoculated into mice with systemic MLV-induced leukemia when given either alone or in combination with chemotherapy. Following chemotherapy, inoculation of the allogeneic serum produced up to 95% survivors when experiments were terminated after 100 days as opposed to approximately 30% in the group treated with drug alone.

2. Immunofluorescence antigens similar to those produced in EBV-infected cells were identified in cells infected with Herpesvirus saimiri (HVS). These include membrane antigens (MA), late antigens (LA) probably identical to viral capsid antigens, and early antigens (EA). MA and LA are produced late in the infection cycle and their expression is inhibited by cytosine arabinoside. In contrast EA is produced early in the infection cycle and in the presence of cytosine arabinoside. Two patterns of nuclear fluorescence have been identified with this antigen. One is referred to as "trabecular", the other as "punctate". It is still not known whether these patterns represent qualitative or quantitative differences. These patterns are in contrast to the LA fluorescence which is diffuse and mainly cytoplasmic. Sera were identified with antibodies to MA and LA but not EA while other sera contained antibody against all three antigens. These sera were from marmosets, owl monkeys, and squirrel monkeys infected experimentally or naturally with HVS. The results from titrations of serial serum samples taken from animals of all three species at different times after HVS infection indicate that the production of antibodies against EA is disease-related. In contrast to the antibody response to MA and LA which has occurred in all marmosets and owl monkeys studies so far, regardless of the presence or absence of neoplastic disease, antibodies to EA have only been demonstrated in the sera of animals with evidence of lymphoproliferation. The sera of adult squirrel monkeys contained antibody to LA and MA but not EA. However, following experimental infection of squirrel monkeys with HVS, a transient EA response was noted. These preliminary findings indicate that the induction of antibodies to EA may be related to the initiation of lymphoproliferation in the infected animals. Striking parallels have been noted so far in the immunological responses to these antigens induced by an oncogenic primate virus and to those reported in humans with EBV-associated diseases.

3. Cell lines established in this laboratory and sera from a variety of sources have been provided to investigators within and outside NIH.

Significance to Biomedical Research and the Program of the Institute:

1. Investigations on the possible use of immunological factors for controlling tumor growth are of obvious importance to the Program. Recent evidence from the Hellstrom Laboratory indicates that sera containing de-blocking factors can induce tumor regressions. It is important to determine not only what is the nature of the active factor in these sera but also what other tests can be utilized for selecting sera for potential use in therapy.

2. Indirect evidence has accumulated associating EB virus with Burkitt's lymphoma. However, direct evidence is lacking. Studies in a model system involving an oncogenic herpesvirus may provide important information on the role of herpesviruses in the induction of human lymphomas.

Proposed Course:

1. Studies on the use of well-characterized immune serum in the treatment of animal tumors will be continued. Sera will be prepared against tumors induced by other viruses and by chemicals, and the effectiveness of these sera in preventing tumor growth will be evaluated. Efforts will be initiated to fractionate sera and to identify the fractions responsible for transferring immunity.

2. Immunological studies on Herpesvirus saimiri oncogenesis in non-human primates as a model for EBV infection in man will continue. These studies will include (a) the continued investigation on the possible diagnostic or prognostic value of the antibody response to EA; (b) studies on the cellular immune response to HVS-induced antigens and attempts to define the role this response plays in the pathogenesis of HVS-induced disease; (c) determination of the effectiveness of sera with high levels of anti-MA antibodies, immune cells or extracts of lymphoid cells in the control of HVS infection.

3. Collaborative research with other investigators within and outside NIH on problems of mutual interest will continue.

Other Activities:

Dr. Pearson is Executive Secretary of the Immunology-Epidemiology Segment, Project Officer on five contracts and Assistant Project Officer on five contracts within the Special Virus Cancer Program. He is also a member of the Resources and Logistics Advisory Committee and TASK Monitor for TASK 2 of the Frederick Cancer Research Center contract.

Honors and Awards:

None.

Publications:

Bergs, V. V., Pearson, G., Chopra, H. C., and Turner, W.: Spontaneous appearance of cytopathology and rat C-type virus (WF-1) in a rat embryo cell line. Int. J. Cancer 10: 165-173, 1972.

Pearson, G., Orr, T., Redmon, L., and Bergs, V.: Membrane immunofluorescence studies on cells producing rat C-type virus particles. Int. J. Cancer 10: 14-19, 1972.

Pearson, J. W., Pearson, G. R., Gibson, W. T., Chermann, J. C., and Chirigos, M. A.: Combined chemoimmunostimulation therapy against murine leukemia. Cancer Res. 32: 904-907, 1972.

Pearson, G., Ablashi, D., Orr, T., Rabin, H., and Armstrong, G.: Cytoplasmic and membrane immunofluorescence investigations on cells infected with Herpesvirus saimiri. J. Nat. Cancer Inst. 49: 1417-1424, 1972.

Pearson, G. R., Redmon, L., and Bass, L.: Protective effect of immune sera against transplantable Moloney virus-induced sarcoma and lymphoma. Cancer Res. 33: 171-178, 1973.

Rabin, H., Pearson, G., Klein, G., Ablashi, D., Waller, W., and Cicmanec. Herpesvirus saimiri antigens and virus recovery from cultured cells and antibody levels and virus isolations from squirrel monkeys. Proc. 4th International Congress of Primatology, In press, 1972.

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Ablashi, D. V., Loeb, W. F., Pearson, G., Valerio, M. R., Armstrong, G. R., Rabin, H., Kingsbury, E. W., and Heine, U.: Induction of lymphoma in owl monkeys with heated, non-cytopathogenic Herpesvirus saimiri. Nature, In press, 1973.

Serial No. NCI-4883
1. Viral Biology Branch
OASDVO, Division of Cancer
Cause and Prevention
2. Cell Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Immunity to virus-induced tumors and cinematographic analysis of malignant cell in vitro

Previous Serial Number: Same

Principal Investigator: Dr. Charles W. Boone

Other Investigators: None

Cooperating Units: None

Man Years:

Total:	2.5
Professional:	1.5
Other:	1.0

Project Description

Objectives:

1. To evaluate various methods of immunotherapy in animal model systems.

2. To determine mechanisms of the cell-mediated immune response to virus induced, chemically induced, and spontaneous tumors.

3. To isolate and characterize tumor transplantation antigens and tumor seroantigens of potential application to the immunodiagnosis and immunotherapy of human cancer.

4. To compare density inhibition of motility in a variety of tumorigenic and non-tumorigenic cell lines.

5. To explore the relationship between cell motility and cell division.

Methods Employed:

1. thru 5. Animal models of immunotherapy, tumor-graft rejection assays in animals, delayed hypersensitivity assays for tumor associated antigens,

chromatographic and biochemical methods for making purified tumor transplantation antigen, immune cell reconstitution of thymectimized and x-irradiated mice, and time lapse cinematography were techniques applied in these studies.

Major Findings:

The immunogenicity of weak tumor cell antigens (TTA) had been shown to be substantially increased by infection of tumor cells with influenza virus. The Cell Biology Section has now demonstrated that these tumor transplantation antigens can be prepared in cell-free form from tumor cells infected with the WSA strain of influenza virus. The cell-free "flu-TTA" conferred protection to mice against challenge in tumor transplant rejection assays, causing suppression, and in some cases complete regression, of proliferating tumor cells. Vesicular stomatitis virus, and the Hong Kong strain of influenza virus, were also shown to have a TTA augmenting effect.

The area density and binding affinity (ΔF°) of cell surface antigens were measured in a Gross virus induced rat lymphoma, a Moloney virus-induced mouse ascites lymphoma, a virus-induced cat leukemia, and endogenous feline C-type virus infected human tumor cells (RD114 line). Work was completed showing increased exposure of Gross virus-induced cell surface antigens after treatment of viable lymphoma cells with trypsin.

Various antigens induced in human lymphoid cells by Epstein-Barr virus (EBV) were located in cell fractions obtained by sucrose density gradient centrifugation with radioiodinated antibodies. Plasma membrane associated histocompatibility antigen (HL-A) and xenoantigens were also located by the same procedure. The EBV-induced membrane antigens appeared to be loosely rather than covalently integrated into the plasma membrane of EBV-infected cells.

Viral transformants of 3T3 fibroblasts, spontaneous transformants, and chemically induced fibrosarcoma explants all showed markedly reduced density inhibition of motility compared to 3T3 fibroblasts. The degree of the diminution was not well correlated with tumorigenicity. Colcemide decreased fibroblast motility, probably by causing the cell to lose it polarity and so decreasing the directional persistance with which it moves. Cytochalasin reversibly inhibited fibroblast motility and did so at doses ten-fold less than those required to suppress cell division. Procaine hydrochloride reversibly inhibited cell motility and cell division in pharmacologic doses, suggesting that cell motility depends, in part, on ionic permeabilities of the cell membrane.

Significance to Biomedical Research and the Program of the Institute:

The finding that the immunogenicity of tumor cell homogenates can be enhanced by prior infection with influenza virus, and that treatment of growing tumors with "flu-TTA" preparations results in tumor growth suppression and regression, has obvious implications for the immunotherapy of human tumors.

The isolation and characterization of tumor associated surface antigens is important in understanding the carcinogenic process and in the development of immunological methods for the diagnosis and treatment of cancer.

Cinematographic analysis can define the phenotypic attributes of cells that correlate with the malignant state. A better definition of these attributes, e.g., contact inhibition of locomotion, overlapping, and mitosis, and the relationship between substrate adhesivity and malignancy, is much needed in cancer research.

Proposed Course:

The mechanism of the influenza virus-mediated adjuvant effect on tumor transplantation antigens will be intensively studied in the coming year. The isolation and characterization of the Gross virus-induced cell surface antigen will be pursued. Attempts will be made to isolate membrane antigen of non-virogenic lymphoid cells (Raji cells) acutely infected with EB-virus.

Honor and Awards:

None

Publications:

Gail, M.H. and Boone, C.W.: Procaine inhibition of fibroblast motility and proliferation. Exp. Cell Res. 73:252-255, 1973.

Boone, C.W.: Augmented immunogenicity of tumor cell homogenates produced by infection with influenza virus. J. Natl. Cancer Inst. Monogr. 35: 301-307, 1972.

Boone, C.W., Gordon, F. and Kawakami, T.: Surface antigens on cat leukemia cells induced by feline leukemia virus (FeLV): Area density and antibody-binding affinity. J. Virol. (in press).

Boone, C.W., Gerber, P., and Brandchaft, P.: Isolation of plasma membrane antigen from Epstein-Barr virus (EBV)-infected lymphoid cells. J. Natl. Cancer Inst. (in press).

Gillette, R., Boone, C.W., and Swanson, M.H.: Unique homing properties of non-adherant peritoneal cells. Cellular Immunol. (in press).

Gillette, R., Boone, C.W.: Changes in PHA response due to presence of tumors. J. Natl. Cancer Inst. (in press).

Gail, M.H. and Boone, C.W.: Calcium requirement for fibroblast motility and proliferation. Exp. Cell Res. (in press).

Serial No. NCI-4888
1. Viral Biology Branch
OASDVO, Division of Cancer
Cause and Prevention
2. Office of the Chief, VBB
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Molecular Aspects of Viral Oncology.

Previous Serial Number: Same

Principal Investigator: Dr. Timothy E. O'Connor

Other Investigators: Dr. Emerson Chan (NCI-4894)

Cooperating Units: None

Man Years:

Total:	0.3
Professional:	0.3
Others:	0.0

Project Description

Objectives:

To study the biology of known animal sarcoma-leukemia inducing virus complexes and the application of the findings in attempts at isolation of similar human viruses.

Methods Employed:

Methods include the propagation of sarcoma-leukemia virus complexes in mammalian cell cultures and study of the quantitative aspects of interaction of these viruses with susceptible cells.

Major Findings:

1. Animal virus studies: In collaboration with Dr. Emerson Chan, the Snyder-Theilen feline sarcoma-inducing virus had been successfully propagated with cell transformation, in feline, bovine and human cell cultures. Infection of these cells with dilutions of the virus stock beyond the cell-transforming dilution induced resistance to transforming superinfection of the cultures with potent inocula of ST-FSV. These resistant cultures also

shed a non-transforming virus capable of inducing this resistance in fresh cell cultures. These findings indicated that Snyder-Theilen virus stocks contain a mixture of a cell transforming virus and a considerable excess of a non-transforming virus.

Infection of feline embryonic lung cells (FEL) with ST-FSV gave foci of degenerating cells, while infection of bovine embryo trachea cells (BET) gave foci of proliferative cells, permitting virus titration. Stocks of ST-FSV propagated in either feline or BET cells gave "single-hit" focus titration patterns when assayed on the homologous uninfected cells. However, infection of BET cells with stocks of ST-FSV harvested from infected FEL cells gave focus titration patterns indicative of defective sarcoma virus-cell interaction. The focus titration was restored to the apparent "one-hit" type when the cells were simultaneously infected with stocks of the non-transforming virus harvested from infected BET. Foci of transformed cells obtained by infecting BET cells with terminal dilutions of the ST-FSV stock, having defective titration, were propagated and examined for virus yield. Most such foci shed transforming ST-FSV. One focus, however, was propagated to mass cultures which did not shed virus detectable either by cell transformation or by RNA dependent DNA polymerase assays. This transformed cell culture shed a mixture of transforming and non-transforming virus on superinfection with the non-transforming Snyder-Theilen sarcoma virus. These findings indicate that the Snyder-Theilen sarcoma virus, like other presently known mammalian sarcoma viruses is defective in its transforming capacity but that this defectiveness is masked in most infections of cell cultures by an excess of the non-transforming oncornavirus present.

In the course of experiments aimed at recovery of a sarcoma virus from the non-virus-shedding transformed BET cells, the observation was made that the uninfected BET cultures could shed an oncorna-like virus under certain conditions. The nature of this virus is being further examined.

2. Human Virus Candidate Studies: Nine different human sarcoma cell lines were examined for virogenic properties. One of these cell lines was found capable of shedding an oncornavirus under appropriate conditions. The virus has C-type morphology, contains a 70S RNA and DNA polymerase and can infect human, non-human primate, and bovine cells. In some instances, the infection is accompanied by an apparent cellular morphological transformation to a rapidly proliferating cell. The possible human origin of this virus is now being examined by a combination of molecular and serological methods.

Significance to Biomedical Research and the Program of the Institute:

The findings in virology and molecular biology from many laboratories suggest that oncornaviruses may be etiologically involved in human leukemias and sarcomas as they are in animal leukemias and sarcomas. Viruses of the RNA C-type of supposed human origin isolated to date have been found to originate in other species. The unequivocal demonstration of the human origin of a

C-type RNA virus would permit the direct application of virological procedures to the examination of human sarcomas and leukemias. The species of origin and the characteristics of the virus, isolated as described above from an apparently human sarcoma cell culture, require careful examination in the light of these possibilities.

Proposed Course:

Propagation of the C-type RNA virus isolated from a human sarcoma cell line is now being expanded to provide sufficient virus to unequivocally determine species of origin.

Other Activities:

Serves as Coordinator for Molecular Control Program, NCI

Honors and Awards: None

Publications: None

Serial No. NCI-4890

1. Viral Biology Branch
   OASDVO, Division of Cancer
   Cause and Prevention
2. Cell Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Immunochemical and Biochemical Analysis of Cell Lines Derived from Methyl-cholanthrene-Induced Murine Fibrosarcomas

Previous Serial Number: Same

Principal Investigator: Dr. Thomas W. Orme

Other Investigators: Dr. Charles W. Boone (NCI-4883)

Cooperating Units: None

Man Years:

Total:	2.1
Professional:	1.1
Other:	1.0

Project Description

Objectives:

1. To measure quantitatively and to chemically characterize tumor specific cell surface antigens (TSCSA) on murine tumor cells.

2. To define the components of TSCSA responsible for tumor transplant rejection in syngeneic tumor-immune mice.

3. To investigate the relationship between TSCSA associated with virus transformation and TSCSA induced by a carcinogen.

Methods Employed:

Tumor transplantation rejection assays and delayed hypersensitivity assay; carbohydrate analysis by gas liquid chromatography and paper chromatography; analysis of solubilized TSCSA by gel permeation chromatography, ion exchange chromatography, electrophoresis affinity chromatography, and preparative differential centrifugation.

Major Findings:

Immunogenic cell surface membranes containing TSCSA have been prepared by sucrose density gradient centrifugation. TSCSA has been solubilized by KCl extraction and shown to inhibit the binding of anti-TSCSA to tumor cells. The partial purification of soluble TSCSA by gel permeation at high KCl concentrations has been achieved.

Carbohydrate chemotyping of six different methylcholanthrene induced fibrosarcomas has revealed the presence of an unusual monosaccharide tentatively identified as a dideoxyhexosamine.

Significance to Biomedical Research and the Program of the Institute:

The development of methods and reagents for the analysis of cell surface antigens is fundamental to improved immunodiagnosis and immunotherapy of cancer.

Proposed Course:

Work on methylcholanthrene-induced fibrosarcoma antigens will continue as outlined. Studies are also being initiated to characterize the cell surface antigens on Gross virus-infected AKR cells, Moloney virus infected lymphoma cells and Herpes virus type 2 infected Vero cells.

Honors and Awards: None

Publications: None

Serial No. NCI-4892
1. Viral Biology Branch
ASDVO, DCCP
2. Cell Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Mechanisms of the Cellular Immune Response to Tumors

Previous Serial Number: Same

Principal Investigator: Dr. Meera Paranjpe

Other Investigators: Dr. Charles W. Boone (NCI-4883)

Cooperating Units: None

Man Years:

Total:	1.1
Professional:	1.1
Other:	0.0

Project Description

Objectives:

To study mechanisms of the cell-mediated immune response of mice to syngeneic tumors.

Methods Employed:

A radioisotopic foot pad assay for delayed hypersensitivity in the mouse, developed in this laboratory; homing distributions of radioisotopically-labeled tumor immune spleen cells, and immunofluorescence microscopy.

Major Findings.

Specific paralysis of the anti-tumor immune response in the tumor-bearing animal was documented using a recently developed radio-isotopic foot pad assay for delayed hypersensitivity in the mouse. The entire time course of the delayed hypersensitivity (DH) response during tumor growth was analysized and a specific and profound suppression of the DH response was observed after tumors reached a certain size. Lymphoid cells from an immunologically suppressed tumor-bearing animal were still active in adoptive transfer of immunity to normal animals, indicating the existence an immunological blocking mechanism. The presence of a substance in necrotic tumors which specifically suppresses immune response was

established.

Enhancement, rather than suppression, of tumor growth was found to occur when tuberculin extract was admixed with tumor cells and injected into tuberculin sensitized mice. The tumor enhancement occurred with both methylcholanthrene induced fibrosarcomas and SV40 transformed fibrosarcomas. This enhancement phenomenon produced by a nonspecific stimulant of the immune system does not support the "innocent bystander hypothesis" sometimes given in explanation of the suppression of tumor growth with agents like BCG.

Significance to Biomedical Research and the Program of the Institute:

Analyzing the cell-mediated immune response to tumors provides leads to ways of manipulating the immune response to the advantage of the cancer patient.

Proposed Course:

The development of tolerance in the tumor bearing animal and methods for abrogating it, will be studied. Cooperation between different cell types required for the delayed hypersensitivity reaction to tumor cells will be analyzed.

Honors and Awards:

None

Publications:

None

Serial No. NCI-4893

1. Viral Biology Branch
   OASDVO, Division of Cancer
   Cause and Prevention
2. Human Tumor Studies Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: The Isolation and Biochemical-Biological Characterization of C-type Particles from Human and Bovine Cell Lines.

Previous Serial Number: None

Principal Investigator: Dr. George F. Vande Woude

Other Investigators: Dr. Richard Ascione (NCI-4902)
Dr. William G. Robey (NCI-4895)

Cooperating Units: Inside NIH
Dr. Angelo P. Andrese VBB (NCI-4897)
Dr. Timothy O'Connor VBB (NCI-4888)

Outside NIH
Dr. Emerson Chan, Columbia University

Man Years:

Total:	2.6
Professional:	0.7
Other:	1.9

Project Description

Objectives:

To investigate several C-type oncornavirus isolates rescued from a human cell culture and an established bovine cell line. To characterize the biochemical, biological and immunological properties of these particles and to determine their etiology as disease agents.

Methods Employed:

A number of cell lines of human and bovine origin were used in these studies. Virus rescue from established cell lines was attempted by treating cells with iododeoxyuridine (IUdR). Isolates were characterized by determination of their reverse transcriptase (RT) activity using exogenous synthetic and

endogenous templates; by ultracentrifugation in either sucrose or glycerol gradients and by electron microscopy.

Major Findings:

Studies initiated by Dr. Emerson Chan (Serial No. NCI-4894) suggested that virus production was activated by treatment of an established bovine cell line with IUdR. Investigations revealed that the virus particles produced had a density of 1.16 g/ml in sucrose gradients and contained RT activity.

A human tumor cell culture was similarly treated with IUdR. Upon examination of cell culture fluids by electron microscopy, virus particles with C-type morphology were detected. Production of virus is not sustained and only a few particles (about 10^4 per ml) are obtained following activation. The agent bands at a density of 1.16 g/ml in sucrose gradients and contains RT. In another laboratory, the virus was shown to be infectious for normal whole human embryo cells and for human embryonic bone marrow cells, which respond by production of proliferating foci of morphologically altered cells. However, neither culture produced significant amounts of virus as determined by RT activity in culture fluids. Attempts are being made to obtain a cell system which will produce the quantities of virus required for immunological and biochemical characterization. A unique property of the virus is its capability to induce a total cytopathogenic effect in some non-human cell lines and to inhibit the production of C-type viruses in chronically infected, virus-shedding bovine cell cultures. No cytopathogenicity is produced in the parent human cell culture. Mouse fibroblasts pre-infected by the human cell virus isolate do not produce C-type virus when superinfected by Rauscher murine leukemia virus. This inhibitory activity may make it possible to acquire interesting data on the regulation of virus gene expression while its cytopathogenicity for some chronically infected cell lines should provide a system for biological assay.

Significance to Biomedical Research and the Program of the Institute:

The identification of a human C-type particle as an etiological agent of cancer is of prime importance to the goals of the SVCP and NCI. The further characterization of the biochemical, biological and immunological properties of this agent may provide insight in approaches to cancer control or prevention.

Proposed Course:

A cell line capable of producing the C-type virus from human tumor cells in large quantities currently occupies the highest priority. Once a suitable cell line has been identified, preparation of virus on a large scale will be implemented at the Frederick Cancer Control Center. Investigations will be continued on the nature of the inhibition of heterologous C-type virus replication by pre- or post infection with the IUdR-rescued human isolate.

This will perhaps reveal an additional property of the sarcoma-leukemia virus complex, in addition to providing a cell system for studying the means for destroying proliferating cells containing sarcoma-leukemia viruses.

Honors and Awards: None

Publications: None

Serial No. NCI-4894

1. Viral Biology Branch
OASDVO, Division of Cancer
Cause and Prevention
2. Human Tumor Studies Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Studies on DNA (Herpesvirus saimiri) and RNA (feline sarcoma) tumor viruses.

Previous Serial Number: Same

Principal Investigator: Dr. Emerson Chan

Other Investigators: Dr. Timothy O'Connor (NCI-4888)

Cooperating Units: None

Man Years:

Total:	0.4
Professional:	0.2
Other:	0.2

Project Description

Objectives:

To complete studies under way on known animal sarcoma-leukemia inducing virus complexes and to apply these findings towards the isolation of similar human viruses.

Methods Employed:

Sarcoma-leukemia virus complexes were propagated in selected mammalian cell cultures by established methods.

Major Findings:

Assay for reverse transcriptase (RT) activity as an indicator of virus production was applied to laboratory cultures of normal bovine and of human tumor origin. The results indicated the presence of low enzyme activity in culture fluids after treatment of both bovine and human cells with halogenated uridine, suggesting the release of viruses of the RNA C-type. The release of C-type virus from the human culture was verified after the principal investigator left NCI to work at another laboratory.

Significance to Biomedical Research and the Program of the Institute:

These observations were the result of previous work under this project. The results obtained in both bovine and human cells require additional study to determine the identity of the agents detected.

Proposed Course:

The principal investigator is continuing his studies on the human culture at another laboratory.

Honors and Awards: None

Publications: None

Serial No. NCI-4895

1. Viral Biology Branch
OASDVO, Division of Cancer
Cause and Prevention
2. Human Tumor Studies Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Biochemical and Immunological Characterization of Oncornaviruses-specific proteins.

Previous Serial Number: None

Principal Investigator: Dr. William G. Robey

Other Investigators: Dr. George F. Vande Woude (NCI-4893)
Dr. Richard Ascione (NCI-4902)

Cooperating Units: Inside NIH
Dr. Angelo P. Andrese VBB (NCI-4897)

Man Years:

Total:	1.5
Professional:	0.8
Other:	0.7

Project Description

Objectives:

To develop biochemical methods to identify, isolate and characterize both intracellular and cell-free oncornaviruses, their subparticles, as well as capsid and noncapsid protein moieties. These methods will serve as a tool for examining the molecular events associated with viral replication.

Methods Employed:

Propagation of oncornaviruses and focus-helper assays for the detection of propagated and purified viruses were conducted in cell cultures. Other assays used were: Agarose gel double-diffusion for the detection of viral antigens; electron microscopy for the quantitation and detection of viruses and their subparticles; polyacrylamide gel electrophoresis and guanidine HCl-agarose chromatography for the purification and identification of virus-specific proteins; DNA (host cell derived) cellulose chromatography of virus specified proteins; in vitro labeling of purified viruses and their subparticles (e.g. cores) using acylating reagents specific for ε-amino

lysine and N-Terminal amino acids; scintillation spectroscopy and autoradiography.

Major Findings:

Examinations of a number of highly purified C-type RNA viruses have indicated the presence of more than forty polypeptides in each preparation ranging in molecular weight from several hundred thousand down to several thousand. Relative to the few major components, the majority are present in minute quantities. The method described below has been developed so that these minor components, labeled in vitro, may be purified by conventional techniques.

Purified avian myeloblastosis virus (AMV) has been labeled with ^{14}C-maleic anhydride (MA) to greater than 10^6 CPM/mg of virus protein. Under the conditions of labeling, both viral integrity and morphology are maintained and there are no obvious changes in the SDS gel electrophoretic mobilities of virion capsid polypeptides due to the addition of maleyl residues. Moreover, the antigenic integrity of the virion polypeptides is preserved as determined by agarose-gel double diffusion against porcine antiserum to detergent-ether treated virus. The sensitivity of this immunological assay has been increased several orders of magnitude by the application of autoradiographic techniques. Also using this method it has been possible to detect preferential labeling of specific virion capsid polypeptides when either intact virus, disrupted virus or core (nucleoid) preparations are used as substrates for acylation. By this procedure, insight into arrangement of proteins in the molecular conformation of the virus can be achieved. This labeling method has been particularly useful for comparing the ^{35}S methionine labeled protein products of cell-free AMV-RNA directed translation with the purified ^{14}C MA labeled proteins of AMV.

Significance to Biomedical Research and the Program of the Institute:

These studies have been undertaken to develop highly sensitive methods to identify small quantities of human tumor viruses and their subparticles in order to further characterize their biochemical and immunological properties as well as for the determination of their role in viral replication.

Proposed Course:

These studies have just been initiated. Studies similar to those described with AMV will be carried out with JLSV-9 cells producing Rauscher murine leukemia virus. In the latter case, the biological activity of the virus will be assayed. The method will have wide application for the purification of immunogenic amounts of virus specific components where limited amounts of virus polypeptides are present.

Honors and Awards: None

Publications: None

Serial No. NCI-4896
1. Viral Biology Branch
OASDVO, Division of Cancer
Cause and Prevention
2. Cell Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Analysis of Gene Controlled Events in Neoplastic Transformation

Previous Serial Number: None

Principal Investigator: Dr. Stephen J. O'Brien

Other Investigators: Dr. Charles W. Boone (NCI-4883)

Cooperating Units: None

Man Years: 1.0

Project Description

Objectives:

Part A - Examine regulation of viral induced surface antigens of feline leukemia.

1. Characterize surface antigens biochemically.

2. Determine mode and kinetics of biosynthesis, e.g. protein activation, stimulated transcription or translation in systems perturbed by inhibitors of various stages of protein synthesis.

3. Determine structural gene locus of antigens:
   a. viral or host origins
   b. locus of gene itself within the genome

4. Characterize nature of genetic regulation of antigen expression.

Part B - Investigate extent and regulation of anomalous gene action following transformation.

1. Examine over 20 randomly selected genes for disfunction in transformed tissue cell lines. These tissues will include 20 chemically transformed lines and a number of virus transformed lines. Furthermore, the same survey

will be performed on a group of these lines following animal passage.

2. Investigate the genetic regulation of aberrant gene products:
   a. mapping structural genes
   b. determine regulation of gene disfunction

3. Determine the linkage or association of malignancy with anomalous phenotypes.

Methods Employed:

1. Differential extraction of surface antigens from cells in vitro by enzymatic digestion and ionic effects without disturbing protein synthetic machinery.

2. Gel electrophoresis (starch, acrylamide and cellulose acetate), and isozyme development.

3. Acrylamide gel isoelectric focusing.

4. Immunological detection of anamolous antigens.

5. Somatic cell hybridization mediated by exogenous Sendai virus.

6. Selective schemes for hybrid formation and allozyme monitoring for segregation analysis.

7. Autoradiographic and cytological techniques.

Major Findings:

1. Feline leukemia cell surface antigen has been fractionated into at least three distinct components on the basis of sensitivity to proteolytic enzymatic extraction.

2. The half life of surface antigen and its messenger RNA transcript have been determined from inhibition curves with cycloheximide and actinomycin D respectively. The antigen decays within six hours of synthesis and is replaced by translation of endogenous mRNA. The mRNA has a half life 2-3 times that of its protein product.

3. The rate of synthesis of antigen following trypsinization occurs at the same rate as the antigen decay as determined in point 2. The replacement or regrowth of antigen is insensitive to Actinomycin D while it is sensitive to cycloheximide. This result is suggestive of long-lived endogenous mRNA.

4. A selective scheme for producing and confirming feline-mouse somatic cell hybrids has been devised, for studying the genetic regulation of antigen production. The procedure involves selective elimination of an H GPRT$^-$ mouse parents in HAT medium and elimination of FLA cells in suspension by aspiration. Hybrids adhere to the surface and grow in HAT

medium.

5. Over ten allozyme systems have been developed from published procedures.

6. Aberrant LDH patterns have been observed in SV40 transformed 3T3 cell lines.

Significance to Biomedical Research and the Program of the Institute:

1. The feline system provides a reliable and informative means to examine aberrant action following neoplastic transformation. The understanding of the virus and host genetic machinery active in a malignant cell is necessary for any meaningful attempt to correct or destroy cancerous tissue.

2. The resolution and characterization of the mutiple antigens of the feline cell surface is an ideal system for projected investigation of immunological protection against malignancy.

3. The survey results will provide considerable information concerning a number of important parameters associated with gene disfunction in neoplasia:

a. the percentage of host genes with anomalous gene products
b. the relative susceptability of different genes
c. the exposure of any pattern of gene disfunction
d. the effect of method of tumor induction
e. the effect of tumor passage

These measurements are essential for the ultimate understanding of neoplasia.

4. The determination of the genetic regulation is of interest in further resolving the nature of genetic dysfunction. This is important in both detection and treatment of malignancy.

Proposed Course:

A thorough study of anomalous gene products and their regulation is in progress. The application of these antigens and isoymes for diagnostic purposes is anticipated.

Honors and Awards:

NIH postdoctoral fellowship 1971-1973
Conferee; Gordon Research Conference, Developmental Biology.
Andover, N. H. 1972
Chairman "Gene action on the Molecular Level"
Session of the 14th Annual Drosphila Research Conference.
Raleigh, N. C. 1972.
Co-Chairman and founder: Mid-Atlantic Drosophila Society.

Publications:

1. O'Brien, S.J. and R.J. MacIntyre 1972. The α-glycerophosphate cycle in D. melanogaster I. Biochemical and developmental aspects. Biochem.

Genet. 7: 141-161.

2. 1972 The α-glycerophosphate cycle in D. melanogaster. II. Genetic aspects. Genetics 71: 127-138.

3. 1972. The α-glycerophosphate cycle in D. melanogaster. III. The effect of "null" mutations at the α-Gpdh-1 locus on viability. Amer. Natur. 106: 967-971.

4. O'Brien, S. J. On estimating functional gene number in eukaryotes. Nature, In press, March, 1973.

Serial No. NCI-4897
1. Viral Biology Branch
OASDVO, Division of Cancer Cause and Prevention
2. Electron Microscopy Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Detection of Virus-induced Intracellular and Cell Membrane Antigens by Immuno-Electron Microscopy

Previous Serial Number: None

Principal Investigator: Dr. Angelo P. Andrese

Other Investigators: Dr. Harish C. Chopra (NCI-4874)
Dr. Walter D. Holder (NCI-4898)

Cooperating Units: Inside NIH
Dr. Adi Gazdar, VLLB, NCI (NCI-4824)

Outside NIH
Dr. D. Fine, Bionetics Research Laboratories, Kensington, Maryland

Man Years:

Total:	2.0
Professional:	1.0
Other:	1.0

Project Description

Objectives:

1. To detect different virus-induced antigens on cell surfaces and intracellular membrane components following transformation of NC-37 cells *in vitro* by woolly monkey virus.

Methods Employed:

Accepted techniques for cell culture, electron microscopy, immunofluorescence, cell mediated cytotoxicity as well as complement-mediated cytotoxicity were used. Immune serum was prepared in rabbits.

Major Findings:

Viral specific antigen(s), detected by the indirect fluorescent-antibody technique appeared within 24 hours following viral infection and prior to the appearance of extracellular virus. The demonstration of this early antigen was dependent on the use of viable cells in the test. At this time, the areas of positive fluorescence were scattered as small discrete points on the cell surface. These gradually increased in area with time. No virus could be detected in the tissue culture medium at this time. The appearance of extracellular virus, 72 hours following infection, intracellular viral antigen was readily demonstrable in fixed cell preparations. This condition persisted until the seventh day post-infection. On the eighth or ninth day of incubation extracellular virus was no longer readily detected, and the number of cells showing positive fluorescence in viable and fixed cell preparations diminished to approximately 20 percent of the cell population. By completely replenishing the cell culture with fresh medium, increased numbers of positive fluorescing cells reappeared within 6 hours.

To confirm these observations, parallel cell cultures were examined by immuno-electron microscopy. At various time intervals following infection, cells were exposed for one hour to ferritin-conjugated rabbit hyperimmune antiserum to woolly monkey virus. Electron micrographs demonstrated the spotty labelling of the cell membrane during the early stages of infection and an increase of membrane labelling with time. Extracellular virus appeared within 72 hours and the outer membrane of the virions were similarly labelled with ferritin.

Quantitative estimates of the number of cells producing virus antigen were obtained by a modified complement-mediated serum micro-cytotoxicity test directed against ^{51}Cr-labeled NC-37 cells infected by the virus. Approximately 10 to 15 percent of cells tested 24 hours after infection were lysed. This number rose to 95 percent by the seventh day of incubation and dropped thereafter to a constant value of about 20 percent.

However, the number of cells lysed again rose to 90 to 95 percent within 24 hours after the infected cell cultures were placed on fresh medium, thereby confirming the observations made by immunofluorescence and immunoferritin labeling procedures.

Significance to Biomedical Research and the Program of the Institute:

These preliminary results confirm, in part other host-virus relationships which exists within the oncogenic RNA virus group of agents. The present observations again reinforce the importance of environmental conditions used in in vitro studies or for the short term cultivation of human material for diagnostic purposes.

Proposed Course:

Further studies will be pursued looking at the possible mechanism of virus potentiation and shut-down in this cell system.

Honors and Awards:

None

Publications:

None

Serial No. NCI-4898

1. Viral Biology Branch
   OASDVO, Division of Cancer
   Cause and Prevention
2. Electron Microscopy Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title:

A. Establishment and characterization of new human carcinoma cell lines and activation, detection, and characterization of virus from these and other breast carcinoma cell lines.

Previous Serial Number: None

Principal Investigator: Dr. Walter D. Holder, Jr.

Other Investigators: Dr. Harish C. Chopra (NCI-4874)
Dr. Angelo Andrese (NCI-4897)

Cooperating Units: Inside NIH
Dr. Jack Gruber, VO, NCI
Dr. Robert Goldberg, VLLB, NCI

Outside NIH
Dr. Jeno Szackes, St. Joseph Hospital, Tampa, Florida
Dr. Richard Binder, Georgetown University Hospital, Washington, D.C.

Man Years:

Total:	1.3
Professional:	1.0
Other:	0.3

Project Description

Objectives:

A. To establish, clone, and characterize cultures of human breast carcinomas and cells derived from pleural effusions of patients with metastatic breast carcinomas.

B. To activate virus from cell cultures of human and animal breast carcinomas.

Methods:

A. Thirteen human breast cancer biopsies, one human Hodgkins biopsy, 2 pleural effusions, and 3 control breast biopsies have been processed. The breast tumors have been predominately scirrhous carcinomas, and mincing followed by collagenase digestion has been found to best free the carcinoma cells from the fibrous stroma. Various media and serum concentrations have been used as nutrients. Best results for isolation of epithelial cells from fibroblasts has been obtained by early subculturing of cells. Characterization of cells which show promise of continued growth will include: electron microscopy with comparison of cells with the original biopsy material, karyotyping, growth in agar, animal inoculations of cells, and reverse transcriptase studies.

B. Activation of virus is being attempted by two methods:

1. Treatment with halogenated uridine and dimethylsulfoxide.
2. Co-culture and inactivated sendai virus-mediated cell fusion.

Major Findings:

A. Cells from two human breast cancer biopsies (HMC-2 and HMC-5), a Hodgkin's Disease biopsy (HHL-1), and cells from a human breast cancer pleural effusion (MCPE-1) are growing well in several clones of each. All are in early passages, however.

B. IUdR, BUdR, and DMSO activation has been attempted on two human breast cancer cultures (HMC-2 and HMC-5), a Hodgkin's Disease culture (HHL-1) and a dog mammary carcinoma culture (TOTH). Examination of these cells by electron microscopy and negative stains of ultracentrifuged tissue culture pellets have been negative for virus. It is too early to evaluate the first of several co-culture and fusion studies.

Significance to Biomedical Research and the Program of the Institute:

With the mouse mammary cancer system as a model for virus induced breast cancer, one must apply this knowledge to human breast cancer. This of necessity must include the establishment of breast cancer cell lines and attempts to activate latent virus or demonstrate viral antigen. The ultrastructural effects of hormones and their effects on virus production remain to be evaluated.

Proposed Course of Project:

A. To continue efforts to establish and characterize human breast carcinoma cell lines and virus activation studies as previously outlined.

B. To examine human breast cell cultures and biopsies by means of immunofluorescence for antigenic relationship to viruses isolated from mammary tumors of other species and candidate human tumor viruses.

C. To determine the effects of progesterone, estrogen, and prolactin on established malignant and non-malignant breast cell lines with particular reference to growth patterns, ultrastructure and virus production.

Honors and Awards:

None

Publications:

None

Serial No. NCI-4899

1. Viral Biology Branch
   OASDVO, Division of Cancer Cause and Prevention
2. Virus & Disease Modification Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Control Through External Inhibitors of Intracellular Biochemical Changes in Tumor Cells and/or Induced by Viral Infection.

Previous Serial Number: None

Principal Investigator: Dr. Takis S. Papas

Other Investigators: Dr. M. A. Chirigos (NCI-4875)
Dr. W. A. Woods (NCI-4877)
Dr. J. W. Pearson (NCI-4878)

Cooperating Units: Inside NIH:
Dr. D. R. Twardzik, VC, NCI
Dr. F. H. Portugal , VC, NCI
Dr. E. L. Kuff, LB, NCI
Dr. R. J. Crouch, LMG, CH

Outside NIH:
None

Man Years:

Total:	2.0
Professional:	1.0
Other:	1.0

Project Description

Objectives:

To investigate the intracellular role of oncorna virus RNA dependent DNA polymerase by using specific inhibitors of these enzymes.

(a) To develop potent inhibitors of reverse transcriptase.
(b) To study the specificity of these inhibitors by comparison with other normal cellular polymerase.
(c) To elucidate the mechanism of action of these inhibitors.

To initiate comparative studies of biochemical properties of RNA dependent

DNA polymerase for different RNA tumor viruses.

Methods Employed:

Avian Myeloblastosis Virus (AMV) obtained from chicken plasma was concentrated and purified by published standard procedures. Other C-type particles were isolated by isopycinic banding in sucrose gradients.

The AMV DNA polymerase was purified approximately 300-fold. The purification steps involved detergent and high salt disruption of the virus, DEAE and phosphocellulose column chromatography and gel filtration.

High molecular weight viral RNA (60-70S) used as template for these studies was isolated by procedures developed in collaboration with Dr. E. Kuff.

Cellular polymerases obtained from cultured cells were fractionated through several column chromatographic procedures.

Major Findings:

1. Inhibition of DNA Polymerase of Avian Myeloblastosis Virus by an Alkaloid Extract from Narcissus Tazetta L.: An Alkaloid extract of the Sacred Lily (narcissus tarzetta L.), a medicinal plant, inhibits the purified DNA polymerase from Avian myeloblastosis virus. The mechanism of action of this inhibitor, differs from that of other known inhibitors. The inhibitor physically combines with the polymerase, it does not affect the binding of the template to the enzyme as demonstrated by classical noncompetitive inhibition kinetics and affects either inhibition or elongation phase of the polymerization reaction. The inhibition is the same whether viral 70S RNA or poly d(AT) is used as template.

2. Inhibition of the DNA Polymerase of Avian Myeloblastosis Virus (AMV) by Pyran Copolymer: Pyran copolymer, a known immunostimulator, was found to be a potent inhibitor of purified DNA polymerase isolated from AMV. Increasing concentration of pyran was followed by a parallel decrease in enzymatic activity; 50% inhibition was obtained at a concentration 25 µg/ml. The inhibitory effect is reversible by further addition of enzyme but not by addition of template to the assay system. The inhibitor binds to the polymerase but not at the template site as demonstrated by noncompetitive inhibition kinetics. The degree of inhibition was not template specific for the templates tested, 70S AMV viral RNA, synthetic hybrid polyrA. oligodT_{10}, synthetic copolymer d(AT) and activated calf-thymus DNA. Although pyran copolymer was a very effective inhibitor of AMV polymerase, it did not at all effect the activity of E. coli B and M. luteus DNA polymerase. The possibility that pyran might be a specific inhibitor of oncornaviral DNA polymerases has interesting ramifications. We are now examining the effect of pyran on other viral and cellular polymerases.

3. Effect of Streptonigrin and Analogs on Oncornavirus Replication and Reverse Transcriptase Activity: The antibiotic, Streptonigrin, was shown to

possess activity in nanogram quantities against Moloney leukemia virus replication in vitro. In microgram quantities it was also effective against the Rauscher leukemia virus in vivo, and markedly inhibited the reverse transcriptase of avian myeloblastosis virus.

Analogs of Streptonigrin were tested, and results showed that minor to major alterations in the structure resulted in progressive decreases in activity.

The antibiotic, Streptonigrin, was shown to be active against leukemia virus by in vivo (using Rauscher leukemia virus), and in vitro (using Moloney leukemia virus) methods, as well as in inhibiting the reverse transcriptase enzyme (from avian myeloblastosis virus) of C-type RNA viruses. The analogs of Streptonigrin tested showed that alterations of the parent structure resulted in varied rates of activity.

4. RNA-dependent DNA Polymerase from a Reptilian Type C Virus: A study was made of the RNA-dependent DNA polymerase from a reptilian type C virus isolated from cultures of spleen cells derived from Russell's viper. Endogenous polymerase activity was dependent on the addition of all four deoxynucleotide triphosphates. Simultaneous detection experiments on sucrose gradients demonstrated both the presence of 70S RNA and reverse transcriptase activity. Unlike the AMV and RLV enzymes, the endogenous activity was significantly inhibited by increasing concentrations of oligo(dT)$_{10}$. A partial purification and characterization of the enzyme was made using phosphocellulose column chromatography and high salt glycerol gradient centrifugation. The enzyme activity elutes from phosphocellulose at 0.22 M KCl, a value comparable to the AMV polymerase yet different from the RLV enzyme (0.40M). The molecular weight as determined from glycerol gradient centrifugation is approximately 109,000 daltons. This value is considerably smaller than the AMV enzyme which is composed to two nonidentifiable subunits of 69,000 and 110,000 daltons each, yet somewhat larger than the RLV enzyme which has a molecular weight of 70,000 daltons. Studies on template specificities and metal ion requirements as a further attempt to distinguish the reptilian reverse transcriptase from other viral polymerases are presently under investigation.

5. Procedure for Isolation of High Molecular Weight RNA from Oncornavirus: In collaboration with Dr. E. Kuff we developed a procedure for extracting 70S from several oncornavirus (AMV, RLV, Reptilian Type C Virus, PK-15 C-type particle). This improved method produces 70S undegraded RNA which is a good template for transcriptase reaction.

6. The C-type particles observed by electron microscopy in PK-15 (pig kidney) cells were demonstrated to have biochemical and biophysical properties associated with the oncorna virus group: density of 1.16 g/ml in a sucrose gradient, 70S RNA, and the RNA dependent DNA polymerase. The group specific interspecies antigen, gs-3, was not present. Evidence of a latent infection with a porcine parvovirus was also obtained.

Significance to Biomedical Research and the Program of the Institute:

Recent advances in the biology of RNA tumor viruses favor the hypothesis that some human cancers may be caused by viruses. Particles isolated from various human tumors have been found to contain reverse-transcriptase activity, a characteristic exhibited by all RNA tumor viruses. Results reported demonstrate a good correlation between the reverse-transcriptase and the induction of malignacy by the RNA tumor viruses. The development of specific inhibitors of reverse-transcriptase activity is, therefore, a useful approach to effective chemotherapy, and to lead to a better understanding of the mechanisms of viral oncogenesis.

Proposed Course:

1. To continue search for natural and synthetic compounds that possess antipolymerase activity.
2. To investigate the mechanism of inhibition of viral and cellular polymerase.
3. To investigate the biological function of ribonuclease H on activity associated with the reverse transcriptase molecule through the use of specific inhibitors.
4. To elucidate biochemical events that occur in the MSC and mutant cells grown in the presence of streptonigrin.

The following questions will be raised:
(a) Whether the difference in streptonigrin sensitivity is due to varying ability of the cell membrane to resist permeability by this drug.
(b) Whether mutant cells develop a mechanism for breakdown or utilization of the drug.
(c) Whether the mutant cells develop streptonigrin resistant polymerase(s).

Honors and Awards:

None

Publications:

None

Serial No. NCI-4900
1. Viral Biology Branch
OASDVO, Division of Cancer Cause and Prevention
2. Experimental Pathology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Immunological Studies on Virus-induced Tumors

Previous Serial Number: None

Principal Investigator: Dr. Minoru Harada

Other Investigators: Inside NIH
Dr. Gary Pearson, VBB, NCI (NCI-4880)
Dr. Hugh Pettigrew, B, NCI

Man Years:

Total:	1.0
Professional:	1.0
Other:	0.0

Project Description

Objectives:

1. To investigate the development of the cellular immune response to virus-induced tumors.

2. To study the effect of well-characterized immune sera on the cytotoxic activity of normal and immune lymphoid cells.

Methods Employed:

1. Mice were immunized with murine sarcoma virus (MSV) or allogeneic tumor cells producing virus. At appropriate times following inoculation, the mice were bled and their spleens removed. The sera were heat-inactivated and titered for antibodies directed against the Moloney virus-induced cell surface antigens by membrane immunofluorescence (MF). Spleen cell suspensions were prepared and tested for cytotoxic activity in a micro-cytotoxicity assay utilizing the release of chromium^{51} from pre-labelled target

cells as indicator.

2. Cellular immunity studies were performed using a newly developed micro-cytotoxicity assay. MSV-induced tumor cells were labelled with chromium^{51} and then incubated with normal or immune spleen cells for 18 hours in Falcon microtiter plates at 37°C. In experiments investigating the interaction of serum with lymphoid cells, the serum was added to the wells after addition of target cells but before the lymphoid cells were added. Following this incubation period, the plates were centrifuged, the supernatants harvested and the radioactivity in the cell-free supernatants determined in a Packard scintillation counter.

Major Findings:

1. A micro-cytotoxicity assay based on the release of chromium^{51} from pre-labelled target cells was developed for studying cellular immunity. The optimal conditions for demonstrating cellular immunity against MSV-induced mouse tumor cells were defined. In the experiments performed, 1-5x10^{3} target cells were added to each well and were incubated in the presence of 5x10^{5}-2.5x10^{6} spleen cells. These ratios produced maximum lymphocyte cytotoxicity. The results of experiments investigating cellular immunity to H-2 and MSV-induced antigens showed that specific immunity could be detected as early as seven days after immunization with H-2 antigens but was not demonstrable against MSV-induced antigens until approximately 14 days post-inoculation. In both cases, immunity could be demonstrated for approximately three weeks after inoculation after which it dropped to undetectable levels. In the experiments designed to investigate the interaction of immune serum with lymphoid cells, it was found that both normal and immune cells acted synergistically with high titered sera in the destruction of tumor cells. The antibody titers were determined by the MF assay. This synergistic effect was particularly striking with normal lymphoid cells and was directly related to the MF antibody titer of the serum. Absorption experiments provided further evidence that antibody was the active serum factor responsible for the enhanced killing activity of normal lymphocytes. These results suggests that immune serum may act synergistically with normal and immune lymphocytes *in vivo* in eradicating antigenic tumor cells since the same sera that were effective *in vivo* were also those active in this *in vitro* system.

Significance to Biomedical Research and the Program of the Institute:

Tumor immunity is mainly mediated through lymphoid cells. However serum has also been reported to be effective in transferring immunity against some experimental tumors. An understanding of the mechanisms

involved in the transfer of tumor-specific immunity will contribute to the development of immunological control measures.

Proposed Course:

1. In vitro studies on the interaction of immune serum with lymphoid cells will continue. The studies in the mouse system will be directed toward defining the factors responsible for mediating the synergistic action. Similar studies will also be initiated with Herpesvirus saimiri.

Other Activities:

None

Honors and Awards:

None

Publications:

None

Serial No. NCI 4901
1. Viral Biology Branch
OASDVO, DCCP
2. Cell Biology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Tumor Cell Surface Antigens: Morphological and Physiological Studies

Previous Serial Number: None

Principal Investigator: Dr. Robert Radin

Other Investigators: Dr. Charles Boone (NCI-4883)

Cooperating Units: None

Man Years:

Total:	1.1
Professional:	1.1
Other:	0.0

Project Description

Objectives:

1. To characterize the chemical conformation of cell surface antigens under varying environmental conditions (confluent vs. sparse culture, osmolarity, temperature, neuraminidase, proteases, and chaotropic ions.

2. To characterize the number and pattern of distribution of cell surface antigens at different times during the cycle of cell division.

3. To characterize the synthesis and turnover of virus coded cell surface antigens.

Methods Employed:

Radio-labeled antibody binding, antibody binding, antibody titrations, biochemical and physical procedures associated with the analysis of chemical composition and physical behavior, and metabolic studies in virus infected cells using isotope tracers.

Major Findings:

Working with feline leukemia virus-infected cat lymphoma cells, the concentration of formaldahyde and gluteraldahyde fixatives that will

preserve structure but not denature surface proteins is being determined. The fixatives will permit stopping physiological changes at any point in the cell cycle; following which, the cell surface antigens can be analyzed at leisure.

Significance to Biomedical Research:

The development of knowledge regarding cell surface antigens, their structure, their physiological behavior, and their relation to viruses and neoplastic transformation, is fundamental to improved immunodiagnosis and immunotherapy.

Proposed Course:

Specific physiological properties of virus-induced cell surface antigens will be sought that can serve as markers for the analysis of relationships between virus genome coding and host genome coding of tumor cell surface antigens.

Honors and Awards:

None

Publications:

None

Serial No. NCI-4902

1. Viral Biology Branch
   OASDVO, Division of Cancer
   Cause and Prevention
2. Human Tumor Studies Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Cellfree Biosynthesis of Oncornavirus-specific polypeptides.

Previous Serial Number: None

Principal Investigator: Dr. Richard Ascione

Other Investigators: Dr. George F. Vande Woude (NCI-4893)
Dr. William G. Robey (NCI-4895)

Cooperating Units: None

Man Years:

Total:	1.3
Professional:	0.8
Other:	0.5

Project Description

Objectives:

To determine the number and molecular properties of the proteins coded by the genetic information of oncornaviruses using a subcellular protein synthesizing system derived from heterologous-noninfected cells which respond to added viral messenger RNA.

Methods Employed:

Cultured cells were produced for use as a source of subcellular protein synthesizing components. These are respectively: Polysomes for translation-initiation factors and ribosomal subunits; partially purified elongation factors; purified oncornaviruses for messenger RNA and stimulatory factors; and purified transfer RNA. These components are purified by differential centrifugation, salt extraction and precipitation, and column chromatography. The translational products of *in vitro* radioactive isotope kinetic analyses are assayed by scintillation spectoscopy and compared to known virion products by polyacrylamide gel electrophoresis and immunodiffusion analyses using autoradiographic methods.

Major Findings:

A subcellular protein synthesizing system has been isolated and partially purified from both Rauscher murine leukemia virus (RLV) infected JLSV-9 tissue culture cells and uninfected, freshly excised rat livers. Although both systems show low levels of endogenous activity in vitro, they are capable of synthesizing proteins over an extended period of time. More importantly, both systems are capable of de novo protein synthesis in response to exogenous messenger RNA. Thus, using partly purified initiation factors derived from either JLSV-9 ribosomes or rat liver ribosomes, a three-fold stimulation of poly-methionine-^{35}S protein was observed using the synthetic methionine coded message poly(AUG). Moreover, a stimulation of ^{3}H-polyphenylalanine incorporation of over ten-fold was observed with poly U as a synthetic message. When avian myeloblastosis virus (AMV) RNA was used as a source of messenger RNA, de novo protein synthesis was stimulated six-fold over the endogenous level. This latter product is now being characterized and compared by immunological and polyacrylamide gel electrophoretic techniques with the following preliminary results. AMV RNA directed proteins in the rat liver cell-free system give indication of reactivity with porcine antisera prepared against detergent-ether disrupted AMV. Gradient polyacrylamide-SDS gel electrophoresis of the AMV RNA directed protein product followed by autoradiography also indicates some virus-specific zones are present when compared to AMV capsid proteins.

Significance to Biomedical Research and the Program of the Institute:

Presumably, oncornavirus interference with normal regulation of protein synthesis is the molecular basis of tumorgenesis. These studies are directed toward the elucidation of the protein gene products of oncornaviruses synthesized in the host cell and to identify proteins which are able to regulate host cell synthesis of macromolecules.

Proposed Course:

These studies have just been initiated. The protein synthesizing system will be optimized for competency in detecting all of the viral genome products. It will then be possible to distinguish the sarcoma viral components and perhaps detect those proteins which specifically interact with DNA from the transformed cells.

Honors and Awards: None

Publications: None

SUMMARY REPORT

VIRAL CARCINOGENESIS BRANCH

July 1, 1972 - June 30, 1973

Introduction

The main thrust for the Viral Carcinogenesis Branch for some years now has been focused on the natural histories of RNA tumor viruses and their apparently critical roles in spontaneous and natural cancer of feral as well as laboratory animals, the ultimate objective of course being the isolation, characterization, and eventual control of similar viruses in man. VCB scientists, as a consequence, have developed a coordinated program aimed at highly targeted goals, each of which is concerned with different aspects of essentially the same question, namely, the nature of the RNA tumor virus and its relationships with its natural hosts. Great emphasis has been on development of highly sensitive and specific assays for not only infectious RNA tumor viruses but for subunits of these viruses. Thus, sophisticated technologies and reagents were developed during the last 10 years and made available for in depth in vitro as well as in vivo studies of at least 10 naturally occurring type C and type B RNA viruses in 9 different species in 3 classes of animals. Although subject to less intensive study, systematic investigations of DNA tumor viruses are pursued as indicated by the newer developments in this field

VCB scientists serve also as managers and project officers on 20 SVCP contracts within the Solid Tumor Segment. In this closely monitored extramural program emphasis has been placed on maximum communication and collaboration between the various scientific leaders of these contracts. At least four Pacific Coast Tumor Virus Group (PACTVIGR) meetings are held each year on the West Coast where 11 SVCP contracts are located.

In the Bethesda, Maryland, area the inhouse VCB serological and small animal trailer laboratory together with long-established service units at Flow Laboratories, Inc., and Microbiological Associates, Inc., provide essential diagnostic services and viral characterization expertise as required by the 20 contract programs (see separate reports of the STS contracts.)

Major Achievements

RNA Tumor Viruses as Natural Causes of Cancer in Experimental Animals. Since the mouse systems (including wild mice) have been best studied and are the most informative, I will focus on the considerable knowledge now available in mouse systems, stressing the various known modes of transmission of type C viruses. The new concepts generated from new findings made particularly during the last year immediately suggest wholly new approaches for attempting to prevent, control and treat cancer. Although the inbred laboratory mouse is the best model for developing these approaches, early

application of the more successful procedures to cancer problems in other animals and in man are quite conceivable.

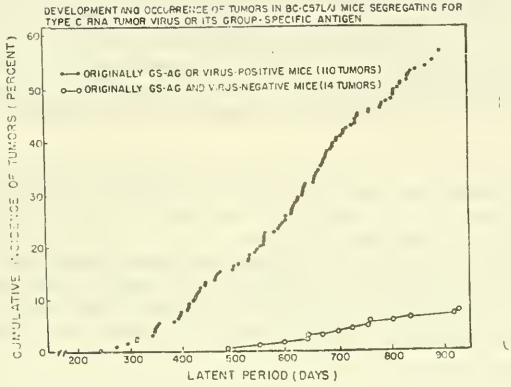
During FY 1973 well-designed in depth studies of the origins and modes of transmission of type C RNA tumor viruses were reported by many virologists, immunologists and geneticists not only within VCB and STS programs, but also by scientists in other Branches of Viral Oncology and in other segments of the SVCP. In virtually every case these studies supported the concept of genetic inheritance of the RNA tumor virus genomes as postulated in the Viral Oncogene Theory proposed in 1969 by VCB scientists. A significant number of reports supporting this theory came from inhouse and grant-supported groups in NCI and in NIAID.

Specific Findings

Evidence for Endogenous Genetic Determinants of Type C RNA Tumor Viruses Linked with Cancer Expressions.
Meier, Taylor and Huebner recently reported genetic linkage of RNA tumor virus and viral gs antigen expressions early in life with development of cancer late in life in F1, F2 and F1 backcross progeny of a cross between two strains of inbred mice which were (a) homozygous for gs expression (AKR/J) and (b) homozygous for lack of expression (C57L/J). It was found that 92% of the cancers observed (mostly in old age, prior to death) occurred in mice whose spleens were gs+ at weaning whereas only 8% occurred in mice which were gs- at weaning. (See chart.) In addition, the gs negative tumored mice turned out to be positive for gs antigen when the tumors themselves were tested; all of the latter occurred late in life. This study, more conclusively perhaps than any other, showed that in mice endogenous structural and regulatory genes were critical determinants of RNA tumor virus expressions and that these genes as well as those responsible for the development of cancer both segregate in Mendelian fashion.

Of greatest importance, however, was the obvious linkage between genes responsible for RNA tumor virus and gs antigens expressed in early life with the cancers which occurred in later life. This work has been recognized by both virologists and geneticists as exceedingly significant and important because it shows (1) that the tumor virus "virogenes" are inherited; (2) that the viral tumor producing "oncogenes" are also inherited; and (3) that these are almost certainly genetically linked natural entities located perhaps on the same chromosome(s). These studies now make it possible to establish soon not only the site but the mechanisms of action of these genes, to determine the effects of immune responses on the occurrences and timing of cancer, and for designing methods for prevention and modification of such tumors.

Evidence that Virus Free Cells in Culture Contain Complete Information for Producing Type C RNA Tumor Viruses.
The evidence that cells of different animal classes and species contain type C RNA tumor virus genomes was greatly extended in FY 1973. Two research groups, Stewart et al. and McAllister et al., reported activation of


DEVELOPMENT AND OCCURRENCE OF TUMORS IN BC-C57L/J MICE SEGREGATING FOR TYPE C RNA TUMOR VIRUS OR ITS GROUP-SPECIFIC ANTIGEN
ORIGINALLY GS-AG OR VIRUS-POSITIVE MICE (110 TUMORS)
ORIGINALLY GS-AG AND VIRUS-NEGATIVE MICE (14 TUMORS)
CUMULATIVE INCIDENCE OF TUMORS (PERCENT)
0
10
20
30
40
50
60
200
300
400
500
600
700
800
900
LATENT PERIOD (DAYS)

endogenous type C RNA virus particles in human tumor cells by mutagenic agents. Unfortunately, as mentioned above, human RNA tumor viruses have not thus far replicated continuously to any useful extent in the cultured normal or neoplastic human cells.

In experimental and certain feral animals a number of well-characterized type C virus strains were produced in large scale tissue culture systems. However, endogenous (inherited) genomes were demonstrated in virus-free cell lines of chickens (Weiss, Hanafusa), pheasants (Hanafusa), many strains of mice (Rowe, Aaronson, Todaro and Aoki), hamsters (Kelloff, Gilden, Freeman and Todaro), rats (Klement and Aaronson), cats (Fischinger, Todaro, Sarma and others) and gibbons (Kawakami). Similar endogenous viruses have also been demonstrated in wild mouse cells (Gardner, Officer and Hartley.)

While most investigators settled on exogenous inducers such as mutagenic agents (IdU and BrdU), radiation and carcinogenic agents to initiate type C RNA virus expressions (Lowy, Teich, Rowe, Klement, Aaronson, and Todaro), graft versus host reactions (GVHR) were also reported in FY 1973 to invariably switch on lymphoma and leukemia-inducing viruses (Schwartz, Cornelius, Armstrong.) Recently Hirsh and Black found that cocultivation of parent and progeny cells from animals subject to GVHR would also switch on RNA tumor virus.

These reports of inherited endogenous RNA virogenes and oncogenes demonstrated in 4 mammalian and 2 avian cell systems, and also in vivo in inbred mice by Meier provide nearly conclusive evidence for widespread inheritance of the type C RNA tumor virus genome.

Evidence that Endogenous Virus Activated in Cells Causes Neoplastic (Cancerous) Changes in Cells.

Freeman, Price and Zimmerman, who had previously shown that RNA viruses when added to mouse, rat and hamster cells serve as determinants of early malignant transformation of cells when treated with chemical carcinogens, made three additional highly significant observations in FY 1973.

1. Normal embryo rat cells treated with BrdU followed shortly thereafter by 3-methylcholanthrene were readily transformed into tumor cells whereas cells untreated with BrdU were not transformed. The transformed cells were shown to contain rat RNA tumor virus (RALV) gs antigen expressions when they were transplanted into newborn rats. Since BrdU is known to produce a switch-on of RALV, which persists in the cells for a week or two (Klement and Aaronson), the presence of RALV at the time of 3MC treatment can be best interpreted as the source of oncogene specific determinants of the 3MC transformation observed by Freeman et al.

2. The importance of precise time sequence relationships between virus infection and 3MC induction of transformation was also provided in experiments reported by Price and Freeman. These studies showed that transformation of

cells only occurred when RNA viruses were replicating at significant easily detectable levels (>1.5 logs) prior to treatment with 3MC. Virus added at the same time or shortly after 3MC treatment failed to result in neoplastic cell transformation.

3. Zimmerman and Price are in the process of reporting that continuous cell lines from a number of genetically different yet well-defined normal murine fibroblast cell lines, when infected with compatible murine RNA tumor viruses, transformed into neoplastic cells at much more rapid rates than the same cell lines which were not infected experimentally. Many of the latter cell lines do not transform spontaneously and they remain free of virus expressions. However those virus negative cells that do transform spontaneously, in most cases, reveal overt expressions of RNA tumor viruses.

The evidence from these 3 experiments are important in that they provide critical data which help to define the role of the oncogenes of type C RNA tumor virus genomes in easily repeated in vitro cell systems.

Further Evidence that Endogenous RNA Tumor Viruses are Oncogenic in vivo. Leukemogenic Effects of B Tropic Type C RNA Tumor Viruses:
Despite the clearcut demonstrations in the Meier et al. host versus graft reaction reports described above, the STV scientists felt it important to show that the generality of natural (wild type) infectious viruses are in fact oncogenic. Thus Peters, Spahn and Kelloff report that infectious RNA tumor virus detected in spontaneous tumors of BALB/c mice (most occurring during old age) were indeed leukemogenic when inoculated into newborn BALB/c's. The viruses which were B tropic, that is, capable of growing in the BALB/c cells, produced tumors, whereas those variants which were N tropic and unable to grown in BALB/c cells did not cause leukemias on transmission.

This experiment proved very important because it shows that RNA tumor viruses, like many other viruses, vary in their pathogenicity; that is, their ability to produce disease. This established once again the importance of host susceptibility and host responses to viruses in general and helps explain paradoxical host-virus relationships that seem to mystify uninformed cancer investigators who expect that viruses in order to be proved as causes of disease must have a one to one overt ratio to the diseases they surely do cause.

It is of considerable importance to note also that (1) Drs. Hartley and Peters have shown that it is possible to convert N tropic viruses to B tropic (disease-producing) infectious viruses, and (2) in similar fashion those with GVHR idnuced leukemias have shown that they are transmissible by type C RNA virus preparations.

Leukemogenic Type C RNA Viruses of the Hamster:
Recently Lane and Huebner confirmed Graffi's report that type C RNA viruses (HaLV's) in hamster lymphomas induced by a hamster specific papovavirus are highly leukemogenic when RNA virus extracts are transmitted to newborns of

all hamster strains. These same workers extended these observations by demonstrating HaLV expressions in virtually all hamster tumors induced by bovine papilloma virus and in most adenovirus and polyoma tumors after several transplants.

These observations are quite important since when considered along with the spontaneous switch-on of HaLV in hamsters carrying defective sarcoma viruses (Kelloff, Gilden), and in cells treated with BrdU (Todaro), it clearly shows that hamsters like mice, rats and chickens also carry the type C RNA tumor virus in the form of endogenous genetically inherited viral genomes, thus increasing the probability that other mammalian vertebrates will be found to have these same arrangements when they are studied in sufficient depth and intensity.

Evidence that Type C Viruses are Responsible for Natural Cancers in Wild (Feral) Mice not Manipulated in the Laboratory.
Earlier studies by Gardner et al. of seven thousand wild mice trapped in several Los Angeles sites and then observed throughout their natural life revealed that virtually all of the mice were switched-off for infectious type C RNA tumor viruses: during their lifetime they developed only 45 tumors. Approximately 30% of those which developed cancer (95% late in life) had RNA tumor virus gs antigen expression detectable in their tumors and spleens. However, the subsequent development of radioimmune assay tests for gs antigen greatly increased the incidence of such antigens. These studies were significant because they showed that wild mice having little or no virus expression also had very little natural cancer.

More recently, a large population (LC) of wild mice was located in the western part of Los Angeles County which is nearly 100% "switched on" for natural RNA virus expressions. During the first 12 months of life these mice developed 20 times the incidence of leukemia and other cancers as did the switched off population described throughout their natural life.

Other studies revealed that feral unmanipulated wild mice have the same general type of RNA tumor viruses as do laboratory strains (identical gs antigens and reverse transcriptase) and analysis of tumors in the "switched-off" and "switched-on" laboratory strains showed that the presence of type C RNA tumor viruses is obviously the critical determinant of cancer in these wild mice. Thus the RNA tumor viruses occurring in natural "wild" ecologies are also the central factors responsible for the large amount of cancer observed in heavily infected populations.

Officer, Gardner and Henderson have shown that the virus present in the LC population is also responsible for a motor neuron disease similar to amyotrophic lateral sclerosis (ALS) in man. The natural incidence is about 10% within a year and it is much higher in mice that also have leukemia.

The typical type C RNA tumor virus after isolation in tissue culture from LC mice produces paralysis within 12 months in 50% of the wild mice injected as newborns. In newborn NIH Swiss mice, the incidence is 100% in 2 to 5 months. Reports of this unique behavior on the part of endogenous wild type RNA tumor viruses has created great interest by investigators of neurological disease everywhere. Considered together with the lupus erythematosis-like disease in NZB mice which is also presumably due to natural type C viruses, such observations greatly increase the importance of genetically and epigenetically transmitted tumor-inducing viruses as causes of non-cancerous chronic diseases.

Evidence from Molecular, Biological, and Biochemical Studies for Type C RNA Tumor Virus Genomes in Normal as well as Tumor Cells.

Evidence that chicken, mouse and hamster sarcoma cells contain RNA and DNA sequences hybridizable with type C virus RNA and DNA has been reported repeatedly by Green and Bishop and also by Spiegelman, Baluda, Roy-Burman, Duesberg, Scolnick and Parks, Hatanaka and Gilden, and others.

Since viral sequences have been found in normal as well as tumor cells, it clearly suggests that transcriptional products of the RNA tumor viruses can, like the translational proteins, also be found occurring as endogenous gene determined factors.

The molecular and biochemical studies are of enormous importance since they can be applied to human normal and tumor cells in efforts to identify hybridizable virus specific sequences that have the characteristics of those found in animal RNA tumor viruses.

Such studies also help provide information on mechanisms of virus expression and their regulation which could lead to novel methods for controlling cancer at the level of the normal and/or cancerous cell.

Evidence that Attempts to Modify Genetic and Molecular Expressions of the RNA Tumor Viruses will Prove Useful in Prevention or Control of Cancer. At the gene control level: Taylor and Meier have reported the identification of a dominant gene in certain strains of mice (C57BL/10Sn and certain cross-breeds) which switches off the gs antigen and of course at the same time the infectious virus. This gene which obviously is a virus regulating gene can now be studied in congenic lines wherein this single gene is either present or absent; thus its effects on both virus expression and tumor can be studied as in the AK X L experiment described above, where a dominant gene linked with a gene for tumor expression was responsible for gs antigen expression (Meier, Taylor and Huebner).

It now seems possible with the use of the C57BL/10Sn males on successive generations of virus and leukemia positive female mice (AKR's) to virtually eliminate both virus and cancer as natural events in these mice. Although this is important for demonstration purposes, showing that cancer is determined by identifiable endogenous factors, Mendelian studies of the

segregation of viral genes and oncogene expressions, regulating genes, linkage groups, should provide many opportunities for modifying murine cancer at the cellular level. Since genes determining immune responses will segregate also in these studies, such studies will also clarify the role of immunological surveillance factors (competence, deficiency and GVHR) in the development of clinical cancer.

Evidence that New Approaches to Control of Cancer Through RNA Tumor Virus Expressions and Immune Responses to These Viruses May Prove of Value to Control of Cancer.

Since the genetic factors which can be manipulated to control and regulate RNA tumor virus expressions and spontaneous cancers in mice are not now applicable to man, other approaches are necessary. The scientists participating in VCB and STS contracts, however, envision the following new approaches to cancer control based on genetic regulation of endogenous oncogenes, virus and/or inhibition of viral oncogene expressions by cell repressors, host immune responses, and chemical inhibitors.

1. Repressors of RNA tumor viruses: Portugal, Twardzik and their associates in VCB have found and isolated a DNA-binding protein in virus-switched-off AKR mouse cells which is present only in much smaller amounts in virus-infected sister cultures. This protein, of about 60,000 molecular weight, has many of the properties of known repressor proteins previously identified in bacterial virus systems. This exciting finding could lead to understanding of the mechanisms by which mammalian cells control gene expressions in general as well as how genetic regulating mechanisms switch off the RNA tumor virus gene expressions and also, as described above, cancer expressions as well. Further research will be aimed at delineation of the effects of proteins with repressor-like properties on the oncogenic activity of viruses.

2. Interferon prevention of chemically induced tumors in mice: Salerno, Whitmire and associates recently reported that interferon given to mice in large doses given 3 X a week starting at 5 days of age and continued for 4 months completely prevented subcutaneous sarcomas and lung adenomas induced in controls by subcutaneous inoculation of 200 ugm of 3MC. Over 60% of the control mice had sarcomas and about 20% had lung adenomas. This effect of antiviral interferon on tumors produced by hydrocarbon carcinogen could have great importance. It should be mentioned, however, that these effects were achieved only when interferon treatment was started in very young mice (5 days of age or less).

3. RNA tumor virus vaccines as preventatives of spontaneous and chemically induced tumors: In a preliminary report, Whitmire and Huebner reported that formalin-killed vaccines made from Kaplan's radiation leukemia virus (RadLV) produced significant reductions in tumor incidences in mice given 3MC subcutaneously. This surprising (unexpected) result is preliminary and remains to be explored in much greater septh. From a theoretical point of view, packaging tumor cell membrane antigen on numerous (billions) of banded

virus particles could possibly provide immune responses which are highly specific for tumor cells and thus prove of value in immunoprevention and therapy of cancer. Fortunately this thesis is quite subject to test in experimental animal systems.

4. Studies of tumor antigens and immune responses: Recently, during studies of autoimmune (lupus-like) diseases in NZB mice, Lerner, Dixon, Gilden, Jensen and Hartley were able to make the following determinations: (a) The NZB virus is N-B tropic and has a different envelope antigen from the usual wild type mouse viruses (AKR-Gross type) in that it resembles the Moloney virus (FMR group). (b) This virus produces potent antibodies that combine with the enormous amounts of the NZB virus antigens in blood plasma which then are deposited in primarily the kidney, producing a generally fatal autoimmune disease resembling lupus-erythrematosis. (c) They found that the NZB mice, unlike other strains of mice, develop antibodies to RNA and DNA of the virus, and various viral enzymes including the reverse transcriptase (Gilden), virus and cellular DNA and RNA. (d) Dixon and Lerner have succeeded in isolating and purifying a major antigen from the NZB plasma and the cell membranes which may represent at the tumor antigen and the antigen responsible for the autoimmune lupus disease in the natural NZB mouse. (e) NZB mice cross bred to BALB/c mice by Lerner and Dixon yield progeny that have less autoimmune disease but which unlike the NZB parents are highly susceptible to the NZB virus, yielding leukemias in 50% in 3 months; thus showing the oncogenic potential of the NZB virus which is almost totally prevented in the hyperimmune natural parental strain.

These studies of immune mechanisms and their independent effects on virion and oncogene expressions emphasize the numerous pleiotropic factors in the development or prevention of cancer and while they help define the critical importance of the RNA tumor virus, they do at the same time reveal the many other genetic, immunological and age factors in spontaneous, natural cancer.

The Critical Importance of Viral Reagents and Characterization of the Transcriptional Properties of RNA Tumor Viruses.

All of the above studies on RNA tumor viruses in the VCB-STS programs (and in other NCI programs as well) have been made possible because of early and recent new developments in technology by in-house VCB program investigators and by contract groups under the direction of Drs. Raymond Gilden, Wade Parks, Gary Kelloff, George Todaro, Padman Sarma, Paul Arnstein, Barge Hampar, and others. Thus Dr. Gilden's group at Flow Laboratories is currently responsible for producing banded RNA virus, various viral vaccines in large volume amounts, viral specific antibodies to gs antigens and reverse transcriptases which are representative of RNA viruses from at least ten different mammalian species. It can be said without contradiction that virtually every major new finding described herein has dependend upon the development of these Flow-produced reagents, and the guidance and the test services provided by Dr. Gilden. In addition, the research contributions of Dr. Gilden and his group at basic viral characterization levels have been both numerous and

highly superior as can be seen from the publications listed on the Flow Contract program report.

RD114 Human Candidate Virus Shown to be a Novel Endogenous Virus of the Cat.

Brilliant detective work on the part of at least four different groups under Drs. Todaro, Sarma, Gilden and McAllister resulted in the isolation of a virus from normal fetal cat cells (in vitro and in vivo) which has all the properties of the RD114 virus. This endogenous (inherited) virus will not grow in the normal or tumor cells in which it becomes switched on, it grows selectively in human as well as other primate cells. It is, however, unexpectedly completely distinct from the three FeLV viruses which are themselves identical except for envelope proteins and cat cell host range. The FeLV viruses and the endogenous (RD114-like) viruses of the cat have no detectable molecular sequences in common and no gs antigen transcriptase or envelope antigens in common. However, both groups of viruses carry DNA sequences (host cell derived) which hybridize with host cell DNA. Thus the RD114 virus represents a novel, inherited, endogenous virus of cats.

Dr. Maurice Green (St. Louis University contract) has shown that RD114 virus DNA hybridizes with Hodgkin's tumor RNA. What this means in terms of etiology is as yet unclear. Attempts to induce human RNA virus by recombination of viral genes from human and mouse cells by transmission of human tumor cells in anti-thymocyte serum (ATS)-treated NIH Swiss mice have been made. Drs. Arnstein and Todaro have grown tumor cells but not normal human cells that readily grow in brains of mice given ATS. Such tumors and the cells derived from them often contain type C RNA viruses which appear to have properties confirmed by both the mouse and human cells.

Viruses in Human Tumor Cells Grown in ATS Treated Mice.

NIH mice and/or their cells have never yielded RNA tumor viruses although they do have viral gs antigen expression in their tissues. Human tumor cells of all types tested, sarcomas, carcinomas, leukemias, are similarly free of type C viruses. The viruses isolated are tropic for human cells, do not grow in mouse cells, but have the gs and RT antigens of the mouse. The envelope antigens inducing neutralizing antibody are entirely different from any of the mouse viruses and can be regarded as presumptive derivatives from human cells. At least five such human-mouse "recombinants" have been induced in human sarcomatous (RD cells and others), carcinomatous, and leukemic cells.

This new technique for deriving human tumor cells for in vitro culture and for switching on presumably recombinant human and animal viruses provides an important wholly new technique for tumors. Specific antigens of these virus particles that can now be grown in large quantities, when purified and banded, can be used in immune response studies as well as for tumor-inducing activity in various cell and animal systems.

Studies of Temperature-Sensitive Mutants of RNA Tumor Viruses as Specific Markers for Virus Replication and Tumorigenesis.

Temperature sensitive (ts) viral mutants provide one of the more definitive ways of proving that type C RNA viral genes are responsible for tumor induction. The ts mutant viruses grow well and produce transformation at a lower than normal temperature, but not at the normal temperature despite equivalent growth rates. Thus the oncogenic response to the mutant viruses is dependent on a specific temperature. This establishes the viral-specific temperature-dependent oncogene as the cause of the neoplastic transformation.

Dr. Peter Vogt of USC has developed ts mutants for the avian sarcoma virus and Dr. Aaronson and others have similar ts mutants for mouse sarcoma virus. One of Dr. Vogt's most significant findings to date has been the demonstration that viral transformed cells stay transformed only by virtue of continued viral genetic activity, thus indicating that viral genes are required not only for the initiation of the transformed state but also for its maintenance.

The Role of Epstein-Barr Herpesvirus (EBV) in Burkitt's Lymphoma and Other Human Lymphomas.

Several important findings were reported by Dr. Berge Hampar, VCB, on the mechanism of Epstein-Barr virus repression and activation in human lymphoblastoid cells which should provide valuable insights into latency by herpesviruses and a possible rationale for implicating them in human neoplasms. Dr. Hampar showed that both EB virus producer and nonproducer cells carry the EB viral genome in a repressed state. Producer cells show spontaneous activation of the repressed viral genome leading to synthesis of virus antigens and cell death. In contrast, nonproducer cells rarely, if ever, show spontaneous viral activation and are considered "virus-negative". The mechanism of IdU activation remains to be determined; however, it appears to involve a disturbance in normal cell DNA synthesis.

Development of the Radioimmune Assay (RIA) as an Extremely Sensitive Test System for RNA Virus Specific Antigens.

Drs. Gilden and Charman of the Flow and USC contracts, and Drs. Parks and Scolnick (VCB) have each developed highly sensitive RIA tests for detection of gs and envelope antigens for mouse, hamster, rat, cat, woolly, and gibbon RNA tumor viruses. These tests represent modifications of techniques developed by Dixon's group (Scripps Inst.) initially for highly purified cellular antigens These tests have greatly amplified the possibilities for detecting small amounts of RNA tumor virus protein expressions and present VCB-STS and the SVCP-supported programs with a powerful new immunological probe into tumor cell systems.

Interactions Between RNA Tumor Viruses and Other Viral Agents.

Dr. Walter Eckhart (Salk Institute) is trying, first, to determine whether infection by a murine leukemia virus overcomes any temperature-sensitive mutation in polyoma; and, second, to determine whether polyoma infection induces the appearance of RNA tumor virus RNA in cells carrying inducible viral genomes.

The work conducted thus far on the synthesis of endogenous RNA tumor virus RNA in BALB/3T3 cells infected with polyoma, or transformed by SV40 viruses, suggests that new RNA virus-specific sequences are transcribed after polyoma infection or SV40 transformation. Fur er studies are in progress to determine whether the new RNA sequences correspond to "oncogenic" information. DNA probes containing sequences corresponding to the sarcoma information of murine sarcoma or leukemia viruses are being used.

RNA Tumor Viruses in Primates.

Continuing studies by Kawakami (University of California, Davis), Parks (VCB), and Gilden (Flow Labs.) of woolly and gibbon type C RNA tumor viruses led to several new observations, as follows: (1) Additional new lymphosarcomas obtained from gibbons were shown to contain type C viruses with the same properties as the original strain. (2) The gs antigens of the woolly and gibbon viruses appear to be antigenically the same. (3) These two observations were made definite by the development of highly specific antiserum to purified gs antigen by Dr. Gilden's group.

These observations are exciting and important because they indicate that during the evolution of primates, their RNA tumor viruses may not have become quite so species-specific as did those of rodent and feline species. This new observation provides some hope that the putative human type C viruses may show antigenic relationships to the viruses of primates.

Proposals for Studies of Immune Responses in human cancer patients to human candidate formalin-inactivated viral vaccines are in preparation. The current candidate viruses will include the several mouse, human and cat-human viruses which show considerable evidence of having viral envelope antigens distinct from those of the usual cat or mouse viruses. We are also interested in cellular membrane antigens specified by these viruses.

Similar formalin-inactivated vaccines (as described in mouse and rat test systems) do produce high titers of both CF and neutralizing antibodies in their respective hosts when the animals are given several immunizing doses.

Initially the safety tested and approved vaccines will be assayed primarily for their immune response capabilities (both cell-mediated and humoral) by collaborating immunologic teams (Gilden, Dixon, Parks, Hellstrom, Herberman). Their possible effects in controlling cancer growth will be also carefully evaluated in several outstanding centers for cancer chemotherapy and surgery (USC, Children's and Orthopaedic Hospitals in Los Angeles).

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Serial No. NCI 4924

1. Viral Carcinogenesis Branch, OASDVO, Division of Cancer Cause and Prevention
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: (A) Determination of neutralizing antibodies in sera of mice immunized with C-type virus vaccines.

(B) Production of C-type viruses for use in viral vaccine studies.

(C) Studies on the natural expression of HaLV in tissues from normal hamsters and hamsters bearing chemically and DNA virus induced tumors.

Previous Serial Number: Same

Principal Investigator: Dr. Gary J. Kelloff

Other Investigators: Dr. Robert J. Huebner
Mr. Worth I. Capps
Mr. W.T. Lane
Mr. Paul Hill

Cooperating Units: Outside NIH:

Dr. Robert L. Peters, Microbiological Associates, Inc., Walkersville, Maryland
Dr. Aaron Freeman, Children's Hospital, Akron, Ohio

Man Years:

Total:	4.0
Professional:	1.0
Other:	3.0

Project Description

Objectives:

(A) To determine the presence of neutralizing antibodies to MuLV in sera of several strains of mice immunized with several strains of MuLV vaccines and in unimmunized controls; and in sera of mice immunized with exogenous viral and nonviral infected cellular vaccines.

(B) Production, characterization, and supply of sucrose band purified type C virus for use as viral vaccines in collaborative studies with Dr. Robert L. Peters, Microbiological Associates, Inc., and with Mr. W.T. Lane, Poolesville field laboratory.

(C) Determine the natural expression of infectious HaLV and HaLV gs antigen in tissues from normal hamsters and hamsters bearing chemically and DNA virus induced tumors.

(D) In vitro assays of neoplastic and normal tissue specimens for infectious type C virus expression.

Methods Employed and Major Findings:

(A) Standard tissue culture methods for virus growth and titration are used including the XC plaque test. The neutralization test is performed by mixing dilutions of test and control sera with standard (100 PFU) doses of several strains of MuLV (usually including AKR-MuLV, RLV, NZB-MuLV, and WM-MuLV). To date the following sucrose banded viral vaccines have been used to immunize the following strains of mice: AKR-LV and RLV have been used to immunize NIH Swiss and BALB/c mice; wild mouse virus has been used to immunize NIH Swiss, C57BL and wild mice; and NZB virus has been used to immunize SJL and C3H/He mice. Test bleeds of NIH and BALB/c mice have shown low-titered neutralizing activity against the immunizing virus AKR-LV or RLV, and although low-titered, this activity has been specific for virus envelope type, attesting to the specificity of the immunologic reactions. The sera from the wild mouse virus and NZB virus immunized mice have currently been pooled according to their complement-fixing reactivity with fresh banded homologous virus and are currently under investigation with the four test viruses. Correlations of the host's immune response, both humoral and cellular, in response to vaccination and the effect on spontaneously and chemically induced tumors in mice are being carried out in collaboration with W.T. Lane, Poolesville field laboratory, and with Dr. Robert L. Peters and other investigators at Microbiological Associates, Inc., Walkersville, Maryland.

(B) AKR-LV and RLV are currently being produced by infection of Swiss mouse embryo fibroblasts with high doses of stock viruses and after a two-week expansion of the cultures, supernatant fluids are harvested every other day. The fluids are clarified, pelleted at 18,000 RPM for 3 hours in a Beckman fixed angle rotor #19, and the virus is banded in sucrose density gradient at 24,000 RPM for 3 hours in Beckman SW25.1 rotor. The virus bands are collected by bottom puncture fractionation and tested by complement-fixation, for infectivity by the COMUL test, and for polymerase activity. The bands are fixed in formalin at 1:4000 for two days and stored in liquid nitrogen. The optimal handling, fixation and storage of this material has been rigorously worked out. Currently the laboratory is producing 12 liters of supernatant fluid per week, which is concentrated to 12 ml of banded virus. The banded product has consistently shown high titers by complement-fixation and infectivity and has been calculated to contain about 3.5×10^{12}

particles/ml or roughly 1.0 mg of virus/ml. This yield is somewhat higher than is usually attainable by harvest of virus from continuous shedding cultures and has proven a very valuable source of virus for vaccine studies and for use as antigen.

(C) A comprehensive study of three strains of hamsters was undertaken to establish the prevalence of hamster type C viruses in normal and neoplastic tissues. Embryonic and postnatal normal tissues from the LSH, NIH and Graffi hamsters were examined for hamster type C virus expression by complement-fixation, electron microscopy, and direct isolation techniques. The only normal tissues found to be positive for HaLV gs antigen expression were early embryonic tissues of the Graffi hamster. In contrast, hamster tumors induced by the chemical carcinogens, 3-methylcholanthrene and DMBA, and by SV40 and polyoma virus, were sometimes found to contain HaLV in the primary tumor and, with transplant passage, a high percentage of these tumors became positive. However DMBA induced tumors of LSH hamsters were an exception. In this case, very few HaLV positive tumors were found, and upon transplant even these became negative. It was concluded that HaLV is widespread in hamster populations, usually in a covert form, and its expression is enhanced in tumors induced by carcinogenic chemicals and DNA viruses. Expression of virus in the tumor is controlled *in situ*.

Significance to Biomedical Research and to the Program of the Institute:

(A) and (B): The vaccine studies are designed to determine whether viral and cellular vaccines can be used in immunoprevention of spontaneously occurring and chemically induced neoplasms. The assessment of the animal's immune response and the production of vaccines are indispensable parts of these studies.

(C): These studies establish that hamsters also have a ubiquitous endogenous type C virus and that chemical carcinogens and DNA viruses behave similarly in that they are both tumorigenic and they both derepress endogenous type C viruses.

Proposed Course:

(A) and (B): Continuation of studies outlined above.
(C): Publication of current findings.

Honors and Awards:

None

Publications:

Kelloff, G., Hatanaka, M., and Gilden, R.V.: Assay of C-type virus infectivity by measurement of RNA dependent DNA polymerase activity. *Virology* 48: 266-269, 1972.

Long, C., Kelloff, G., and Gilden, R.V.: Variations in sarcoma and leukemia virus activity in somatic cell hybrids. Int. J. Cancer 10: 310-319, 1972.

Hampar, B., Gilden, R.V., Kelloff, G., Oroszlan, S., and Simms, D.: Immunofluorescent detection of murine and hamster C-type virus species-specific (gs-1) determinants by monospecific guinea pig sera and interspecies-specific (gs-3) determinants by tumor bearing rat sera. Int. J. Cancer 8: 425-431, 1972.

Serial No. NCI 4925
1. Viral carcinogenesis Branch
OASDVO, Division of Cancer
Cause and Prevention
2. Ecology and Epizoology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Studies of Type C RNA Tumor Viruses

Previous Serial Number: Same

Principal Investigator: Dr. Padman S. Sarma

Other Investigators: Dr. Robert J. Huebner
Dr. D. Verwoerd (Visiting Scientist)
Dr. Paul Arnstein
Mr. Paul Hill

Cooperating Units: Inside NIH:

Dr. Adi Gazdar, VLLB, NCI

Outside NIH:

Dr. M. Gardner, USC School of Medicine, Los Angeles, California
Dr. R. Gilden, Flow Laboratories, Rockville, Maryland
Dr. L. Vernon, Microbiological Associates, Inc., Bethsda, Maryland
Dr. David Allen, Massachusetts General Hospital, Boston, Massachusetts

Man Years:

Total:	9.0
Professional:	2.0
Other:	7.0

Project Description

Objectives:

This project is concerned with basic studies on the isolation and characterization of the biological, antigenic, biophysical and biochemical properties of known model type C RNA tumor viruses and those newly isolated from primate species. A large portion of the effort is directed towards the development and use of sensitive in vitro systems for studying the prevalence and behavior

of overt as well as covert type C viruses. Various approaches are being intensively applied to animal and human tumor systems having covert ("switched off") viral genomes in efforts to "turn on" or rescue overt expressions of the viral genomes.

Major Findings:

RD114 virus is a covert endogenous virus of cats: In past collaborative studies with investigators of other laboratories (Drs. R. Gilden, M. Gardner, R. McAllister) we established the salient antigenic and biologic properties of a suspected human candidate virus, RD114, derived from a cell line of human rhabdomyosarcoma.

In our recent attempts to isolate and characterize vertically transmitted covert, endogenous virus of cats, we discovered that an established cell line of adult cat kidney, Crandell cat cells, contained an infectious virus with properties identical to RD114. We succeeded in inducing the same virus from virus-free sublines of Crandell cells we obtained from two other sources.

The induced RD114-like virus is identical with the RD114 virus in properties such as tropism for human cells rather than cat cells and the antigenic characteristics of the group-specific antigen, the envelope antigen, and the reverse transcriptase enzyme.

In a more recent study, we isolated a RD114-like virus from a thymus tumor of a cat fetus (in collaboration with Dr. M. Gardner).

These studies suggest that cats have a second major group of type C virus entirely distinct from the presently known feline leukemia and sarcoma viruses.

Isolation of RD114 virus and a feline leukemia virus from tissues of cat XC 114 B: The RD114 virus-producing rhabdomyosarcoma cell line was initially established in tissue culture after one in vivo passage in a cat (McAllister et al., 1972). The spleen and bone marrow of this cat were observed to have type C virus particles by Dr. M. Gardner. We succeeded in isolating an infectious RD114-like virus and a feline leukemia virus from both of these tissue specimens. In the light of the new finding that RD114 is of cat origin, it appears that the human cell RD got infected with the vertically transmitted feline RD114 virus.

Studies of prevalence of antibodies in humans and cats against cat RD114 virus: We have thus far been unable to find RD114 virus neutralizing antibodies in 64 cats with or without neoplasia. Similarly we failed to find virus-neutralizing antibodies in the sera of 70 humans with or without cancer and in sera of 12 laboratory workers in this laboratory.

A study of the prevalence of feline type C virus neutralizing antibodies in sera of cats and humans: We have found that close association of humans with cats or handling of feline leukemia and sarcoma viruses by laboratory workers

does not result in the development of detectable type-specific virus neutralizing antibodies in such subjects. We have been unable to detect virus neutralizing antibodies against A, B or C serotypes of feline. This finding indicates that FeLV may not be a threat to humans although previous studies have shown that this virus is highly infectious for human cells in vitro.

Sera of a proportion of normal cats and cats with neoplasia contained demonstrable levels of virus-neutralizing antibodies against FeLV of one or more subgroups of A, B and C. This finding and similar findings of other investigators suggest that the occurrence and mode of spread of feline type C viruses may be analogous to that known for avian type C viruses.

Studies of viral envelope antigens of feline leukemia and sarcoma viruses: We recently described a subclassification of feline leukemia and sarcoma virus into three subgroups of A, B and C. The major envelope antigens responsible for type-specificity were initially detected by the induction of a type-specific viral interference by a leukemia virus against the cell transforming effects of challenge feline sarcoma virus and murine sarcoma virus (MSV) with the viral envelopes of feline leukemia virus (feline leukemia pseudotypes of MSV). We subsequently found that the envelope antigens responsible for type-specific viral interference patterns are also responsible for the development of type-specific virus neutralizing antibodies. Thus, feline type C viruses can be classified into the same subgroups either by viral interference or viral neutralization tests. Several field strains of feline leukemia virus (FeLV) including antigenically mixed types occurring in nature were 'purified' into single antigenic types by cloning techniques and classified into the same antigenic subgroups by viral interference as well as viral neutralization tests.

Studies of the biologic and antigenic characteristics of rat type C viruses: We found that many established strains of rat cells including cell lines transformed by RSV release type C virus particles with the gs-1 antigen of rat type C viruses. We recently characterized the Bergs virus from Wistar-Furth cell line. Whereas most rat type C viruses we have studied are non-infectious, the Bergs virus was infectious in vitro for normal rat cells. We were able to prepare rat gs-1 antigen and corresponding antibodies with the Bergs virus. We recently characterized three other rat viruses derived from virus-producing rat cells obtained from Bob Peters (WR-9), Victor Bergs (BV-1) and from Dr. Lennette. All of the three appear to be completely noninfectious for a variety of cell species. It appears the WR-9 virus, although it is noninfectious, is quite suitable for rat gs-1 antigen production.

Control of group-specific antigen synthesis by a new strain of murine sarcoma virus (Gazdar strain): Hamster cells transformed by the Gazdar strain of murine sarcoma virus (Gz-MSV) release 'noninfectious' sarcoma virus particles containing the species-specific group-specific antigen (gs-1) of the mouse type C viruses. On trans-species rescue of this defective murine sarcoma virus (MSV) from hamster cells with feline leukemia virus (FeLV), the 'pseudotype' cell-transforming virus recovered has the envelope antigenic

and host range characteristics of FeLV and the gs-1 antigenic markers of mouse as well as cat type C viruses. The presence of the mouse gs-1 antigenic marker in Gz-MSV and the rescue of this marker from hamster tumor cells with FeLV strongly suggest that the Gz-MSV differs from the other presently known strains of MSV in its innate capacity to direct the synthesis of gs-1 viral protein component of the mouse leukemia group.

Studies of a chicken flock with a high incidence of solid tumors: In a collaborative study with Drs. Henderson and Gardner (USC) we studied a flock of chickens with a high incidence of solid tumors after vaccination with live Marek's disease Herpesvirus vaccine. We isolated infectious avian leukosis virus from over 80% of tissues of normal as well as vaccinated chickens. This is an unusually high incidence of infectious virus and it appears that the solid tumor development may be due to a co-carcinogenic effect of Herpesvirus and infectious leukosis virus.

Significance to Biomedical Research and to the Program of the Institute:

Studies performed during the reporting period were directed in part towards getting definitive and much needed information on the delineation of the occurrence and characteristics of as yet undetected as well as newly discovered type C viruses of man, cat, rat and mouse. These and other model tumor virus systems were intensively studied to better understand the biologic and antigenic similarities and interrelationships between the viruses of various species. These studies, as well as studies of the expression of the vertically transmitted endogenous tumor virus genomes, have been successful and provide tools for further studies of the natural occurrence and spread of leukemogenic and/or sarcomagenic viruses in homologous and heterologous species, including man.

Our studies and those of others (Todaro and Fischinger) have now established that RD114 virus can no longer be considered a human candicate type C virus. This virus appears to be a covert endogenous virus of cats. The absence of demonstrable neutralizing antibodies against this virus in humans suggests that man may not be at risk of infection with this virus.

The re-isolation of RD114 virus from the tissues of the original cat yielding RD114 virus containing RD cells provided confirming evidence on the cat derivation of this virus.

Our virological and sero-epidemiological studies of feline leukemia and sarcoma viruses provided evidence on the occurrence of multiple antigenic types of these viruses in nature, very often in the same host. Man does not appear to be at risk of infection with these viruses.

The viral envelope antigens of feline type C viruses could be identified by viral interference and viral neutralization tests and either of these tests could be used for a subgroup classification of these ciruses as described recently.

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Diverse strains of rat type C viruses were predominantly found to occur in the noninfectious state, with a viral density lower than that observed for type C viruses of other species. In collaborative studies with Dr. D. Verwoerd (see separate report), evidence was obtained that apparently normal rat cells of three rat strains contained covert, vertically transmitted type C viruses demonstrable by induction techniques. This observation lends further support for the viral 'oncogene' hypothesis.

Proposed Course:

Virological and immunological studies are being performed to detect the presence of covert type C viral genome in human cells. The natural history of RD114 virus in cats and other felines will be investigated. Collaborative studies are planned to elucidate the type C viral etiology of bovine leukemia and turkey leukosis.

Honors and Awards: None

Publications:

Sarma, P.S. and Log, T.: Viral envelope antigens of feline leukemia and sarcoma virus. Proc. Fifth Int. Symp. Comp. Leuk. Res., in press.

Sarma, P.S. and Log, T.: Subgroup classification of feline leukemia and sarcoma viruses by viral interference and neutralization. Virology, in press.

Sarma, P.S., Log, T. and Gazdar, A.: Control of group-specific antigen synthesis by the defective Gazdar murine sarcoma virus genome. Virology, in press.

Sarma, P.S., Tseng, J., Lee, Y.K., and Gilden, R.V.: A 'covert' C type virus in cat cells similar to RD-114 virus. Nature, in press.

Sarma, P.S., Gazdar, A.F., Turner, H.C., and Kunchorn, P.D.: Gazdar strain of murine sarcoma virus. Biologic and antigenic interaction with the heterologous hamster host. Proc. Soc. Exp. Biol. Med. 140: 928-933, 1972.

Sarma, P.S., Sharar, A.L. and McDonough, S.: The SM strain of feline sarcoma virus. Biologic and antigenic characteristics of virus. Proc. Soc. Exp. Biol. Med. 140: 1365-1368, 1972.

Rabin, H., Theilen, G.H., Sarma, P.S., Dungworth, D.L., Nelson-Rees, W.A. and Cooper, R.W.: Tumor induction on squirrel monkeys by the ST strain of feline sarcoma virus. J. Nat. Cancer Inst. 49: 441-450, 1972.

Allen, D.W., and Sarma, P.S.: Identification and localization of avian leukosis group-specific antigen within "leukosis-free" chick embryos. Virology 48: 624-626, 1972.

Serial No. NCI 4926
1. Viral Carcinogenesis Branch
OASDVO, Division of Cancer
Cause and Prevention
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: (A) Transcriptional Controls During Endogenous Type C Viral Infection.
(B) Alterations of Polysome Profiles During Transformation.

Previous Serial Number: Same

Principal Investigator: Dr. F.H. Portugal

Other Investigators: Dr. Josephine Simonds
Dr. Daniel Twardzik
Miss Marianne Oskarsson
Mr. Patrick Mulroy

Cooperating Units: Inside NIH:

Dr. Robert J. Huebner, VCB, DCCP, NCI
Dr. Wallace Rowe, LVD, NIAID
Dr. Janet Hartley, LVD, NIAID

Outside NIH:

Dr. Hans Meier, The Jackson Laboratory, Bar Harbor, Maine
Dr. Leonard Hayflick, Stanford University, Stanford, California

Man Years:

Total:	2.0
Professional:	1.0
Other:	1.0

Project Description

Objectives:

(A) To determine the mechanism regulating transcription of endogenous type C viral information.

(B) To determine the characteristics of the endogenous transcribed type C viral genome product, namely the viral polysome.

Serial No. NCI 4926

Methods Employed:

(A) AKR cell lines are labelled by adding isotopic amino acids to the cell culture media. Extracted proteins are passed over DNA-cellulose columns, and proteins binding to the column are eluted with increasing concentrations of salt. The respective fractions are analyzed by gel electrophoresis.

(B) Polysomes are isolated from the spleens of different genetic strains of mice by discontinuous and linear sucrose gradients. Polysome products are determined following in vitro protein synthesis and immunoprecipitation by gel electrophoresis.

Major Findings:

(A) A protein of molecular weight 80,000-90,000 has been found which decreases in cells undergoing spontaneous activation of endogenous type C viral information. This protein which binds to homologous mouse DNA represents approximately 50% of an elution fraction which blocks transcription of DNA into RNA product in vitro.

(B) A polysome fraction has been isolated that can be translated in vitro. At least one protein is virus specific and reacts with gs-3 antisera.

Significance to Biomedical Research and to the Program of the Institute:

(A) The significance of this project is in understanding the biochemical mechanism for the natural occurrence of leukemias and sarcomas in the absence of overt viral infection.

(B) Elucidation of the protein regulating transcription and the product made by viral polysomes should enable increased surveillance by immunological methods in the progress of these diseases.

Proposed Course:

(A) The P_1 protein is being prepared in large quantity and its physical, chemical and immunological characteristics are being studied in detail.

(B) The polysome fraction producing gs-3 protein is also being isolated in quantity. The size of the RNA message is being studied as are the number and type of other proteins produced.

Honors and Awards:

None

Publications:

Portugal, F.H., Simonds, J., Twardzik, D.R., Mulroy, P.F., and Oskarsson, M.: Comparison of DNA-binding proteins from mouse cells in culture. Biochem. Biophys. Res. Commun., in press.

Serial No. NCI - 4927
1. Office of the Associate Scientific Director, Viral Oncology
2. Division of Cancer Cause and Prevention
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Electron Microscopic Survey and Ultrastructural Studies of Viruses in Various Human and Animal Tissues

Previous Serial Number: Same

Principal Investigator: Dr. Victor H. Zeve

Other Investigators: None

Cooperating Units: Dr. Adeline Hackett, Naval Biological Laboratories, Oakland, California
Dr. Murray Gardner, University of Southern California, Los Angeles, California
Dr. Robert Nathan, Jet Propulsion Laboratories, Pasadena, California
Dr. Stuart Aaronson, VLLB, NCI

Man Years:

Total:	1
Professional:	1
Other:	0

Project Description

Objectives:

(1) Search for type B and C RNA viruses in human milk as well as other cancer and normal human tissues.

(2) To develop mew methods of rapid and positive identification of A, B, and C type viruses by utilizing unstained materials subjected to image intensification and image enchancement techniques.

(3) To clarify and define structures which are similar to A, B, and C type viruses in negatively stained preparation of human milk.

Methods Employed:

Samples of human milk were collected in various ways and subjected to several techniques for electron microscopic studies. It was anticipated that the usual collection procedures, storage and preparation of milk samples greatly altered the ultrastructural appearance of "virus like" particles in human milk. This work is being done in cooperation with Dr. Murray Gardner, U.S.C. and at the Jet Propulsion Laboratories, California Institute of Technology, Pasadena where computer analysis of negatively stained materials were carried out. In addition to negatively stained preparations, thin sections of human milk pellets were studied utilizing image intensification and storage of unstained images on magnetic tape. These tapes were computer analyzed by image enhancement to determine the effect of phase grain and heavy metal staining on the appearance of "virus like" structures.

Major Findings:

Several ultrastructural changes can be observed in milk preparations which have been collected and stored for various lengths of time and in various fixatives. Frozen milk as opposed to milk collected and immediately mixed with chilled Glutaraldehyde appear to give more satisfactory results. The identification of A, B, and C type virus by negative stain techniques is highly unreliable for identification of virus in milk samples. Positive identification of virus in milk can only be made by examining thin sections at the present time.

Studies in collaboration with Dr. Adeline Hackett on candidate human cell lines continue to give negative results. At present, polymerase assays appear to be a more sensitive method of screening cell lines for the presence of virus.

Studies in collaboration with Dr. Robert Nathan, Jet Propulsion Laboratories continue to investigate by image intensification and computer enhancement of unstained materials to determine how much structural information is masked by lead and uranyl acetate staining. Comparative studies of various C type viruses continues.

Significance to Biomedical Research and the Program of the Institute:

The association of Type A, B, or C virus to human breast cancer must still be determined. If a B type particle is the etiologic agent in this cancer then a positive identification must be made by thin section techniques rather than negative stain preparations which give inconclusive results. Any method which would enable us to more clearly identify viruses occurring in human cancers must be investigated. At present the visualization of virus particles in the E.M. is the only method of associating virus to human cancers. The importance of positively identifying virus like structures in human cancer is extremely important for the continued efforts along the lines of cancer cause and prevention.

Serial No. NCI - 4927

Proposed Course:

Major emphasis will focus on positive identification of virus like structures in human milk and biopsies of other types of cancer. Studies will be performed at the Frederick Cancer Research Center to study new instrumentation and techniques to clarify ultrastructural findings.

Serial No. NCI-4928

1. Viral Carcinogenesis Branch, OASDVO, Division of Cancer Cause and Prevention
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Progress Report
July 1, 1972 through June 30, 1973

Project Title: Immunological Studies of Virion-Associated Antigens in Natural Tissues

Previous Serial Number: Same

Principal Investigator: Dr. Wade P. Parks

Other Investigators: None

Cooperating Units: Inside NIH:

Dr. Robert J. Huebner, VCB, NCI
Dr. Edward M. Scolnick, VLLB, NCI
Dr. George J. Todaro, VLLB, NCI

Outside NIH:

Dr. Murray Gardner, University of Southern California, Los Angeles, California
Dr. Raymond Gilden, Flow Laboratories, Inc., Bethesda, Md.
Dr. Thomas Kawakami, University of California, Davis, Ca.
Dr. Hans Meier, Jackson Laboratory, Bar Harbor, Maine
Dr. John Verna, Meloy Laboratories, Springfield, Va.

Man Years:

Total:	1.0
Professional:	1.0
Other:	0.0

Project Description

Objectives:

(1) Combining previous work with the syncytium-forming viruses with an increasing capability to detect and measure other potentially oncogenic viruses in natural tissues, to investigate the following systems:

a) Immunological relationships of mammalian Type-C viruses
b) Natural expression of Type-C viruses in tissues and tumors from mice, cats and primates including man
c) Mouse mammary tumor virus expression

(2) To characterize viral surface proteins involved in natural immune reactions and cell surface proteins associated with transformed cells.

Methods Employed:

A wide variety of technical procedures are essential to any comprehensive investigation of the interaction between viruses, cells and host immune responses. Standard biological assays include measurements of XC plaque formation, transformed foci determinations, the ability of cells to grow in a variety of conditions to distinguish transformed from "normal" cells, etc. The interphase between the biological aspects of these problems and biochemical analyses include polymerase (reverse transcriptase) determinations, sucrose density gradients, nucleic acid hybridization studies and the technical consequences thereof. Electron microscopy continues to be a key adjunct to our efforts. Immunologically, the minimum procedures include a variety of column procedures, immunoelectrophoresis, radioimmune precipitation, animal work, complement fixation, immunodiffusion and other measures of antigen-antibody interaction. Finally, the preparation of purified virus on an adequate scale necessitates large scale tissue culture facilities, containment technology and ultracentrifugation capabilities. All of the above methods were central to the accomplishment of our objectives and were subjected to repeated evaluation, improvement and ultimately standardized quality control.

Major Findings:

(1) Immunological relationships of mammalian Type-C viruses has been examined primarily in two ways. First using antisera to mammalian Type-C viral reverse transcriptases, we showed that the recently discovered primate viruses are very closely related immunologically. Conversely, the RD-114 virus appeared to be unrelated to any previously described mammalian Type-C virus. A virus isolated from a rhesus monkey mammary tumor, the Mason-Pfizer monkey virus (MP-MV) was unrelated to either known Type-C viruses or the primate syncytium-forming ("foamy") viruses. Consequently, the three known primate virus groups (Type-C, MP-MV and foamy) are distinct from one another and yet share in the biological consequences of reverse transcription. Since any or all of these viruses may be found in human neoplasia increased knowledge about them is needed.

As an example, a virus, recently reported in the literature, from a transformed brain culture of a patient with Creutzfeldt-Jakob disease was studied. On examination this virus cannot be distinguished from the monkey virus, MP-MV. Since cross-contamination between known MP-MV

and the human culture is possible, we must await other isolates before drawing conclusions as to the host of origin of this putative human virus. Nevertheless, we have recently noted the presence of morphologically similar particles to the MP-MV in cultures sent to the SVCP by Russian virologists. Further study is obviously necessary to evaluate the significance of these findings.

Second, we have purified the major internal protein of mouse, cat, woolly, gibbon and RD-114 viruses to homogeneity. These proteins have both intra- and interspecies antigenic determinants common to all mammalian Type-C viruses and all are approximately 30,000 daltons in size. These are the viral gs antigens or VP3 based on their relative migration in polyacrylamide gel electrophoresis. By establishing species specific radioimmunoassays for the respective viral gs antigens we could show that the primate viruses are highly related, that RD is distinct from previously described mammalian Type-C viruses, thus independently confirming our observations with the viral polymerase. We showed that the primate Type-C viruses and RD-114 virus had the interspecies or gs-3 antigenic determinant(s) but that this reactivity was distinct from the reactivity of mouse, cat, rat or hamster viral gs antigens. The radioimmunoassay for the interspecies reactivity detects reactivity in purified pig Type-C virus, leukemic bovine cells and rabbit lymphosarcoma tissues.

Natural expression of the mouse gs antigen was found in all mouse tissues examined. This strongly suggests that a continuous synthesis of this polypeptide is an integral part of the murine cell macromolecular synthesis. Although further substantiating the existence of viral information in all murine cells, this information does not answer the question of the function of this protein (other than holding the virion together) nor its role in natural tumors.

Measurement of feline viral antigen in cat tissues and tumors revealed a very high correlation of particles and antigen as well as an excellent correlation of antigen with tumors. RD-114 antigen was not detected in over 200 feline tissues examined by radioimmunoassay.

Studies with radioimmunoassays for gs-3 or primate viral information in human tumors is still not conclusive, but has generally been negative.

Studies of the mouse mammary tumor virus system have shown the following:

1) Four different cell lines positive for MTV and negative for Type-C virus have been established.
2) Morphologically similar clones of transformed epithelial cells can be found which differ over 200-fold in virus expression although they have identical amounts of MTV genetic information of DNA.
3) The major internal protein of MTV is 52,000 daltons and can be isolated in pure form iodinated and then employed in radioimmunoassays.

4) High and low mammary tumor incidence strains show marked differences in the levels of antigen in their mammary glands and are being studied for genetic segregation.
5) Synthesis of mammary tumor virus is apparently a differentiated cell function.

2. Viral glycoproteins of the murine Type-C viruses have a molecular weight of ~50,000 daltons and >80,000 daltons. They constitute less than 10 percent of the virion protein and may be involved in neutralization of the virus in tissue culture. These proteins appear to be located on the surface of the cells, analogous to the localization of proteins responsible for transformation by viruses.

Significance to Biomedical Research and to the Program of the Institute:

(1) The ability to identify viruses and to detect viruses in tissues is basic to establishment of etiologic associations and to an ultimate approach to prevention or treatment. Given the potential importance of viruses to diseases including cancer, the approach outlined herein offers one of the more important directions open to viral oncology at the present time.

(2) Ultimately an understanding of natural protection, immunization or possibly even causation will derive from studies of membrane proteins. This area will be the continuing focus for localization of "transforming proteins(s)" as well as host immune responses.

Proposed Course:

(1) Continued evaluation of viral expression relative to natural diseases with Type-C and Type-B viruses.

(2) Characterization and development of immunological methodologies to study cell membrane proteins.

Honors and Awards:

None

Publications:

Livingston, D. M., Scolnick, E. M., Parks, W. P., and Todaro, G. J.: Affinity chromatography of RNA-dependent DNA polymerase from RNA tumor viruses on a solid phase immunoadsorbent. Proc. Nat. Acad. Sci. U.S.A. 69: 393-397, 1972.

Parks, W. P. and Scolnick, E. M.: Radioimmunoassay of mammalian Type-C viral proteins: Interspecies antigenic reactivities of the major internal polypeptide. Proc. Nat. Acad. Sci. U.S.A. 69: 1766-1770, 1972.

Serial No. NCI-4928

Parks, W. P., Scolnick, E. M., and Gilden, R. V.: Immunological studies of mammalian Type-C viral proteins. In Day, S. B. and Good, R. A. (Eds.): Membranes and Viruses in Immunopathology. New York, Academic Press, 1972, pp. 339-354.

Scolnick, E. M., Parks, W. P., and Todaro, G. J.: The reverse transcriptases of primate viruses as immunological markers. Science 177: 1119-1121, 1972.

Livingston, D. M., Parks, W. P., Scolnick, E. M., and Ross, J.: Affinity chromatography of avian Type-C viral reverse transcriptase. Virol. 50: 388-395, 1972.

Tronick, S. R., Scolnick, E. M., and Parks, W. P.: Reversible denaturation of the DNA polymerase of Rauscher leukemia virus. J. Virol. 10: 885-888, 1972.

Parks, W. P., Livingston, D. M., Todaro, G. J., Benveniste, R. and Scolnick, E. M.: Radioimmunoassay of mammalian Type-C viral proteins. III. Detection of antigen in normal murine cells and tissues. J. Exp. Med. (In press), 1973.

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Serial No. NCI-4929

1. Viral Carcinogenesis Branch, OASDVO, Division of Cancer Cause and Prevention
2. Solid Tumor Virus Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Herpesviruses: Immunologic and Biologic Studies in Relation to Human Tumors

Previous Serial Number: SAME

Principal Investigator: Dr. Berge Hampar

Other Investigators: None

Cooperating Units: Inside NIH:

None

Outside NIH:

Dr. L. M. Martos, Flow Laboratories, Inc, Rockville, Maryland
Dr. J. G. Derge, Flow Laboratories, Inc., Rockville, Maryland
Dr. R. V. Gilden, Flow Laboratories, Inc., Rockville, Maryland
Dr. R. Lerner, Scripps Clinic and Research Foundation, La Jolla, California

Man Years:

Total:	2.0
Professional:	1.0
Other:	1.0

Project Description

Objectives:

(1) To study the mechanism of herpesvirus latency in human cells, with emphasis on the Epstein-Barr virus associated with Burkitt's lymphoma.

(2) To determine the relevance of herpesviruses associated with human tumors.

(3) To study the relationship between herpesviruses and Type-C viruses in dually infected human cells.

Methods Employed:

Standard tissue culture and virologic methods were employed.

Major Findings:

(1) Sequence of Spontaneous Epstein-Barr Virus (EB Virus) Activation:

The P3HR-1 producer line of human lymphoblastoid cells was made resistant to 5-bromodeoxyuridine (BrdU) and the sequence of EB virus activation studied. The P3HR-1(BU) cells are negative for the enzyme thymidine kinase (dTK). When spontaneous virus activation occurs, however, dTK appears in the activated cells.

Virus activation is initiated during the cells' S phase and is prevented by ara-C or hydroxyurea added to stationary phase (G-1) cells. The DNA synthesis required for initiation of the activation sequence apparently involves normal cell DNA, and occurs in P3HR-1(BU) cells which are dTK negative. Virus activation is followed by synthesis of the early antigen (EA) complex in the absence of additional DNA synthesis. This is followed by another cycle of DNA synthesis where both cell and viral DNAs are made and where the cells are now dTK positive. This cycle of DNA synthesis is inhibited by ara-C, but is not inhibited by concentrations of hydroxyurea which inhibit DNA synthesis in non-activated cells. Synthesis of viral DNA is followed by synthesis of the viral antigen (VCA) complex and cell death.

(2) Identification of Critical Period of Cell DNA Synthesis for EB Virus Activation by 5-iododeoxyuridine (IdU):

Both EB virus producer and non-producer cells carry the EB viral genome in a repressed state. Producer cells show spontaneous activation of the repressed viral genome leading to synthesis of virus antigens and cell death. In contrast, non-producer cells rarely, if ever, show spontaneous viral activation and are considered "virus-negative." Previous studies described activation of the repressed viral genome in non-producer cells treated with thymidine analogues.

Studies were carried out to determine whether IdU activation of EB virus in human cells required incorporation of the analogue during a specific period in the cells' S phase. One producer cell line (EB-3) and one non-producer cell line (Raji) were selected for study. The cells were synchronized by the double thymidine blocking technique and were treated with 20 µg per ml of IdU for intervals of 30 or 60 min. and virus activation was assessed by immunofluorescence. Both cell lines showed a peak of virus activation in cells treated with IdU at 60 min. into the cells' S phase. Incorporation of IdU during other periods

of the S phase resulted in little or no activation when compared to control cells. The critical period for IdU incorporation to activate virus occurred prior to the periods of maximum drug incorporation. The results suggested that cell DNA made at approximately 60 min. into the S phase contains unique sequences which control repression and activation of EB virus. In the case of EB-3 cells, IdU activation resulted in synthesis of both EA and VCB (in 50 percent of the activated cells), while activated Raji cells showed synthesis of only EA.

The mechanism of IdU activation remains to be determined; however, it appears to involve a disturbance in normal cell DNA synthesis. This is manifested by a reduction in the rate of cell replication and DNA synthesis, and is seen in all samples which incorporate IdU regardless of whether activation occurs. Consequently, a mere disturbance in cell DNA synthesis is not sufficient in itself to activate virus, since it must occur at a critical period during the cell's S phase.

Activation of EB virus in both producer and nonproducer cells can be achieved with relatively short periods of drug treatment (30 to 60 minutes). In the case of nonproducer Raji cells, synthesis of EA in activated cells persists for only a short time after drug treatment (1 to 3 days) and the cells then regain a nonproducer status. These findings make it unlikely that activation by thymidine analogues is due to a heritable mutational change. In contrast to short-term drug treatment, nonproducer Raji cells treated for prolonged periods (several weeks) with increasing conentrations of drug show synthesis of EA, VCA and virus particles which persist. This conversion of a non-producer cell to a producer cell probably involves a heritable mutational change induced by the incorporated analogue.

(3) Fusion of Human Lymphoblastoid Cells with Type C Viruses and Activation of EB Virus:

Previous studies described the establishment of carrier cultures in EB virus producer and nonproducer cells infected with murine (KiMSV) and feline sarcoma viruses. Subsequent studies by other workers described carrier cultures with feline leukemia viruses (FeLV). Studies were undertaken to determine the effect of Type C viruses on human lymphoblastoid cells.

Three human cell lines (P3HR-1, WI-L2, and Raji) when infected with either RD-114 virus or FeLV showed syncytia involving up to 20 percent of the cells within 2-3 hrs post-infection. The formation of syncytia was due to cell fusion rather than endomitosis as shown by autoradiography. When isotope labeled and unlabeled cells were mixed and infected by Type C virus, the syncytia which appeared within 3 hrs post-infection showed both labeled and unlabeled nuclei in the same cell. The percent fused cells decreased over a 7 day period to control cell levels. This was followed within 2-3 weeks by another cycle of fusion involving up to 20 percent of the cells. From this point on carrier cultures formed which showed continued synthesis of Type C virus in the absence of further cell fusion.

Type-C viruses other than RD-114 and FeLV were also tested for their ability to fuse human lymphoblastoid cells. Neither RLV, RaLV or HaLV induced fusion when tested at comparable input doses as employed with RD-114 and FeLV. Whether higher input doses of these viruses or the use of different host cells for propagating virus stocks would have resulted in cell fusion cannot be excluded. Consequently, we would only conclude that RD-114 and FeLV can fuse human lymphoblastoid cells and would not exclude similar effects by other Type-C viruses.

Cell fusion by RD-114 virus was also tested after inactivation with B-propiolactone (BPL). Virus was inactivated with BPL (1:2000 dil.) and residual infectivity tested on permissive RD and KB cells. In no case was residual infectivity detected. When lymphoblastoid cells were infected with BPL-inactivated RD-114 virus, syncytia were evident during the first 24 hours post-infection similar to those observed with live virus. The syncytia disappeared within 5-7 days with no evidence of virus replication. At 3-4 weeks post-infection, however, a second cycle of syncytia appeared followed by replication of live virus and the establishment of carrier cultures. The appearance of live virus following infection with BPL-inactivated virus has yet to be explained, but two possibilities may be considered. First, the BPL treatment may not have inactivated all virus. This combined with the possibility that human lymphoblastoid cells are more sensitive to RD-114 virus than either RD cells or KB cells could account for the infectivity observed with BPL inactivated virus. Second, infectious RD-114 virus may have appeared following recombination of viral genetic material in cells infected with BPL-inactivated virus.

Additional studies were carried out with RD-114 and FeLV carrier cultures to determine their susceptibility to fusion following re-infection. The RD-114 carrier cultures were resistant to fusion induced by either RD-114 virus or FeLV; the FeLV carrier cultures were resistant to fusion induced by FeLV but were still susceptible to fusion induced by RD-114 virus. This suggests a possible sharing of antigens between these two presumably feline viruses and also demonstrates a significant difference between the two.

Cells infected with either live or BPL-inactivated RD-114 and live FeLV were tested for production of EB virus antigens. Both producer P3HR-1 and non-producer Raji cells showed EB virus antigen synthesis in syncytia suggesting activation induced by cell fusion. In the case of P3HR-1 cells, both EA and VCA were made in fused cells while Raji cells showed synthesis of only EA. Following the disappearance of syncytia, the percentage of virus positive cells in P3HR-1 cultures returned to control levels, while the Raji cultures were again EB "virus-negative" despite the continued production of RD-114 virus. This suggests that Type-C viruses have no direct effect on the replication production of EB virus, although they may indirectly affect EB virus by causing cell fusion.

Serial No. NCI-4929

Significance to Biomedical Research and the Program of the Institute

Elucidating the mechanism of EB virus repression and activation in human lymphoblastoid cells should give some insight into latency by herpesviruses and a possible rationale for implicating this virus group in human neoplasms.

Proposed Course:

Studies will continue relating to persistency of EB virus and other herpesviruses in human cells. Attempts will be made to expand the present in vitro studies to fresh human tissues which would be more representative of the state of the virus in vivo.

Honors and Awards:

None

Publications:

Hampar, B., Derge, J. G. Martos, L. M., Tagamets, M. A. and Burroughs, M. A.: Sequence of spontaneous Epstein-Barr virus activation and selective DNA synthesis in activated cells in the presence of hydroxyurea. Proc. Nat. Acad. Sci., U.S.A. 69: 2589-2593, 1972.

Girardi, A., Hampar, B., Hsu, K. C., Oroszlan, S., Hornberger, E., Kelloff, G., and Gilden, R. V.: Intracellular localization of mammalian Type-C virus species-specific (gs-1) and interspecies-specific (gs-3) antigenic determinants using the indirect immunoperoxidase technique and light microscopy. J. Immunology (In press).

Hampar, B., Derge, J. G., and Martos, L. M.: Sequence of spontaneous Epstein-Barr virus activation in human lymphoblastoid cells. IV Lepetit Colloquium. North Holland, Dutch Company. (In press).

Serial No. NCI 4930

1. Viral Carcinogenesis Branch
   OASDVO, Division of Cancer
   Cause and Prevention
2. Field Studies Unit
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Biology of tumor viruses in naturally occurring neoplasias and neoplastic cells.

Previous Serial Number: Same

Principal Investigator: Dr. Paul Arnstein

Other Investigators: Dr. Robert J. Huebner
Dr. Padman S. Sarma
Dr. George Todaro
Dr. Stuart Aaronson

Cooperating Units: Outside NIH:

Dr. E.H. Lennette, California State Department of Public Health, Berkeley, California
Dr. M. Gardner, USC School of Medicine, Cancer Research Laboratory, Los Angeles, California
Dr. R. McAllister, Children's Hospital, Los Angeles, California
Dr. Paul Price, Microbiological Associates, Inc., Bethesda, Maryland
Dr. Raymond Gilden, Flow Laboratories, Rockville, Maryland

Man Years:

Total:	2.5
Professional:	1.0
Other:	1.5

Project Description

Objectives:

1. Select most important malignant tumor types, obtain available cell cultures or original viable specimens. Confirm persistent neoplastic nature by reproduction of appropriate tumors in anti-thymocyte serum (ATS)-treated or otherwise immune suppressed mice.

2. Attempt to activate tumor viruses (or their genomic expressions) in transplanted human tumors growing in the immunologically deficient host; additionally, apply chemical inducers to increase chances of activation.

3. Modify the ATS-treated mouse system to compare protective potency of potential anti-cancer vaccines.

Methods Employed and Major Findings:

In vivo tumorigenesis tests: The method of transplantation of human tumors to ATS-treated mice remains essentially as reported in the previous (1972) report and is reproduced below:

> "Immunosuppression of NIH Swiss mice by administration of ATS has made it possible to replicate certain transformed and tumor-derived cells, as well as some original tumor tissue, from heterologous hosts. The technique is based on Eric Stanbridge's article (Nature 221: 80, 1969) demonstrating the growth of HeLa cells in mice. Beginning with this known tumorigenic cell line, we have applied the technique to other cell and tumor types and added the following refinements: (a) all attempts at tumor cell growth are subjected to pathological examination by our veterinary pathologist; (b) all tumor-suspect lesions in ATS-treated mice are removed, grown up in culture and submitted for karyology (with the aid of Dr. W. Nelson-Rees, NBL); (c) all successful tumors induced and removed are examined here, as well as submitted to appropriate cooperating units for detection of viral activation (either complete virus or viral products such as gs antigens, RNA-directed polymerase enzyme, envelope antigens)."

Excellent correlation of type of tumor reproduced in the ATS mice with the tumor from which the culture originated was observed when human tumor-derived cell cultures having "malignant" in vitro morphology were used at a dosage of 2-3 x 10^6 subcutaneously or 2-3 x 10^5 intracerebrally, or both. The "takes" were as follows:

> 5/6 sarcoma cultures transplanted as progressive sarcomas, confirmed histopathologically;
>
> 13/15 carcinoma cultures transplanted as progressive sarcomas, confirmed histopathologically.

The same transplantation method failed to reproduce tumors if the tumor-derived cells had an in vitro morphology resembling "benign" (normal) cells, even if the tumor of origin was highly malignant. Of 14 such cultures tested, none produced progressive tumors.

Results with suspension cultures were not as clear-cut: about one-half were transplantable intracerebrally; the brain histopathology of the "takes" was always described as lymphomatous infiltrate.

In all instances where tumors were reproduced in the ATS mice, human cells karyologically and morphologically identical to the "input" were isolated in vitro.

Direct transplants of recently removed surgical specimens gave a low rate of "takes". Technical difficulties, including errors in selecting the optimal portion of the amputated tissue and subsequent delays in transportation to the laboratory may have been partly responsible. These difficulties may be diminished in attempts now in progress. Results of completed direct transplants had a "take" rate of 4/19.

Viral isolations from transplanted tumors: NIH Swiss albino mice have been used for all transplanted tumors reported here; this strain had never yielded type C virus spontaneously. One viral isolate of the C type has been confirmed and characterized by collaboration with Dr. Todaro. This agent, coded ATS 124, was isolated from the 3rd mouse $\rightarrow$ mouse passage of RD sarcomas. This agent has the polymerase and the gs-1 antigen of murine leukemia virus, but an entirely different host range: it grows best on human and primate tissue culture cells. (Interestingly, RD cells previously yielded a new virus, RD114, after transplantation to fetal kittens by McAllister.) Four other type C viruses (one in collaboration with Dr. Todaro, the other three with Dr. Aaronson) have been detected in cultures derived from mouse-borne human tumors and are in the process of characterization.

Vaccination experiment: A pilot experiment performed to determine whether ATS mice may be used to test protection afforded by tumor virus vaccines has been completed. It was designed to test RD114 virus vaccine (banded, concentrated C-virus, formalin inactivated). Groups of mice, vaccinated with two doses of the RD114 vaccine or with a placebo, were subsequently challenged with either subcutaneous (s.c.) or intracerebral (i.c.) viable RD cells, while on ATS treatment (3×10^6 cells s.c. or 3×10^5 cells i.c.). A summary of results:

	Total vaccinates	Total controls
s.c. tumors	2/11	5/13
i.c. mortality	3/13 (23%) (2 histopathologically confirmed sarcoma cerebri)	9/14 (63%) (7 histopathologically confirmed sarcoma cerebri)

The results suggest protection against lethal i.c. sarcoma mediated by the vaccine. The possibility that the protection may be nonspecific should be excluded in future experiments.

Significance to Biomedical Research and to the Program of the Institute:

The system of replicating human tumors on immunosuppressed (ATS-treated or otherwise) mice, and later possibly other hosts, is furnishing a new way to experiment with solid human tumors of several histopathologically characteristic types, without actually involving human subjects. Should the activation of cryptic viral genomes by transplantation (and subsequent chemical induction)

prove to be a regularly occurring phenomenon, numerous new candidate human viruses will become available in relatively short time. In addition, the effect of chemical, immunologic or physical influences on the progression of tumor growth can be measured directly on human solid tumor tissue.

There is evidence that NIH Swiss mice can be vaccinated and subsequently resist a usually lethal malignant cell challenge, in spite of ATS immunosuppression. The protective effect observed in the pilot experiments may have been partly due to anti-species (anti-human) antibody: the vaccine virus had been harvested from human (RD) cells.

Nevertheless, these experiments suggest that (1) specific "anti-transplant protection" immunity could be produced with appropriate vaccines, and that (2) this immunity could be demonstrated in spite of subsequent ATS immunosuppression, provided adequate controls are incorporated.

Thus the ATS-treated mouse system may become a valuable screening tool for potential anticancer vaccines.

Proposed Course:

(a) Continue transplantation and reproduction of important tumor types:
(b) Continue collaborative viral detection studies from human tumor cells recovered from transplanted tumors:
(c) Design and perform additional vaccine experiments.

Honors and Awards:

None

Publications:

Todaro, G., Arnstein, P., Parks, W., Lennette, E., and Huebner, R.: A type C virus in human rhabdomyosarcoma cells after inoculation into antithymocyte serum treated NIH Swiss mice. Proc. Nat. Acad. Sci. U.S., in press.

Serial No. NCI 4931

1. Viral Carcinogenesis Branch
   OASDVO, Division of Cancer
   Cause and Prevention
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Syncytial assay for RD114 virus, utilizing the KC cell.

Previous Serial Number: Same

Principal Investigator: Dr. Kenneth Rand

Other Investigators: Dr. Cedric Long, Flow Laboratories, Rockville, Md.

Cooperating Units: Inside NIH:

Dr. Robert Huebner, VCB, NCI

Man Years:

Total:	1.0
Professional:	1.0
Other:	0

Project Description

Objective:

To find a syncytial assay for the RD114 virus.

Methods Employed:

Cell lines to be tested for the ability to form syncytia in response to RD114 virus were co-cultivated with the RD114 cell line, using standard tissue culture techniques as described by Klement et al. (PNAS 1969) for the XC test.

Major Findings:

Since the XC cell line is a Rous sarcoma virus transformed tumor line, it seemed quite natural to look at the Rous sarcoma virus transformed human brain tumor line KC (118 MG-EH) developed by Ponten and MacIntyre in 1968 (Acta. Path. Microbiol. Scand. 74: 465, 1968). In fact, abundant syncytia were produced when RD114 and KC were co-cultivated. This finding was easily developed in a semi-quantitative assay for the RD114 virus, very similar to that described by Klement et al. for the XC line. Since virtually no syncytia are formed when human cells producing other type C viruses (e.g., woolly

monkey, gibbon ape, feline leukemia virus, and Kirsten-MSV(RLV) are co-cultivated with KC cells, the phenomenon appears to be specific.

We have subsequently set out to investigate the mechanism of syncytia formation. The following major points have emerged:

1. Infectious virus is not required. RD114 virus which has been inactivated with 1:2000 B-propiolactone can still fuse KC cells despite complete lack of infectivity.

2. The KC cell can be productively infected with RD114 virus, and when this is done, no cytopathic effects are observed in the chronically infected cultures. Furthermore, the chronically infected cultures are completely refractory to fusion by RD114 virus. The virus produced by these cultures is also incapable of fusing fresh KC cells. However, when virus produced by KC cells is tested, it contains RD114 gs-1 measured by complement fixation with monospecific anti-RD114 gs-1 (Oroszlan et al., PNAS 1972), and RD114 RNA-dependent DNA polymerase measured by loss of RDP activity with anti-RD114 RDP antisera (Long et al., Nature New Biol. 241: 147, 1973). When the RD114 virus produced by KC cells is grown in RD cells or in a human diploid fibroblast (HSEM, Flow Labs), and then co-cultivated with fresh KC cells, typical syncytia result. Thus, in addition to the species-specificity, there is host cell specificity for syncytia formation of KC by RD114.

3. Virus alone is sufficient to produce syncytia, since banded virus works very well directly on a monolayer of KC cells. It does, however, have to be in high titer. Treatment of the virus with enzymes and physical agents which destroy its structure also destroy its syncytia forming ability. The RD114 virus is sensitive to heat (56°C), ether, trypsin, and sonication, but stable with regard to Neuraminidase, DNase, RNase, and PHospholipase A.

4. Cellular synthesis of DNA, RNA and protein is not necessary for syncytia formation, and the reaction proceeds even in the presence of ARA-C, Actinomycin D, and Cycloheximide.

Significance to Biomedical Research and to the Program of the Institute:

The KC cell line can be used as a convenient assay for RD114 virus which requires no special reagents. It has at least as much sensitivity as measuring gs-1 by complement fixation, and as much as measuring RNA-dependent DNA polymerase in the culture media.

Proposed Course:

The syncytia formation phenomenon can be utilized to isolate one of the viral envelope proteins. Preliminary experiments have shown the RD114 virus disrupted in Guanidine-HCl by boiling for 3 minutes will inhibit formation of

syncytia by intact virus. Furthermore, when RD114 virus disrupted in this manner is fractionated on a Guanidine-Agarose column, two adjacent fractions with inhibitory activity were isolated in the 40-80,000 MW range. These fractions had no toxicity and KC cells were still able to be fused by Sendai virus. The mechanism probably involves binding by the fragment or partially purified protein to the cell membrane in place of the intact virus, preventing the virus from forming a bridge between adjacent cells. This observation is being repeated using different protein separation procedures.

Honors and Awards:

None

Publications:

Rand, K. and Long, C.: Syncytial assay for the putative human C-type virus, RD114, utilizing human cells transformed by Rous sarcoma virus. Nature New Biol. 240: 187-190, 1972.

Serial No. NCI 4932
1. Viral Carcinogenesis Branch
OASDVO, Division of Cancer
Cause and Prevention
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: (A) Changes in DNA binding protein in a cell culture spontaneously producing oncogenic RNA virus.

(B) Effect of fetal development on DNA polymerase activity in AKR mice.

Previous Serial Number: Same

Principal Investigator: Dr. Josephine Simonds

Other Investigators: Dr. Franklin Portugal
Dr. Daniel Twardzik
Miss Marianne Oskarsson
Mr. Patrick Mulroy

Cooperating Units: Inside NIH:

Dr. Janet Hartley, LVD, NIAID
Miss Marilyn Lander, LVD, NIAID

Outside NIH:

Dr. Eugene Zimmerman, Microbiological Associates, Inc., Bethesda, Maryland
Dr. Paul Price, Microbiological Associates, Inc., Bethesda, Maryland

Man Years:

Total:	2.0
Professional:	1.0
Other:	1.0

Project Description

Objectives:

(A) To identify the mechanism by which the information to produce virus is repressed in cloned AKR cells derived from 14-17-day embryos. In these cells neither virus nor viral antigens can be detected although every cell is potentially able to produce virus either spontaneously or by chemical induction.

(B) To survey DNA polymerase activity in fetal mice to ascertain normal patterns of template preference, metallic requirements, inhibitory and stimulatory substances and conditions, and changes with development; to use the above information as a data base for experimental work in the effect of oncogenic viruses on DNA polymerase activity; to use purified DNA polymerase enzyme obtained in these experiments in other related experiments; example, reaction with repressor protein extracted from negative AKR cell culture.

Methods Employed:

(A) The AKR system was selected because it seemed to be a clear-cut case of repression of viral information not complicated by considerations of rescue of "defective" virus. The system was in very delicate balance because each cell had the potential to spontaneously produce virus. Consequently, the cells had to be treated with extreme care and monitored continuously for spontaneous production of virus. In order to do this, a cell culture laboratory was set up.

In this research it is necessary to demonstrate the presence of a molecule which is present in a ratio of approximately $1:10^6$. Polyacrylamide disc electrophoresis is a very powerful analytical method which I hoped to use to detect a repressor protein in the virus negative cell line; SDS acrylamide gel systems were set up in our laboratory, and these techniques were disseminated.

DNA was extracted from 14-17-day embryos of AKR/J mice in the same stage from which the AKR cell cultures were derived. The method of extraction of DNA was by selective adsorption of DNA by hydroxylapatite from a cell homogenate, a method which I had found in research for my doctoral project to be very efficient with small quantities of DNA and especially suited to my purposes because the DNA is subjected to a minimum of chemical extractions and no precipitation which might selectively remove the very sequences to which the repressor protein might bind. The negative and positive cell cultures (the positive cell culture had been established when a cloned negative cell line spontaneously produced virus) both in late log phase of growth were labelled respectively with tritiated and ^{14}C essential amino acids. Neither glycine nor any cyclic amino acids were used because of storage and conversion problems concerned with these amino acids and a desire to minimize the role of collagen in our study. Cold amino acids were substitutes for those omitted. Nucleic acids were removed from the cell homogenates by phase partition extraction in a polyethylene glycol-dextran T500 system.

The protein fractions from both cultures were combined and passed over the DNA-cellulose column under conditions which minimize nonspecific binding. Subsequently the proteins which bound to the DNA were eluted with increasing salt concentration. The eluates were concentrated and these samples were analyzed on SDS polyacrylamide disc gels.

(B) S_{30} preparations have been made of 10 and 12 day embryos and preliminary analyses have been made on the 10 day preparation.

Serial No. NCI 4932

Major Findings:

(A) Preliminary findings show a difference in one protein peak of the low salt elution. No differences were noted in any other peaks of any of the three elutions. The protein which shows the difference is present in higher concentration in the negative cell line.

The position of the protein on the gel indicated a molecular weight of approximately 86,000 relative to the migration of protein standards run simultaneously.

It was demonstrated that the material of this low salt elution was inhibitory to DNA polymerase activity. In all of the above characteristics this protein conforms to the classical model of a repressor protein.

(B) A preliminary survey shows that DNA polymerase activity can be shown in murine embryonic material.

Significance to Biomedical Research and to the Program of the Institute:

(A) The above findings constitute a beginning in the search for a biological substance which has an inhibitory effect on the oncogenic process. It may be possible to isolate and further characterize this protein. At that time it could be used in animal experiments.

(B) Work on DNA polymerases of normal cells and oncogenic cells has been given a tremendous impetus by the discovery of reverse transcriptase. It has now become imperative to establish the role in normal cells of the various DNA polymerase activities in order to understand the special role of the viral enzymes and their relation to the oncogenic process.

Proposed Course:

(A) These preliminary findings have been accepted for publication. Since the preliminary work was done, Dr. Portugal and Mr. Mulroy have taken over this project.

(B) We intend to conduct an exhaustive study of the DNA polymerase activities in normal and oncogenic cells. We hope to be able to show changes in these activities in the course of development of mammalian embryo.

Honors and Awards:

None

Publications:

Portugal, F.H., Simonds, J., Twardzik, D.R., Mulroy, P.F., and Oskarsson, M.: Comparison of DNA-binding proteins from mouse cells in culture. Biochem. Biophys. Res. Commun., in press.

Serial No. NCI 4934

1. Viral Carcinogenesis Branch
   OASDVO, Division of Cancer
   Cause and Prevention
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Studies on expression of the viral genome in murine sarcoma virus transformed nonproducer cells.

Previous Serial Number: None

Principal Investigator: Dr. Joel S. Greenberger

Other Investigators: Dr. Stuart A. Aaronson
Dr. John R. Stephenson
Dr. Steven R. Tronick

Cooperating Units: Inside NIH:
Dr. Robert J. Huebner, VCB, NCI

Outside NIH:
Dr. Garth R. Anderson, Hazleton Laboratories, Vienna, Virginia

Man Years:

Total:	1.0
Professional:	1.0
Other:	0

Project Description

Objectives:

1. To characterize murine sarcoma virus (MSV)-specific cellular antigens and develop immunologic methods of stimulating tumor regression in animals with (MSV) nonproducer and other kinds of tumors.

2. To study cellular regulation of type C virus induction and the mechanisms of action of inducers of these viruses.

3. To study cellular genetic factors required for the expression of transformation by tumor viruses by obtaining and analyzing morphologic revertants of MSV nonproducer cells which contain normal sarcoma viruses.

Methods Employed:

Standard tissue culture techniques for in vitro work. Animal facilities for several hundred mice and standard in vivo tumor immunologic techniques.

Major Findings:

(1) A method of study of MSV-specific cellular antigen(s) is in development using an in vivo system. Studies of the immunogenicity of MSV nonproducer cells and therapy of established growing tumors of MSV nonproducer origin are in progress. (2) The mechanism of action of IdU and other inducing agents is being studied using a highly sensitive in vitro assay of virus induction. (3) A method has been developed for the isolation and selection of MSV nonproducer cell flat revertants. These clonal lines are now being grown. (4) Drug resistant cell lines are being established for hybridization studies of MSV nonproducer cells. (5) The differences in antigenicity and tumorigenicity between MSV producer and nonproducer cells are being studied in an animal model.

Significance to Biomedical Research and to the Program of the Institute:

The MSV transformed nonproducer is a model of human cancer in that no virus production is detectable in the presence of malignant transformation. Studies of the expression of virus information in this system will hopefully increase our understanding of the role (if any) of oncogenic human viruses in human cancer.

Proposed Course:

(1) To characterize and isolate MSV nonproducer virus-specific cellular antigens. (2) To maximize IdU induction and apply these techniques to human cell lines. (3) Genetic experiments with MSV nonproducer flat revertants to establish the number of genetic factors required for transformation. (4) Hybrid cell line development (mouse-human). (5) A method of treatment of established tumors caused by MSV nonproducer cells in mice will be developed.

Honors and Awards:

None

Publications:

None

Serial No. NCI 4935
1. Viral Carcinogenesis Branch
OASDVO, Division of Cancer
Cause and Prevention
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Biochemical Studies on Viral Carcinogenesis.

(A) RNA-dependent DNA polymerase from a reptilian type C virus.
(B) Translation of AKR-MuLV RNA in an E. coli cell-free system.
(C) Changes in cellular DNA polymerase activities after infection of monkey cells with Herpesvirus saimiri.

Previous Serial Number: None

Principal Investigator: Dr. Daniel R. Twardzik

Other Investigators: Dr. Franklin Portugal
Miss Marianne Oskarsson
Dr. Josephine Simonds

Cooperating Units: Inside NIH:

Dr. Takis Papas, VBB, NCI
Dr. D. Rogerson, LNE, NIAMD
Dr. D. Ablashi, VLL, NCI
Mr. Gary Armstrong, VLL, NCI

Outside NIH:

Dr. J. Lempf, Electronucleonics, Inc., Bethesda, Maryland

Man Years:

Total:	1.75
Professional:	1.0
Other:	.75

Project Description

Objectives:

(A) To partially purify and characterize the RNA-dependent DNA polymerase from a reptilian type C virus and compare its physical and biochemical

properties with the RNA-dependent DNA polymerase found in the avian (AMV) and murine (RLV) type C oncogenic viruses.

(B) RNA from avian myeloblastosis virus (AMV) has been shown to direct the synthesis of several proteins, one of which is identified by immunodiffusion as a group-specific antigen. Rauscher leukemia virus (RLV) RNA also has been recently reported to stimulate the incorporation of amino acids into protein in an E. coli cell-free system. The objective of this study was to translate the AKR-MuLV genome in an E. coli cell-free system and identify the in vitro synthesized products as viral-related. Experiments were designed to determine if the 35S viral RNA subunit could also be translated in the E. coli cell-free system.

(C) Infection of a continuous African green monkey kidney cell line (Vero) with Herpesvirus saimiri (HVS) causes a suppression in cellular DNA synthesis and maximum cytopathic effects (CPE) were observed between 5 to 7 days after initial infection. The mechanism, however, by which this inhibition of cellular DNA synthesis after infection is achieved has not been reported. The objective of this study was to examine DNA-dependent DNA polymerase activities in normal monkey cells and in these cells after infection with HVS.

Methods Employed:

(A) Virions were detergent disrupted and the labelled products of the endogenous reaction were analyzed by sucrose gradient centrifugation. Kinetics of the polymerization reaction and requirements for maximum enzymatic activity were determined. Phosphocellulose chromatography and glycerol gradient centrifugation were used to partially purify and characterize the RNA-dependent DNA polymerase. The response of the enzyme to the synthetic template $rAdT_{10}$, divalent metal requirements, and temperature optimum were determined.

(B) Preincubated S-30 supernatants were prepared from an RNase I deficient strain of E. coli and reaction mixtures were optimized for in vitro protein synthesis in response to homologous F2 and QB bacteriophage messenger RNA. AKR-MuLV RNA was extracted by SDS-phenol method and examined by sucrose gradient centrifugation and polyacrylamide gel electrophoresis. Labelled protein synthesized in vitro in response to AKR-MuLV RNA was examined by SDS polyacrylamide gel electrophoresis and patterns were compared to those obtained on analysis of proteins of disrupted AKR-MuLV virions. The molecular weights of the in vitro synthesized products were determined using known standard marker proteins. The immunological reactivity of proteins synthesized in vitro in response to AKR-MuLV RNA was tested using MuLV antisera; normal Fischer rat sera was used as a control.

(C) DNA-dependent DNA polymerase activities in S-100 supernatants from normal and HSV-infected Vero cells were examined by DEAE-cellulose and hydroxylapatite chromatography. The partially purified cellular DNA polymerase activities (I and II) were compared for heat stability, divalent

cation requirements, inhibition by p-chloromercuribenzoate, and template specificity. Mixing experiments were performed to determine if specific inhibitors of the DNA polymerase reaction were present in HVS-infected Vero cells, 4 and 8 days after initial infection.

Major Findings:

(A) Simultaneous detection experiments demonstrated that reptilian type C virions contain a DNA polymerase which utilizes 70S RNA as a template. The RNA-dependent DNA polymerase requires all four deoxynucleotide triphosphates for activity and demonstrates an absolute requirement for a divalent cation. The enzyme elutes from phosphocellulose at 0.22 M KPO_4 as does the RNA-dependent DNA polymerase from AMV, and is different from the murine (RLV) enzyme which elutes at 0.40 M KPO_4. The estimated molecular weight as determined by glycerol gradient centrifugation is 109,000 daltons. The reptilian type C viral polymerase is therefore smaller than the avian (AMV) enzyme, molecular weight 180,000 daltons, and somewhat larger than the murine (RLV) viral polymerase, molecular weight 70,000 daltons. The temperature for optimal enzyme activity was 40-45°C; optimum magnesium and manganese concentrations were determined to be 10 mM and 2 mM respectively when $rAdT_{10}$ was used as template.

(B) High molecular weight RNA isolated from the oncogenic type C murine leukemia virus, AKR-MuLV, stimulates the incorporation of radioactive amino acids into protein in the E. coli cell-free system. Initial difficulty in obtaining nominal stimulation of amino acid incorporation was traced to poor viral preparations containing mostly degraded low molecular weight RNA. RNA extracted from AKR-MuLV viral preparations which demonstrated high endogenous reverse transcriptase activity was found to give maximum stimulation of amino acid incorporation. Analysis of the in vitro synthesized protein products by SDS polyacrylamide gel electrophoresis demonstrated the synthesis of at least three proteins corresponding in molecular weight to several authentic viral proteins. Positive immunoprecipitation tests also confirm the translational product as AKR-MuLV related. Although at least 18 proteins were found on analysis of disrupted murine leukemia virions, only three were synthesized in vitro in response to MuLV RNA in the E. coli cell-free system.

(C) Uninfected Vero cells contain two DNA polymerase activities separable by DEAE-cellulose chromatography. These two activities exhibit differences in heat inactivation, divalent metal requirements, inhibition by p-chloromercuribenzoate and salt. One of these DNA polymerase activities, Peak I, is significantly reduced in Vero cells examined 4 and 8 days after infection with HVS. Mixing experiments demonstrate that infected cells do not contain an inhibitor of the DNA polymerase reaction and suggest that this reduction in a host DNA polymerase activity is a result of HVS infection.

Significance to Biomedical Research and to the Program of the Institute:

(A) This study now provides some of the biochemical and physical properties of the RNA-dependent DNA polymerase from a reptilian type C virus and permits a comparison of this enzyme with the avian (AMV) and the murine (RLV) type C viral DNA polymerase. The role of RNA-dependent DNA polymerases and type C tumor viruses in neoplasia is difficult to assess; however the reverse transcriptase and other ancillary core enzymes provide a mechanism for the transfer of information from RNA to DNA and integration of viral information into the host genome and thus adds support to the theory of the oncogene.

(B) Proteins synthesized in vitro in response to type C viral RNA can be specifically labelled and therefore easily characterized using biochemical and immunological techniques. Viral gene products can then be identified and antigens expressed in neoplasia and other proteins responsible for cell transformation can be determined to be of either viral or host origin. In this respect, enzyme assays could detect the in vitro synthesis of reverse transcriptase and other specific virions enzymes. Drugs regulating the synthesis of viral gene products could also be developed.

(C) Herpesvirus saimiri (HVS) has been shown to induce malignant lymphoma and/or lymphocytic leukemia in various species of non-human primates. This study demonstrates a reduction in a host enzyme activity after infection with this DNA virus. This reduction in a cellular DNA polymerase activity correlates with the suppression of host DNA synthesis observed after HVS infection. Studies on the biochemistry of viral-induced changes in the host cell will provide much information on the mechanism of viral-induced carcinogenesis.

Proposed Course:

(A) Initial characterizations of the reverse transcriptase from a reptilian type C virus have been done. Kinetic studies on the mechanism of polymerization require large amounts of enzyme and are easier done with the avian (AMV) viral polymerase. This project has been terminated and a manuscript is in preparation.

(B) The limiting factor in these experiments is obtaining native, undegraded high molecular weight RNA in large quantities. The high input of viral RNA needed to obtain enough protein product for biochemical analysis makes the heterologous E. coli type C viral translational system unfeasible. The low levels of amino acid incorporation in response to type C viral RNA and the fact that only three relatively low molecular weight proteins were synthesized in vitro are indicative of the various restrictive and modulating factors involved in the efficiency of translation in heterologous systems. The use of homologous mammalian translation systems and the preparation of viral RNA in large quantities is being done by Dr. Maurice Green (St. Louis University) and associates (35 professionals), making a continuation of this project redundant. A manuscript is being submitted for publication.

(C) Studies are in progress to (a) correlate the initial reduction in DNA polymerase activity with time after infection; (b) verify that the reduced DNA polymerase activity is nuclear in origin; and (b) examine cellular DNA polymerase activities in mouse cells after type C virus infection.

Honors and Awards:

None

Publications:

None

Serial No. NCI 4936
1. Viral Carcinogenesis Branch, OASDVO, Division of Cancer Cause and Prevention
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Studies of Mouse Leukemia Viruses

Previous Serial Number: None

Principal Investigator: Dr. Douglas R. Lowy

Other Investigators: Dr. Natalie Teich
Dr. Sisir Chattopadhyay, LVD, NIAID
Dr. Wallace P. Rowe, LVD, NIAID
Dr. Janet W. Hartley, LVD, NIAID

Cooperating Units: Inside NIH:

Dr. Authur S. Levine, DCT, NCI
Dr. Joseph Pitha, GRC, NICHD

Outside NIH:

Dr. Paula M. Pitha, Johns Hopkins School of Medicine, Baltimore, Md.

Man Years:

Total:	1.0
Professional:	1.0
Other:	0.0

Project Description

Objectives:

To study the biology and biochemistry of murine leukemia viruses both in the intact organism and in tissue culture. Sensitive quantitative assays of infectious virus, viral antigens, and viral-specific nucleic acids are utilized in pursuing these studies.

Serial No. NCI 4936

Methods Employed:

Tissue culture procedures, using plaque and complement fixing antigen induction, and fluorescent antibody techniques. Measuring incorporation of halogenated pyrimidines into DNA through the use of isotopically labeled compounds, and liquid scintillation techniques. Cellular DNA and RNA purification, synthesis of viral DNA probes utilizing the endogenous reverse transcriptase activity of murine leukemia viruses (MuLV), and nucleic acid hybridization with separation of single- and double-stranded DNA by hydoxyapatite chromatography.

Major Findings:

1. Activation of MuLV by IdU and BrdU.

In our laboratory, screening of various compounds had shown that the two thymidine analogs 5-bromodeoxyuridine (BrdU) and 5-iododeoxyuridine (IdU) are by far the most efficient inducers of MuLV in virus-negative cell lines derived from AKR mouse embryos. Current investigations of the mechanism of virus induction by BrdU and IdU suggest that incorporation of either halogenated pyrimidine is a prerequisite to viral activation. This has been demonstrated by using isotopically labeled analog under a variety of conditions and correlating incorporation into DNA with viral activation; also, analog-treated cells have been found to have their rate of induction enhanced when treated with visible light, ultraviolet light, or x-irradiation.

2. Virus-specific DNA in Mouse Cells.

Isotopically labeled single-stranded viral DNA probes have been synthesized from purified AKR and Kirsten MuLV utilizing the virion associated endogenous reverse transcriptase in the presence of actinomycin D (method of E. Scolnick). Utilizing this probe, DNA reassociation kinetics with a variety of cellular DNA's have been carried out as described originally by Britten and Kohne. Significant differences in hybridization have been noted between DNA from uninfected NIH (low leukemic) and AKR (high leukemic) mouse embryo cells. These differences have been noted with both AKR and Kirsten probes. A cloned wild mouse embryo cell line from our laboratory which has an unusually high degree of sensitivity to non-tissue culture adapted MuLV has been found to contain the smallest amount of hybridizable material of the various mouse cells tested.

3. Polyvinyl Compounds as Inhibitors of MuLV.

See Serial No. NCI 4937, Dr. Natalie Teich, Principal Investigator.

Serial No. NCI 4936

Significance to Biomedical Research and to the Program of the Institute:

The discovery of the ability of the thymidine analogs IdU and BrdU in inducing MuLV has been applied to cells from many other species and has confirmed the endogenous presence of viral genomes in these systems as well. It appears to be a promising method for the induction of some virus genomes in human cells as well. The experimental proof of the existence of naturally occurring endogenous virus genomes may have fundamental implications for understanding viral induced disease, especially cancer. The findings of Rowe and Hartley strongly suggest that the IdU virus induction is closely related to spontaneous appearance of virus in vivo and that further investigation of the IdU induction phenomenon may have relevance to spontaneous appearance of virus in the whole animal.

The nucleic acid hybridization studies confirm the reports of others that cellular DNA contains sequences which are complementary to viral nucleic acids. These findings, when combined with results from many other laboratories using a variety of approaches, suggest that endogenous RNA virus genomes are present in cells as DNA, probably integrated into the host genome. Furthermore, our studies suggest that mouse strains differ in the number of copies of virus genomes per cell, a situation which differs from the results reported with chicken strains.

The studies of the vinyl polymers represent an initial attempt to selectively inhibit viral replication by utilizing compounds which may inhibit the viral reverse transcriptase. Our findings demonstrate the importance of analyzing compounds in terms of their ability to inhibit viral replication, their effect on the reverse transcriptase, and their effects on non-viral cellular metabolism. It is hoped that this approach will prove useful in the testing of other potential antiviral compounds.

Proposed Course:

Studies on the mechanism of IdU and BrdU activation will be continued. We plan to determine whether analog incorporation during a critical portion of the cell cycle is necessary and whether critical sites of cellular DNA must be analog-substituted for viral activation. In addition, nucleic acid hybridization studies will be carried out to determine the kinetics of viral-specific RNA production during IdU virus induction. Finally, it will be determined if IdU mediated induction is associated with viral-specific sequences going from high to low molecular weight DNA.

Further definition of the viral DNA probes will permit a more quantitative analysis of the number of viral genome copies in different mouse cells, as determined by DNA reassociation kinetics. This investigation will be coordinated with the animal genetics experiments of Rowe and Hartley to determine if the virus inducing genes Akv-1 and Akv-2 represent structural viral genes.

Serial No. NCI 4936

Honors and Awards:

None.

Publications:

Rowe, W.P., Lowy, D.R., Teich, N., and Hartley, J.W.: Some implications of the activation of murine leukemia virus by halogenated pyrimidines. Proc. Nat. Acad. Sci. 69: 1033-1035, 1972.

Teich, N., Lowy, D.R., Hartley, J.W., and Rowe, W.P.: Studies of the mechanism of induction of infectious murine leukemia virus from AKR mouse embryo cell lines by 5-iododeoxyuridine and 5-bromodeoxyuridine. Virology 51: 163-173, 1973.

Pitha, P.M., Teich, N.M., Lowy, D.R., and Pitha, J.: Inhibition of murine leukemia virus replication by polyvinyluracil and polyvinyladenine. Proc. Nat. Acad. Sci., in press.

Serial No. NCI 4937
1. Viral Carcinogenesis Branch
OASDVO, Division of Cancer
Cause and Prevention
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Studies of Mouse Leukemia Virus Induction

Previous Serial Number: None

Principal Investigator: Dr. Natalie M. Teich

Other Investigators: Dr. Douglas R. Lowy, VCB, NCI
Dr. Sisir K. Chattopadhyay, LVD, NIAID

Cooperating Units: Inside NIH:

Dr. Arthur S. Levine, DCT, NCI
Dr. Josef Pitha, GRC, NICHD

Outside NIH:

Dr. Paula M. Pitha, The Johns Hopkins School of Medicine
Baltimore, Maryland

Man Years:

Total:	1.0
Professional:	1.0
Other:	0

Project Description

Objectives:

The objectives of this project are to utilize sensitive virological and molecular biological techniques to study: (1) the mechanism of induction of endogenous MuLV by the halogenated pyrimidines; (2) the extent to which viral genes are integrated and transcribed in inducible and noninducible cells; and (3) the effect of synthetic polyribonucleotides on virus replication.

Methods Employed:

Tissue culture assays; fluorescent antibody techniques; nucleic acid hybridization procedures, including isolation of cellular and viral RNA, DNA and synthesis of viral DNA probes using the endogenous RNA-dependent DNA polymerase

Major Findings:

Activation of MuLV by IdU and BrdU: A screening of many compounds led to the discovery that two thymidine analogs, IdU and BrdU, are by far the most effective inducers of endogenous MuLV from AKR cell cultures. Several observations point to the necessity for incorporation of analog into DNA for virus induction to occur. (1) Inhibition of DNA synthesis, by serum deprivation or cytosine arabinoside treatment, prevented virus induction. (2) Preventing analog incorporation by simultaneous thymidine treatment inhibited virus induction. (3) Enhancement of analog incorporation by simultaneous treatment with fluorodeoxyuridine increased virus induction. (4) Exposure of analog-treated cells to ultraviolet light, visible light, or X-irradiation also enhanced the induction phenomenon. (5) Experiments using synchronized cell cultures demonstrate that the cells are most susceptible to IdU induction during the early part of the S phase.

Nucleic acid hybridization studies: Precise quantitative studies are now in progress to determine the number of viral genome copies present in embryos and in tissue culture lines derived from several mouse strains. Using a single-stranded DNA probe synthesized from viral RNA, we have been able to detect differences in the hybridizing fractions of DNA from AKR embryos compared to those from the non-leukemic, non-inducible NIH embryos. In addition, the degree of hybridization varies with each specific viral DNA probe; different hybridization curves are obtained using AKR viral DNA compared to Kirsten viral DNA.

Studies with polyvinylnucleotides: Studies by other investigators had established that poly A and poly U could inhibit MuLV reverse transcriptase activity as well as the replication of MuLV in tissue culture. The synthetic vinyl analogs of these compounds were also found to inhibit MuLV replication. However, kinetic and temporal studies show that these compounds do not specifically block reverse transcriptase activity *in vitro* and can block virus replication at later stages of the infectious cycle.

Significance to Biomedical Research and to the Program of the Institute:

The efficiency with which the halogenated pyrimidines can induce endogenous MuLV from mouse cells and endogenous viruses from other systems can be applied to the study of the natural transmission of these viruses *in vivo* and *in vitro*. The use of nucleic acid hybridization should reveal information about the state of the viral genome in the cells and the degree to which the viral genes are transcribed. These procedures can also provide insight about the mechanism of virus induction by the thymidine analogs and whether the analogs work on virus-inducing loci or on integrated viral genomes.

Proposed Course:

Data from synchronized AKR cell cultures show that virus induction is associated with IdU incorporation during the S phase. Using synchronized cells and the sensitive hybridization methods, one should be able to

elucidate more information on the mechanism of induction by IdU; whether host "control" sequences are shut off, virus-inducing loci are turned on, or complete viral genomes are excised from an integrated state in the chromosomal DNA.

Honors and Awards: None

Publications:

Rowe, W.P., Lowy, D.R., Teich, N., and Hartley, J.W.: Some implications of the activation of murine leukemia virus by halogenated pyrimidines. Proc. Nat. Acad. Sci. U.S. 69: 1033-1035, 1972.

Teich, Natalie, Lowy, Douglas R., Hartley, Janet W., and Rowe, Wallace P.: Studies of the mechanism of induction of infectious murine leukemia virus from AKR mouse embryo cell lines by 5-iododeoxyuridine and 5-bromodeoxyuridine. Virology 51: 163-173, 1973.

Pitha, Paula M., Teich, Natalie M., Lowy, Douglas R., and Pitha, Josef; Inhibition of murine leukemia virus replication by polyvinyluracil and polyvinyladenine. Proc. Nat. Acad. Sci. U.S., in press.

Serial No. NCI 4938

1. Viral Carcinogenesis Branch
   OASDVO, Division of Cancer
   Cause and Prevention
2. Office of the Chief
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: In Vitro Replication of Murine Sarcoma Viruses

Previous Serial Number: None

Principal Investigator: Dr. Fuw G. Duh

Other Investigators: Dr. Robert J. Huebner
Mr. Paul R. Hill

Cooperating Units: Outside NIH:

Dr. J. S. Rhim, Microbiological Associates, Inc., Bethesda, Md.
Dr. M. L. Vernon, Microbiological Associates, Inc., Bethesda, Md.
Dr. C. F. Demoise, Microbiological Associates, Inc., Bethesda, Md.

Man Years:

Total:	1.0
Professional:	1.0
Other:	1.0

Project Description

Objectives:

To determine the in vitro host range of murine sarcoma virus (MSV); to isolate nonproducer clones of MSV transformed cells; to activate RNA tumor viruses from virus-free, nonproducer cells by chemical treatment; to activate type C viruses from tumors induced by chemically or spontaneously transformed cells.

Methods Employed and Major Findings:

Murine sarcoma virus (MSV) replication and assay. The Kirsten strain of MSV (Ki-MSV) (Kirsten, Mayor, J. Nat. Cancer Inst. 39: 311, 1967) stock [supernatant fluids from a Ki-MSV transformed rat cell line No. 58967 (Klement et al., J. Nat. Cancer Inst. 47: 65, 1971)] was a gift from Dr. V. Klement, Childrens Hospital, Los Angeles, Calif. The virus preparation was

passed through 0.45 u HA membrane filter (Millipore Co.) before being used. Cells tested were cultures of guinea pig, canine, rabbit, porcine, avian, feline, bovine, simian and human origin. The replication of virus in infected cultures was determined by the following methods: (1) examined for the presence of morphological alteration; (2) assayed for CF antigen reactive with murine leukemia virus group reactive rat serum; (3) assayed for RNA dependent DNA polymerase activity; (4) examined by electron microscopy for the presence of type C virus particles, and (5) assayed for ^{3}H-uridine incorporated virus particle. Data so far obtained showed that Ki-MSV replicates and transforms the cultures of guinea pig embryo, canine embryo, porcine kidney, feline embryo, rabbit kidney, bovine embryonic kidney, and human tumor cells and that these morphologically altered cells contained both infectious virus and gs antigen. The present results indicate that members of the murine sarcoma-leukemia virus complex, particularly Ki-MSV, exhibit a wider host range than was hitherto believed.

Nonproducer clones of murine sarcoma virus transformed guinea pig embryo cells. We recently reported that guinea pig embryo (GPE) cells can be transformed by Ki-MSV, and that the transformed cells contain both infectious virus and group-specific (gs) CF antigen of the murine sarcoma-leukemia virus group (Rhim et al., Virology 48: 841, 1972). Of 12 foci selected at limiting MSV dilution, 5 were found to release both MSV and murine leukemia virus (MuLV). Two focus-derived lines, however, did not release any virus though morphologically they were indistinguishable from virus-releasing MSV transformed GPE lines and produced tumors when transplanted into newborn guinea pig. Assay of culture fluids of the "nonproducer" (NP) clones on a variety of host cells revealed no biological activity. No reverse transcriptase activity was demonstrable in these lines. Immunological tests gave no evidence of mouse gs antigens. The co-cultivation of these NP cells with a "helper" MuLV releasing GPE cells resulted in the rescue of the MSV genome with the antigenicity indentical to that of the leukemia virus used.

Activation of RNA tumor viruses from virus-free, guinea pig nonproducer cells following chemical treatment. Particles resembling guinea pig leukemia virus were activated from guinea pig nonproducer cells following 5-bromodeoxyuridine or 5-iododeoxyuridine treatment. These particles were approximately 100 mu in the mature form and had a density of 1.16-1.17 g/ml, the density characteristic of murine sarcoma-leukemia virus complex. They contained RNA dependent DNA polymerase activity.

Activation of a type C RNA virus from tumors induced by rat kidney cells transformed by a chemical carcinogen. A cell line derived from Fischer rat kidney was treated with 7,12-dimethylbenz(a)anthracene (DMBA) or dimethyl sulfoxide (control). Cells treated only with the carcinogen underwent morphologic transformation in vitro and produced progressively growing transplantable tumors when injected into homologous hosts. Although the transformed cell line was negative for infectious virus before inoculation into animals, a type C RNA virus was activated from a cell line derived from the tumor. Since infectious type C viruses are not usually demonstrable in

rat tissues of normal or tumor origin, we suggested that the chemical treatment and activation of the virus may be related.

Significance to Biomedical Research and to the Program of the Institute: Studies on the host range of Ki-MSV indicated that Ki-MSV replicates and transforms the cultures of guinea pig, canine, rabbit, porcine, feline, bovine, and human tumor cells. In addition, isolation of nonproducer clonal lines from Ki-MSV transformed guinea pig embryo cells and chemical induction of the endogenous RNA tumor virus from virus-free, nonproducer cells have been described. Thus, isolation and characterization of endogenous guinea pig, rabbit, bovine, canine, porcine, feline, rat and human type C RNA viruses or genomes can be studied using Ki-MSV transforming virus. MSV nonproducer cells would provide an excellent system for induction of endogenous type C virus, study of the process of induction, and for the rapid screening of chemical carcinogens and other compounds for their ability to induce RNA tumor virus for virus-negative cells. Such studies have obvious implications for chemical and viral carcinogenesis. It will be also helpful to serve as a model for induction or isolation of human RNA tumor viruses or genomes.

Proposed Course:

Continue and amplify the above studies, particularly: (1) determine whether the transformed cells produce tumors when inoculated into the immunosuppressed (ATS-treated) homologous hosts; (2) to further characterize the transformed cells; (3) to select nonproducer clones from Ki-MSV transformed mammalian cells, particularly of human tumor origin; (4) to isolate endogenous type C RNA viruses from nonproducer cells by means of chemical induction or cocultivation techniques.

Honors and Awards:

None.

Publications:

Rhim, J.S., Demoise, C.F., Duh, F.G., and Cho, H.Y.: Transformation of guinea pig embryo cells by a murine sarcoma virus. Virology 48: 841-843, 1972.

Rhim, J.S., Duh, F.G., Cho, H.Y., Elder, E., and Vernon, M.L.: Activation of a type C RNA virus from tumors induced by rat kidney cells transformed by a chemical carcinogen. J. Natl. Cancer Inst. 50: 255-261, 1973.

Duh, F.G., Vernon, M.L., and Rhim, J.S.: In vitro transformation of canine embryo cells by murine sarcoma virus (Kirsten). Proc. Soc. Exp. Biol. Med. in press.

Serial No. NCI 4939

1. Viral Carcinogenesis Branch
   OASDVO, Division of Cancer
   Cause and Prevention
2. Ecology and Epizoology Section
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Studies on rat C-type viruses.

Previous Serial Number: None

Principal Investigator: Dr. D.W. Verwoerd

Other Investigators: Dr. Padman S. Sarma, VCB, NCI
Dr. Lee Vernon, Microbiological Associates, Inc.

Cooperating Units: Inside NIH:

Dr. Robert J. Huebner, VCB, NCI

Man Years:

Total:	1.0
Professional:	1.0
Other:	0

Project Description

Objectives:

The presence of C-type particles in rat tumors produced by chemical carcinogenesis or of spontaneous origin have been described by various investigators. A few transplantable tumor cell lines producing virus particles have been obtained, but almost without exception these virus particles are non-infective for cell systems tested. The consequent lack of an assay system precluded a serological comparison or classification of the various rat viruses so far. In another type of study, pseudotypes of murine sarcoma viruses and rat helper viruses have been obtained by IdU treatment of non-producer cell lines, suggesting the presence of an oncogene in the rat. The present study was therefore an attempt to develop methods for the rescue and assay of the various rat viruses in order to characterize them in greater detail.

Methods Employed:

Normal rat embryo cells were treated with 5-iododeoxyuridine (IdU) to induce viral production. Presence of viral particles was determined by electron microscopy, assays for reverse transcriptase and density determinations in

sucrose gradients. Assays for rat gs antigen by means of complement fixation were also carried out.

Major Findings:

Reverse transcriptase activity could be induced in primary cultures of normal rat embryo fibroblasts derived from three commonly used laboratory rat strains by treatment with IdU. The presence in the cells of particles resembling C-type viruses at the peak of reverse transcriptase activity could be demonstrated electron-microscopically and labelled uridine was incorporated into material banding at a density of 1.14 in sucrose gradients, the density of the known rat C-type viruses. Attempts to isolate infectious virus have so far been unsuccessful, however. Further studies to find a susceptible cell system, or to rescue defective viruses by means of complementation with helper virus are being pursued.

Further culture of the IdU-treated cells under appropriate experimental conditions invariably lead to morphological transformation of the cells. The absence of equivalent transformation in control cultures suggests a relation between the induction of C-type particles in, and subsequent transformation of, the embryonic rat cells.

Significance to Biomedical Research and to the Program of the Institute:

The induction of C-type virus production in embryonic rat cells confirm previous indications of the existence of an oncogene in the rat. It further constitutes an experimental technique to overcome the apparently highly effective repression of this gene in the rat, reflected by the very rare natural expression of the viral genome. A further study of this phenomenon could possibly serve as a model for the human situation where repression of a possible C-type oncogene can be expected to be very effective too.

Proposed Course:

A detailed study of the characteristics of the IdU-induction of C-type particles in embryonic rat cells will be made in an attempt to clarify the mechanism involved. Attempts to rescue the induced virus by means of either complementation by helper virus or by finding a suitable host cell will be continued. If successful, it will enable us to study the endogenous rat viruses in greater detail and possibly to shed some new light on the natural history of these viruses.

Honors and Awards:

None

Publications:

None

Serial No. NCI/NIAID 71 D
1. Viral Diseases
2. Viral Oncology
3. Bethesda, Maryland

PHS-NIH
Individual Project Report
July 1, 1972 through June 30, 1973

Project Title: Studies of Mouse Leukemia Viruses

Previous Serial Number: Same

Principal Investigators: Dr. Janet W. Hartley, LVD, NIAID
Dr. Wallace P. Rowe, LVD, NIAID

Other Investigators: Dr. Sisir K. Chattopadhyay, LVD,NIAID
Dr. Douglas R. Lowy, NCI
Dr. Bernice Moll, LVD, NIAID
Dr. Marshall D. Sklar, LVD, NIAID
Dr. Natalie M. Teich, NCI

Cooperating Units: Inside NIH:

Dr. Arthur S. Levine, DCT, NCI
Dr. Nelson A. Wivel, MB, NCI

Outside NIH:

Dr. Theodore Bremner, Howard University, Washington, D.C.
Dr. Edward A. Boyse, Sloan Kettering Institute for Cancer Research, New York, New York
Dr. E. Stockert, Sloan Kettering Institute for Cancer Research, New York, New York
Dr. H. Ikeda, Sloan Kettering Institute for Cancer Research, New York, New York
Dr. H. Sato, Sloan Kettering Institute for Cancer Research, New York, New York
Dr. Frank Lilly, Albert Einstein College of Medicine, New York, New York
Dr. John C. Parker, Microbiological Associates, Inc., Bethesda, Maryland

Man Years:

Total:	15.1
Professional:	6.8
Other:	8.3

Project Description

Objectives:

To utilize sensitive virus isolation and quantitation procedures for murine leukemia and sarcoma viruses in studying the natural history of infection with these agents and the biology of their growth in tissue culture.

Methods Employed:

Tissue culture procedures, using plaque and focus assays and complement fixing antigen induction; complement fixation; fluorescent antibody techniques; starch-gel electrophoresis for marker isozyme locus assays; nucleic acid hybridization.

Major Findings:

Genetics of MuLV infection: Studies on the transmission of infectious MuLV to the progeny of the high leukemia, high virus AKR mouse crossed and back-crossed to various low virus Fv-1^n and Fv-1^b strains have indicated that the capacity to produce infectious virus is determined by either of two unlinked chromosomal loci, designated as Akv-1 and Akv-2, which appear to be integrated genomes of the virus. One of the loci, Akv-1, has been located in linkage group I (chromosome 7), 12 map units from the Gpi-1 locus, with a gene order of c-Gpi-1-Akv-1.

That these loci represent integrated virus genetic information is suggested by the finding that the host range of the virus detected in crosses to Fv-1^b strains, which are genetically restrictive for the virus from AKR, was almost always that of AKR virus.

Preliminary data on the transmission of infectious virus in matings of low virus Fv-1^n mice to another high MuLV strain, C_3H/Fg, indicate that at least 3 virus-inducing loci are segregating in these crosses.

The Fv-1 locus of the mouse, the major determinant of the biology of MuLV infection, has been located in linkage group VIII (chromosome 4), very closely linked to the Gpd-1 locus.

Activation of MuLV by IUDR: Close correlation has been found between segregation patterns for infectious MuLV in post-natal animals and for induction of virus in embryo cells treated with 5-iododeoxyuridine (IdU). Activation of virus in individual embryo cell cultures has proved to be a sensitive technique for studying virus segregation in restrictive or low penetrance crosses.

All Fv-1^n strains of mice which produce infectious virus produce only N-tropic virus, while Fv-1^b strains produce B-tropic or both N- and B-tropic virus. As first reported by Aaronson *et al.*, the virus induced by IdU or BdU

treatment of BALB/c cells (a B-type strain) has been invariably N-tropic. We are studying the host range characteristics of virus induced by IdU treatment of embryo cells of two B-type strains of mice (B10.D2, old; and B10.Br) which in vivo produce predominantly B-tropic virus. By use of a highly sensitive wild mouse embryo cell line (see below) we can now consistently recover N-tropic virus from all B10.D2 and B10.Br embryo cultures tested to date, and B-tropic virus has been found on several occasions.

Screening of various mutagens, nucleic acid analogs, and chemical carcinogens has shown that the two thymidine analogs IdU and BdU are by far the most effective inducers of MuLV in AKR cell cultures. Studies of the mechanism of virus activation by IdU indicate that incorporation of the analog into cellular DNA is essential. Inhibition of DNA synthesis and blocking of analog incorporation prevented induction, while induction could be enhanced by simultaneous treatment with fluorodeoxyuridine, or by exposure of analog-treated cells to visible light or X-irradiation.

Development of cell line highly sensitive to MuLV infection: A wild mouse embryo cell line has been obtained which has the unusual property of sensitivity to both N- and B-tropic MuLV host range variants. In addition, the line is more sensitive than reference secondary mouse embryo cultures to MuLV, particularly for detecting virus in mouse tissue extracts or virus activated from embryo cell cultures. The line was developed by cloning from a feral mouse embryo cell line derived from a single fetus.

Serological classification of MuLV isolates: Virus neutralization studies have continued on isolates of MuLV from various sources. All isolates from naturally infected inbred mice tested to date, with the exception of the agent isolated by Lerner et al. from NZB mice, have shown relationship to Gross virus. The Lerner virus, in contrast, is apparently more closely related to virus of the FMR subgroup. Further testing of viruses isolated from feral mice indicates that these viruses comprise a subgroup distinct from the Gross-AKR and FMR subgroups.

Significance to Biomedical Research and the Program of the Institute:

Studies of the natural history of MuLV infection have now developed to a stage in which the biology of both exogenous and endogenous infection can be studied with increasing depth, with possibilities for contributing to the understanding of oncogenesis.

Establishment of chromosomal locations of integrated virus genetic material in various strains of mice may eventually permit the determination of whether inheritance of a particular viral genome is associated with development of a particular type of tumor, especially if closely linked non-viral markers could be used to detect viral genes of varying degrees of defectiveness.

the discovery of the effectiveness of the thymidine analogs in inducing production of infectious MuLV has led to wide application in studies of endogenous MuLV in mouse cells and of endogenous virus in other systems. The development of such relatively efficient systems may lead to understanding of the process of virus induction.

The development of the feral mouse embryo cell line with high sensitivity to both N- and B-tropic MuLV provides a tissue culture system superior to those used previously for detecting and quantitating MuLV. This line is of particular value in assaying field specimens and for recovering and assaying IdU-induced virus, especially in restrictive systems.

Proposed Course:

Studies on the genetic transmission of MuLV and MuLV-related antigens will be continued with the aim of determining the location of the Akv-2 gene, clarifying the relationship between the known loci specifying various types of MuLV genetic information or control, and determining whether viral genes with specific chromosomal locations in one strain of mice are similarly located in others.

Further comparisons are planned of the host range and other characteristics of viruses activated from embryo cells of various inbred strains of mice. Of particular interest to us is the biology of B-tropic MuLV infection which will be explored further in both in vitro and in vivo studies.

Honors and Awards:

Dr. Wallace P. Rowe - Rockefeller Public Service Award

Invited Lectures:

Dr. Wallace P. Rowe - The Gustav Stern Memorial Lecture in honor of Peyton Rous
Fifth Annual G. Burroughs Mider Lecture
13th G.H.A. Clowes Memorial Lecture

Publications:

Rowe, W.P., Lowy, D.R., Teich, N., and Hartley, J.W.: Some implications of the activation of murine leukemia virus by halogenated pyrimidines. Proc. Nat. Acad. Sci. 69: 1033-1035, 1972.

Rowe, W.P.: Studies of genetic transmission of murine leukemia virus by AKR mice. I. Crosses with Fv-1^n strains of mice. J. Exp. Med. 136: 1272-1285, 1972.

Rowe, W.P., and Hartley, J.W.: Studies of genetic transmission of murine leukemia virus by AKR mice. II. Crosses with Fv-1^b strains of mice. J. Exp. Med. 136: 1286-1301, 1972.

Teich, N., Lowy, D.R., Hartley, J.W., and Rowe, W.P.: Studies of the mechanism of induction of infectious murine leukemia virus from AKR mouse embryo cell lines by 5-iododeoxyuridine and 5-bromodeoxyuridine. Virology 51: 163-173, 1973.

Rowe, W.P., Hartley, J.W., and Bremner, T.: Genetic mapping of a murine leukemia virus-inducing locus of AKR mice. Virology 178: 860-862, 1972.

Rowe, W.P., Humphrey, J.B., and Lilly, F.: A major genetic locus affecting resistance to infection with murine leukemia viruses. III. Assignment of the FV-1 locus to linkage group VIII of the mouse. J. Exp. Med., in press.

Ikeda, H., Stockert, E., Rowe, W.P., Boyse, E.A., Lilly, F., Sato, H., Jacobs, S., and Old, L.J.: Relation of chromosome 4 (linkage group VIII) to MuLV-associated antigens of AKR mice. J. Exp. Med., in press.

PROGRAM MANAGEMENT SEGMENT

Dr. J. B. Moloney, ASDVO, DCCP, NCI, Chairman

Dr. Louis R. Sibal, OASDVO, DCCP, NCI, Executive Secretary

LITTON BIONETICS, INC. (NIH-NCI-E-72-3294), BETHESDA, MARYLAND

Title: Operation and Maintenance of the Frederick Cancer Research Center at Frederick, Maryland

Contractor's Project Director: Dr. Robert E. Stevenson

Project Officer (NCI): Dr. William W. Payne

Objectives:

A. To establish and, if necessary, develop large-scale virus production and purification capabilities to meet NCI program requirements.

B. To conduct developmental research relative to virus production of those oncogenic or suspected oncogenic viruses for which no established protocols exist or for which existing protocols have failed to consistently provide a suitable product.

C. To prepare special viral diagnostic and test reagents including developmental research to either improve established techniques or improvise new ones as necessary.

D. To evaluate potential hazards associated with research activities in viral oncology and chemical carcinogenesis and to develop adequate protective measures in support of the NCI Office of Biohazards and Environmental Health.

E. To develop and implement a safety and environmental control program for the Frederick Cancer Research Center (FCRC) and to support applied and basic studies that will be performed by the Office of Biohazards and Environmental Control.

F. To establish, maintain and operate an Advanced Systems Laboratory for research in viral oncology, which shall contain the most modern equipment and safety features available for special programs conducted by NCI and visiting scientists.

G. To operate an animal farm for the breeding of rodents to meet the needs of research programs at FCRC and for shipment to other NCI operations as production permits.

Major Findings:

A. Virus Production. The initial objective of this task was to develop methodology to produce and purify 150 liters of Rauscher leukemia virus per week. Staffing, except for a purification department head, is completed as are facility renovations. Virus production reached 75 liters per week in January, 100 liters per week in February and 150 liters per week in March. This level of productivity is to be maintained through June 1973.

B. Developmental Research. This task has undertaken the development of production protocols for Epstein-Barr virus, gibbon ape virus and mouse mammary tumor virus. Staffing and facility renovations are essentially at completion; initial studies began in November 1972. Two electron microscopes have been installed and are operational in support of Task 1. The biological quality control area is in routine operation to furnish product description of Task 1 virus production.

C. Viral Diagnosis and Test Reagents. The program assigned to this task is to purify gs antigens and RNA-dependent DNA polymerase and to prepare specific antisera against these components. The laboratory has been in full operation since February 1973 and has finalized protocols to accomplish objectives of this task. Upon demonstration of the purity of antigen materials, antisera will be prepared in guinea pigs, rabbits, goats and burros. As with Task 2, quality control techniques have been developed and implemented in support of Task 1 virus production.

Associated with this task, at the present time, are two NCI intramural research programs dealing with the isolation and characterization of cell surface antigens and antibodies associated with various neoplasms and studies on compounds that can be used to prevent or treat cancer by immunostimulation and/or chemotherapy. Nononcogenic and oncogenic virus-host cell relationships are also under investigation. More detailed reports on intramural research will be found elsewhere (see reports of Dr. Boone and Dr. Chirigos).

D. Environmental Control and NCI Office of Biohazards and Environmental Control (OB&EC).

1. Environmental Control. A complement of ten was recruited for Task 4 to accomplish both the safety control program and to carry out an applied research program in support of FCRC operations including viral oncology. Safety programs were established for controlling the hazards related to biological agents. These activities include the following of all engineering plans and maintenance operations, training programs for personnel, the publishing of regulations covering biological, chemical and industrial safety, and the testing of equipment, e.g. biological safety cabinets.

2. OB&EC. Task 5 provided and maintained laboratories, animal holding and administrative space for use of the NCI OB&EC in Building 550. Materials and supplies, facility renovation, equipment and travel were provided as was a technical support staff of six scientific and technical employees. A number

of applied research programs are being carried out in cooperation with Task 4 to evaluate potential hazards associated with research activities in viral oncology.

E. Advanced Systems Laboratory. Viral Oncology's input in Task 6 consisted of (i) the establishment of a complete electron microscope laboratory in which a research program related to the finite structure of cancer cells was initiated under Dr. Victor Zeve, (ii) laboratory support for Dr. Sabin's and Dr. Tarro's work on nonviral herpesvirus-associated antigens, and (iii) a cooperative program with Dr. Paul Shapshak, LBI, on the localization and characterization of binding sites on neoplastic cell surfaces.

F. Animal Breeding. Foundation stock animals were received from the Genetic Center of VRB, DRR, where the animals had been caesarian derived and strictly maintained under barrier conditions. Production colonies have been established for three strains of guinea pigs, six strains of mice and one strain each of rabbits and rats. After the build-up of breeding stocks animals were made available to various programs including those of viral oncology.

Significance to Biomedical Research and the Program of the Institute:

The Viral Oncology projects that have been initiated at FCRC will greatly assist in providing a wide variety of biological resources that are necessary for NCI programs in virology, immunology, chemotherapy, molecular biology, genetics, and electron microscopy. They will also provide additional important information in the area of biological hazards and environmental control. Finally, the establishment of the Advanced Systems Laboratory will provide an exceptionally well equipped flexible laboratory environment for investigations that require special attention and/or for studies to be conducted by invited foreign scientists.

Proposed Course:

Programs will continue to be carried out in a manner similar to that outlined above. Further characterization of effort in the Advanced Systems Laboratory will be forthcoming.

Date Contract Initiated: June 26, 1972

Current Annual Level: $1,708,000

UNIVERSITY OF NORTH DAKOTA (PH43-66-8) GRAND FORKS, NORTH DAKOTA

Title: Quantitative Studies on the Transmission of Feline Oncogenic RNA Viruses and Selected Herpesviruses by Certain Bloodsucking Arthropods

Contractor's Project Director: Dr. Robert G. Fischer

Project Officer (NCI): Dr. George J. Burton

Objectives: To determine whether certain oncogenic viruses can be transmitted from infected animals to healthy susceptible animals by bloodsucking arthropods. Specific objectives are: (1) To determine virus levels in arthropods which have fed on experimentally infected donor animals of known titer. (2) To determine whether the disease caused by each oncogenic virus can be transmitted biologically, i.e. by transfer of the virus via the salivary glands of the infected arthropod. (3) To determine the quantitative distribution of the virus among various organs and tissues in those arthropods demonstrating suitably high virus levels. (4) To determine whether the disease caused by each virus can be transmitted mechanically through interrupted feeding.

Major Findings: (a) Friend leukemia virus (FLV) complex. In collaboration with Dr. Willie Turner (Howard University), the XC test was used as a sensitive assay for Friend leukemia virus viability in flies. After 7, 11, and 14 days in the fly the insect-passaged material was inoculated into Balb/c mice. Leukemic spleens yielded FLV titers greater than 10^5 PFV/ml; after 21 days the XC titer was less than $10^{2.5}$ PFV/ml. Simple bioassay procedures failed to detect after 2 days in the stablefly. Both the LLV and SFFV components of FLV survived up to 11 days in the insect, with probable viral replication. Virus degradation occurred between 11 and 14 days in the insect. All biological transmission experiments were negative.

(b) Herpesvirus Saimiri. In collaboration with Dr. L. Falk (Rush-Presbyterian St. Luke's), viability of H. saimiri was studied in the stablefly, the cat flea, Aedes and Anopheles mosquitoes, and the cone-nose bug. The HVS genome failed to survive beyond 6 hours in the stomach of any of the insects tested. All mechanical transmission attempts on insect mouthparts, when feeding on cotton-topped and white-lipped marmosets, were negative.

(c) Marek's disease virus. In collaboration with Dr. Jack Frankel (Life Sciences), mechanical transmission of Marek's disease by stableflies, cone-nose bugs, and Aedes mosquitoes was effected in 11 out of 18 LSI chickens kept in isolators. The insects had been pooled to determine whether a large amount of virus could be transferred from donor to recipient chickens on stylets of the mouthparts. Results obtained with S-line chickens were negative.

(d) Murine cytomegalovirus (Henson strain). In the stablefly this virus level falls to an undetectable level between 5 and 18 days after the viral blood meal, reappears subsequently, and in some cases may still be detected 29 days postfeeding. The virus probably replicates in the insect. Similar results were obtained with the Aedes mosquitoes with the virus reappearing

at 28 and 40-48 days postfeeding. Mechanical transmission of MCMV was also effected by stableflies in 11/53 Balb/c mice, as shown by challenge with $100xLD_{50}$.

Significance to Biomedical Research and the Program of the Institute:

Viruses may be transmitted biologically or mechanically by arthropod vectors. Mosquitoes, biting flies, fleas, ticks, and mites may suck blood from both man and domestic animals (including birds) closely associated with man. It is, therefore, important to investigate whether oncogenic viruses present in infected animals can be transferred to man, and remain viable and replicate in man. If such a vector is found, measures can be taken to control or prevent contact between the vector and human or animal hosts.

Proposed Course:

Insect transmission experiments will be continued using Marek's disease virus, murine cytomegalovirus (Henson strain), and feline oncogenic viruses, with mosquitoes, flies, fleas, and other arthropods. Additional species will be used as test insects in transmission experiments, when available in sufficient numbers.

Date Contract Initiated: October 27, 1965

Current Contract Level: $25,000 (Extended to December 31, 1973 without additional funds.)

BIOHAZARD CONTROL AND CONTAINMENT SEGMENT

Dr. Alfred Hellman, OASDVO, Division of Cancer Cause and Prevention, Chairman
Dr. W. Emmett Barkley, OASDVO, Division of Cancer Cause and Prevention, Vice Chairman

The contract programs administered by the Biohazard Control and Containment Segment are designed to identify potential biohazards which may be associated with facilities, equipment and procedures used by virus cancer investigators and to provide guidance and technical assistance to these investigators in order to improve their capacity for performing research with minimum hazards to the laboratory worker and the community and maximum protection against contamination. A major contract effort with the Dow Chemical Company has provided environmental control and laboratory safety services to all contractors of the SVCP. These services include conducting surveys of laboratory facilities to identify potential biohazards and to recommend practical corrective actions, designing equipment and developing improved procedures for the safe handling of oncogenic viruses, certifying safety equipment and assisting in the design of laboratory facilities. The equipment certification service has demonstrated that over 60 percent of all laminar flow biological safety cabinets tested to date failed to meet performance specifications and had to be modified in order to provide adequate safety for the user.

A basic safety training course on the principles of biohazard and injury control has been developed through a contract with the University of Minnesota. This course is presented four times annually to laboratory workers engaged in cancer virus research. These courses provide an opportunity to investigators and technicians to learn the fundamentals of laboratory safety including the correct usage of biological safety hoods, personnel protective devices, disinfection and sterilization techniques and the principles of biological, physical, chemical and radiological hazard control.

An evaluation of potential aerosol exposure hazards created by biochemical and biophysical procedures used in virus-tissue culture laboratories has been conducted by the Naval Biological Laboratory. Tests have been performed to quantitate the aerosol output of the blender, sonic homogenizer, pipetting procedures and the zonal centrifuge. Results of these tests demonstrated that the blender, sonic homogenizer and pipetting procedures are capable of producing detectable aerosols which can be inhaled by the laboratory worker.

Basic research studies are in progress to: (1) evaluate the effect of a selected stress situation on induction of viral disease or cancer in situ, (2) investigate the immunological response of laboratory animal hosts to oncornaviral antigens and (3) elucidate the role of latent virus infection of laboratory animals. Aerosol exposure of mice to selected environmental insecticides which mimic estrogenic activity induces the expression of information found in murine leukemia virus. Studies initiated at the Southwest Foundation for Research and Education to determine if oncornaviruses could pass the placental barrier demonstrated the presence of C-type virus particles in the syncytiotrophoblast. This observation is being pursued in detail.

THE DOW CHEMICAL COMPANY (PH43-65-1045)

Title: Research and Development of Biohazard Containment Facilities

Contractor's Project Director: Mr. Cyril B. Henke

Project Officer (NCI): Dr. W. Emmett Barkley

Objectives:

1. In collaboration with the NCI Office of Biohazard and Environmental Control, the contractor performs Biological Safety and Environmental Control surveys of SVCP contractor operations to evaluate laboratory practices, safety equipment and facilities and to assess the effect of contractor operations on laboratory safety and environmental quality.

2. The contractor assists the Office of Biohazard and Environmental Control in the preparation and dissemination of safety equipment specifications, facility design criteria, operations quidelines and safety procedures. The contractor also prepares engineering design drawings and specifications for containment systems required to meet specific program needs.

3. The contractor operates a safety equipment certification program to assure safe operation of SVCP safety and containment equipment.

4. The contractor assists the Office of Biohazard and Environmental Control in the review of plans and specifications for SVCP renovation and new facility construction projects.

Major Findings:

The contractor participated in eleven site visits, provided extensive consultation support to six SVCP contractors, certified over 100 laminar flow biological safety cabinets and has reviewed facility design criteria for a number of NCI contractors and grantees. The contractor has assisted the NCI, OB&EC in evaluating laboratory safety problems identified during the eleven completed site visits. Individual site visit reports were prepared to provide necessary quidelines to assist contractors in implementing the NCI Minimum Standards of Biological Safety and Environmental Control. Design drawings and specifications have been prepared for laminar flow biological safety cabinets, small batch laboratory biowaste inactivation systems and other specialized containment devices. A special ventilated enclosure for the Beckman Model L series centrifuge has been designed, fabricated, installed and evaluated. Recommendations for safe operation of the KII centrifuge were prepared. A subcontract has been initiated to investigate aerosol hazards created during normal and abnormal operating conditions of the KII centrifuge.

A manual entitled, "Laminar Flow Biological Safety Cabinets: A Training Manual for Biomedical Investigators" has been written, printed, bound, and distributed to SVCP contractors. The contractor, in a collaboration with

the Division of Research Services, NIH, has prepared a film strip based on this manual. The film strip is accompanied by a narration on a tape cassette. One hundred fifty sets of the strip and narration will be distributed to interested laboratories throughout the country.

The equipment certification program has demonstrated that over 60 percent of laminar flow hoods fail to provide adequate personnel protection.

Significance to Biomedical Research and the Program of the Institute:

This contract contributes biological safety and environmental control expertise to the SVCP. This expertise is used to improve the quality and safety of the cancer research laboratory environment. The contractor functions as an integral part of the NCI Office of Biohazard and Environmental Control.

Proposed Course:

The contractor will continue to provide technical assistance to the SVCP contractors on problems of environmental control, personnel safety and product protection. The contractor will participate on approximately twenty-five Biohazard Safety and Environmental Control surveys. The contractor will review all plans and specifications for renovation and new facility construction projects supported by contracts awarded by DCCP. Review and approval will be based on compliance with accepted engineering design, biological safety and environmental control practices.

Date Contract Initiated: June 25, 1965

Current Annual Level: $250,000

UNIVERSITY OF MINNESOTA (NIH-NCI-E-72-2066)

Title: Development and Presentation of Course in Contamination and Physical Hazard Control

Contractor's Project Director: Dr. D. Vesley

Project Officer (NCI): Dr. W. Emmett Barkley

Objectives:

The objective of this project is to present four courses on the principles of biohazard and injury control in the biomedical laboratory. Course participants are drawn from personnel from other cancer laboratories associated with the NCI contracts and grants programs.

These courses provide an opportunity to scientific investigators and laboratory technicians involved in cancer virus research to learn the fundamentals of laboratory safety including the correct usage of biological safety hoods, the adequacy of protective clothing and other personal

protective devices, disinfection and sterilization techniques and the principles of biological, physical, chemical and radiological hazard control.

Major Findings:

Four courses have been presented; two at the University of Minnesota from June 12-16 and October 16-19, one at the Naval Research Biological Laboratories in Oakland, California from August 21-24, 1972, and one at the NIH from December 12-14, 1972. One hundred fifty investigators and technicians have attended these training courses.

The contractor has developed a course manual and compiled a "handout packet" of up-to-date reference material. The course manual includes lecture outlines, references, data tables, and other supporting information for each of the course lectures. Another section of the manual details laboratory exercises and instructions for the laboratory portion of the course.

The course has succeeded in stimulating the participants to think positively about laboratory safety problems. Follow through efforts by the Office of Biohazard and Environmental Control have demonstrated an increased awareness of laboratory safety objectives among those scientists who have attended the course. Most important, however, has been an increase in laboratory safety practices by many course participants.

Significance to Biomedical Research and the Program of the Institue:

The training program provides a most effective mechanism for disseminating current biological safety information. Course participants benefit directly by learning biological safety and environmental control methods and techniques. Thes program benefits the institute by increasing the general safety awareness of the laboratory participant and his associates.

Proposed Course:

The contractor will present four courses in 1973. Because the simplified logistics of presenting the course in Minneapolis rather than on the road contributes to the course quality, three courses will be presented in Minneapolis and only one out of state at Fort Detrick at approximate three month intervals in June, September, October and December.

The contractor will re-evaluate the course content thoroughly on the basis of evaluations received from the four 1972 courses before plans are finalized for the new series. The laboratory sessions particularly will be reviewed carefully and revised as necessary for maximum impact. The experience and responsibility level of the majority of course attendees might dictate other course changes. In addition, handouts and references will be reviewed constantly and updated as new materials become available.

In addition, other efforts will update audiovisual aids and obtain new materials for displays. Lecturers will be encouraged to update and revise lecture outlines and content after each course.

Date Contract Initiated: December 10, 1971

Current Annual Level: $88,000

NAVAL BIOLOGICAL LABORATORY (FS-57)

Title: Studies of Environmental and Physiological Factors Influencing Virus-Host Interaction

Contractor's Project Directors: Dr. R. L. Dimmick
Dr. M. A. Chatigny

Project Officers: Dr. A. Hellman
Dr. A. K. Fowler
Dr. W. E. Barkley

Objectives:

This contract has three objectives, they are:

1. Virus Laboratory Hazards Evaluation. The objective of this section of the proposal is to evaluate the extent of possible hazards involved in biochemical and biophysical procedures used in virus-tissue culture laboratories.

2. Studies on Environmental Effects on Physical and Biological Characteristics of Viral Aerosols. The objective of this section of the proposal is to provide survival data of both "model" and oncogenic viruses as related to environmental parameters (e.g. temperature, RH, RH changes, and trace chemicals - decontaminants) for use in Section 1, and to evaluate the importance of end-spectrum (0.1 to 0.5 mu) (5 to 15 mu) particles on virus-host interaction considering both the hazard to humans and animals and the potential for cross contamination.

3. Host-Virus Interactions. The objective of this section is to evaluate the effect of selected stress situations (physiological as by hormonal imbalance, immunological as by concurrent infection or biochemical, as by exposure to injurious chemical vapors of aerosols) on induction of viral disease or cancer in situ, and to evaluate the role airborne particle size might play in such interactions.

Major Findings:

1. Laboratory Hazards. Tests of the aerosol output of those methods and techniques of major interest and usage in the research laboratory have been substantially completed, and sufficient knowledge of data treatment has been gained to permit using additional published data to create tables of spray factors.

2. Environmental Effects on Aerosols. The project has encountered difficulty in obtaining a sufficiently high titer of Rauscher Murine Leukemia Virus (RLV) to permit quantitative assay of aerosols, but at 30% and at 70% relative humidity, at room temperature, some virus remained viable in the air as long as 2 hrs. In addition to a program of evaluation of aerosol outputs from various laboratory operations, we have conducted a limited scope program of in situ testing of verticll laminar flow hoods (VLF). This testing is a logical follow-on to previous work as a measure to determine the manner of application of the biological risk data previously acquired, estimation of the protective factors, protective equipment, and provision of equipment selection criteria for the investigators.

Most VLF units tested provided excellent product protection but leaked from the inside to the outside through filters or casings. Only in rare cases do the units meet manufacturer's advertised performance specifications. Safety hoods have inherent leakages through the work entries. These can be computed and the risks of their use defined. Leakage through case defects is of varying magnitude and must be controlled before prediction of the protection factor of the equipment can be defined.

VLF hoods currently in use must be afforded substantial repair, or modification (or replacement) before they will be suitable for use with materials with any presumptively pathogenic or oncogenic materials. A program for hood upgrading and standardized testing procedures is a prerequisite for definition of "in use" protective factors. We will continue to test equipment both as a service and to define upgrading requirements in preparation for definition of protection factors.

3. Host Virus Interaction. The dosage of inhaled and retained insectisides was 60 to 100mg/kg of body wt. Mice were held either 4 to 6 weeks after inoculation with RLV (0.5 ml intraperitoneal), then necropsied. Two experiments with DDT have been completed. Exposure to DDT caused a uterotropic effect and influenced the course of the subsequent leukemia disease.

The same general affect was noted in experiments with Lindane, except that the response was more significant. Exposure to Lindane alone produced a significant decrease in size of the thymus.

A preliminary experiment with Methoxychlor caused mean uterine weight of Balb/c mice to nearly doubled and the increase in spleen weights of inoculated and exposed mice, compared to unexposed inoculated mice as above, was significant at the 99%, (t-score) level.

4. Disinfectants. One-half ml of a 1000 ppm solution of NHC could be added to a 5 ml tissue culture dish without adversely affecting Hela cells.

In a preliminary experiment with polio virus the titer was decreased from 2×10^8 pfu/ml to less than 1×10^2/ml in a 10 minutes exposure to NHC solution (2500 ppm).

Significance to Biomedical Research and the Program of the Institute:

An understanding of the survival and clearance rate for virus aerosol will allow the development of a rational and hopefully less expensive means for control of cross infection. Similarly development of mathematical models will permit the more precise evaluation of facilities, on the basis of risk. Transmission of chemicals that are found in the environment, which have been demonstrated by Hellman and Fowler to induce activation of tumor virogenic markers (g.s. and reverse transcriptase), make an understanding for their potential to activate such markers by the respiratory route very important in the evaluation of risk either as a primary or co-inducing factor.

Proposed Course:

We propose to continue studies of specific problems in control of laboratory biohazards generated in work with oncogenic viruses and infected tissue cultures. We will study synergistic effects of selected chemicals and viruses on oncogenic susceptibility, and the effect of physiological stress (as related to changes in immune response) on susceptibility. As adjuncts to this work we will study survival of selected airborne viruses in varied environments and optimize methods of assay.

Date Contract Initiated: March 1, 1971

Current Annual Level: $115,000

OHIO STATE UNIVERSITY (PH43-65-1001)

Title: Biohazard Control and Containment in Oncogenic Virus Research

Contractor's Project Director: Dr. D. Yohn

Project Officers (NCI): Dr. A. Hellman
Dr. A. K. Fowler

Objectives:

The purpose of this research program is to elucidate the possible hazards to man and animals from exposure to oncogenic viruses and their infectious nucleic acids. The possibility that animal hosts are able to respond immunologically to oncornaviral antigens is being investigated and efficacy of various vaccine preparations is being tested.

This program for the past year has had essentially five components. These can be identified as follows: 1) analysis of human sera for antibodies to mammalian RNA tumor virus antigens, 2) evaluation of killed virus vaccines against feline RNA tumor viruses, 3) evaluation of a nucleic acid-free vaccine from feline sarcoma tumor cell membranes against feline sarcoma virus, 4) characterization of virus induced melanomas in cats and 5) *in vitro* assays.

Major Findings:

The pilot studies were considered to be of sufficient potential importance to warrant more definitive studies on sera from patients with rhabdomyosarcoma, osteogenic sarcoma, reticulum cell sarcoma, lymphosarcoma, liposarcoma and breast cancer. Consequently it was the considered judgment of SVCP staff that these studies should be repeated with, if possible, an antiserum specific for the mammalian interspecies antigen, gs-3. The use of such an antiserum would preclude the criticism that more than a single antibody-antigen reaction was at least potentially available to be inhibited by the human sera. Accordingly a major porportion of this years effort was directed toward preparation of such an antiserum.

A small molecular weight protein of approximately 13,000 MW has been isolated from KT-FeLV that cross-reacts with antisera to FeLV and MuLV core proteins. Antisera prepared to the protein cross reacts in CF and in immunodiffusion with FeLV and MuLV. This antisera was absorbed with sheep and human A and B RBC and used in complement fixation inhibition (CFI) tests for antibodies to the interspecies antigen in feline and human sera. In feline sera the antibodies appeared following virus infection and prior to tumor formation.

In humans, antibodies in relatively high titer, $\geq$ 1:32, were found in a highly significant number of sera from patients with lymphosarcoma (70%), osteosarcoma (41%), reticulum cell sarcoma (57%) and rhabdomyosarcoma (32%). Similar titers were found in only 6% of normal sera. Approximately 23% of sera from patients with acute lymphocytic leukemia or with breast cancer were reactive.

Vaccination regimes employing newborn kittens is nearing completion. All 14 cats in the UV-inactivated G-FSV vaccine treated group have been necropsied while 5 of 14 remain in the formalin inactivated G-FSV vaccine treated group. Of the remaining cats one has no tumor, 2 have demonstrated tumor regression, one has demonstrated tumor regression with reappearance of tumor and one shows a progressing tumor. Two of 15 cats remain in the unvaccinated control group, both of which have demonstrated tumor regression.

Results in the UV and formalin treated G-FSV vaccine groups were essentially identical. Ten of 14 cats (71%) in both groups have succumed as a result of tumor progression while of the remaining 4, three have demonstrated tumor regression and 1 has not developed any tumor. In unvaccinated control kittens, 54% (8/15) demonstrated tumor progression while 46% (7/15) either developed no tumors or developed tumors which regressed. Greater tumor incidence in the vaccinated groups than in the unvaccinated group may reflect possible enhancement. Certainly it is apparent that vaccination did not provide protection.

Vaccination had no protection for kittens challenged with Snyder Theilen - tumor homogenate since only one of 5 kittens demonstrated tumor regression. All 12 unvaccinated control kittens died as result of tumor formation.

Vaccination of pregnant queens and challenge of their litters within 1 week after birth has resulted in the most successful of the vaccination regimens. Three of 12 cats have not formed tumors while an additional 4 have demonstrated tumor progression. Only 3 of the 12 cats in this group have demonstrated tumor progression (25%) with an additional 2 giving rise to tumors which subsequently regressed then reappeared. At present 4 cats remain alive in this group; one with no tumor, two with regressed tumors and one with a progressing tumor which had earlier regressed. The control group of 13 cats from unvaccinated queens have been challenged but it has been too soon for tumors to have appeared.

Tumor latent period and mean survival time differences for the various vaccine regimens being evaluated. Latent periods for the G-FSV challenge of vaccinated kittens do not appear to be significantly different from that of the unvaccinated group.

The differences in tumor latent period observed between the vaccinated kitten and vaccinated queen regimen groups does not appear significant. It is interesting to note however, that consistently greater latent periods are observed in cats challenged at 5 days after birth, while those challenged at 8 days appear to give rise to tumors in a shorter time. The significance of this will be evaluated in future experiments.

Challenge with the ST tumor homogenate appears to give rise to tumors in a significantly shorter time interval than does challenge with G-FSV. This is consistent with the virulence differences between these viruses, as is evidenced by the substantially shorter survival times for both the vaccinated and unvaccinated groups.

Mean survival time for the progressor groups among the kitten immunized with killed G-FSV and challenged with G-FSV (149 ± 37 and 179 ± 44) are clearly shorter than the regressor or no tumor groups. Such a clear cut difference is not readily apparent within the unvaccinated control group.

The use of nucleic acid free tumor virus vaccine by using tumor cell membrane, has not demonstrated any significant degree of protection on subsequent virus challenge.

Significance to Biomedical Research and the Program of the Institute:

Development of a diagnostic test for maligna t disease prior to clinical evidence would be of great benefit for subsequent determination in the type of therapy to be used. Development of a vaccine against a C-type RNA virus in a model system such as the cat could provide some rational as to the potentials for developing such vaccines against human disease if ever a viral induced malignancy can be identified in man.

Proposed Course:

Evaluation of the significance for the reactive human cancer seras will be evaluated on the basis of control seras appropriately selected. The presence of neutralizing antisera in cats previously immunized will be determined. In order to permit a greater degree of immunological

competency to develop prior to virus challenge, a feline leukemia virus isolated by Dr. Hardy, will be used. This virus will cause disease in adult cats, thereby permitting adequate vaccination regimes to be developed, since other feline tumor virus to date required inoculation into cats during the first week of life.

Date Contract Initiated: June 22, 1965

Current Annual Level: $257,000

SOUTHWEST FOUNDATION FOR RESEARCH AND EDUCATION (NIH-71-2348)

Title: Study of Latent Virus Infection and Transmission

Contractor's Project Director: Dr. S. S. Kalter

Project Officer (NCI): Dr. Alfred Hellman

Objectives:

It is the purpose of this contract to study various aspects of the role of latent virus infections of laboratory animals and cultures of their organs as a source of contamination of experimental materials, laboratory personnel and vaccines.

Major Findings:

During the first third of this contract year, studies on the inactivation of H. hominis types 1 and 2 by ultraviolet light were carried out. Briefly, these studies demonstrated the ability of a cell to replicate herpes-viruses was quickly inactivated. This occurred more rapidly in diploid than heteroploid cells. When infected cells were irradiated at different periods during the replicative cycle of the virus it appeared virus inactivation occurred more readily during the latent period.

During this period, studies were initiated on several oncornaviruses with the anticipation that infected cells may be capable of crossing species barriers and initiating tumors or leukemia in a foreign host. Studies were also initiated to determine if oncornaviruses could pass the placental barrier. This work is being carried out in baboons. Pregnant animals were inoculated intravenously with either herpes- or pox-viruses and the fetal tissues, including the placenta, were examined for the presence of virus, by virus isolation and examination by the electron microscope. In the course of the electron microscopic studies no herpes- or pox-virus particles were observed but a number of C-type virus particles were seen in the syncytiotropho last. Because of the importance of these findings to the Special Virus Cancer Program and the epidemiology of virus induced cancer, at the direction of the project officer, the entire effort of this contract was redirected toward the study of placental and fetal tissues for evidence of viral particles.

Significance to Biomedical Research and the Program of the Institute:

The presence of C-type particles in "normal" primate tissue has for the first time been visualized. The significance of their presence can only be conjectured at this time, however, the fact that they are present in relatively large quantities at a time of endocrinological stimulation further adds significance to the induction of C-particles information by hormones (Hellman and Fowler) and may be significant for propogation of the species. Finding such particles in "normal" tissue permits one now to determine whether they really have any direct cause and effect relationship in inducing malignancy in any primate.

Proposed Course:

All efforts will be directed in this laboratory and that of the project officer to determine the function that these particles have in primates as well as the possible universal presence of such C particles at particular stages of host development.

Date Contract Initiated: June 3, 1971

Current Annual Level: $57,400

SUMMARY REPORT

BREAST CANCER VIRUS SEGMENT

July 1, 1972 through June 30, 1973

Introduction

The observation of particles in human milk which resemble mouse mammary tumor virus (MMTV) has triggered most of the work in the Breast Cancer Virus Segment. The objectives of the Segment which follow from this observation are:

1. Confirm and extend the observation of B-type particles in human milk and correlate its occurrence in high and low risk breast cancer groups.

2. Develop and standardize tests for detecting such particles, especially by electron microscopy and reverse transcriptase measurement.

3. Isolate from milk or from tissue culture sufficient human milk particles to produce immunologic reagents for screening.

4. To develop methods of *in vitro* production of MMTV for development of immunologic reagents.

5. To grow functional human and mouse breast cells in culture as target cells for these viruses.

6. To test *in vivo* the ability of new animal breast cancer viruses to produce mammary carcinomas, e.g. Mason-Pfizer monkey virus.

Progress

During this year studies on three contracts at Michigan Cancer Foundation, Institute for Medical Research, and Georgetown University have revealed that there is no correlation between the presence of reverse-transcriptase activity and virus or virus-like observation of particles in human milk. These particles are physically degraded by action of the cream; the mechanism of damage has not yet been identified. RNAase activity naturally present in milk co-sediments with the particles and destroys the template for the reverse-transcriptase; methods of inhibiting RNAase activity are under development. Pending the development of reliable methods for reverse-transcriptase determinations in milk, correlation of particles in human milk to various risk levels for breast cancer will have to be postponed and previous surveys will have to be repeated. The incidence of positive milks by reverse-transcriptase now ranges from 17% to 75% among the various laboratories.

Keydar has reported preliminary results that indicate it may be possible to replicate the human milk agent in primary tissue cultures taken from the breast area of neonates. The particles were detected primarily by reverse-

transcriptase but were also seen on EM examination. However, none of the cultures maintained "particle" production for extended periods. Confirmation of these observations is high in priority for next year.

The homology found by Spiegelman between MMTV RNA and RNA from human breast cancer, has stimulated more work on production of MMTV in vitro. Keydar has reported development of a mouse cell line which apparently produces MMTV in vitro. However, this line has not yet been studied in detail. Another line has been established by the Detroit group which forms "domes" in culture, a characteristic of primary mouse cultures which produce MMTV. This dome-forming line has not been studied for MMTV production. It does have estrogen receptors and therefore is likely to be a functional breast cell. One of the impediments to the study of in vitro MMTV production has been the lack of a convenient assay system. A sensitive and specific radio precipitation assay has been developed by Cardiff.

Attempts are under way to grow cultures of functional breast cells from human neonates to repeat here the work of Keydar. Several cultures have been produced but not yet tested. New methods for growing human breast cancer cells have been studied in several laboratories and have resulted in the isolation of several lines, one of which produces "domes". These lines will have to be studied closely for susceptibility to, and production of, the human milk agent.

Moore has produced further evidence that human breast cancer sera can neutralize MMTV in the in vivo tumor production test. This is consistent with Spiegelman's homology experiments. The radioimmune assay should help to clarify this observation in the coming year. The Netherlands group has developed a rapid in vivo assay for MMTV which may also accelerate such work.

The Netherlands group also has evidence for vertical transmission of MMTV in GR mice as contrasted with milk transmission in other strains. Vertical transmission is more consistent with the occurrence of breast cancer in humans than is milk transmission, according to preliminary evidence by the Michigan group. Linkage of cancers through the paternal side was as strong or stronger than through the maternal lineage.

As soon as immunologic reagents have been prepared to either the mouse or human agent, they can be tested against a panel of human breast cancer sera and controls being collected at Memorial Hospital.

The type of particle seen routinely in human milk resembles that of Mason-Pfizer virus. Innoculation of monkeys and later stimulation to lactation by hormones has not produced any tumors; however, animals have not been observed for a sufficient time. A neutralization test for Mason-Pfizer virus is under development. This test may allow testing for neutralizing ability of human breast cancer sera and the distribution of the virus in monkey populations. Viruses similar to Mason-Pfizer have been identified morphologically in several normal monkey fetuses.

R-35 virus was claimed to increase the incidence of malignant but not benign tumors in rats. Confirmation studies are under way. The in vitro transformation of rat breast cells by R-35 virus was not repeatable and earlier reports must be discounted.

UNIVERSITY OF CALIFORNIA, DAVIS, CALIFORNIA (NO-1-CP-3-3253)

Title: *In Vitro* Cultivation of Human and Mouse Mammary Tumor Viruses

Contractor's Project Director: Dr. Robert D. Cardiff

Project Officer (NCI): Dr. Robert H. Depue, Jr.

Objectives:

1. To study the production of mouse mammary tumor virus (MTV) *in vitro* in order to develop concepts, techniques, and reagents that might be applicable to larger scale production of this virus and of potential human breast cancer viruses.

2. To study human breast tissue under various experimental conditions developed from the mouse mammary model for the presence of possible human mammary tumor viruses.

Major Findings: Densely crowded primary cultures of mouse mammary tumor cells form 3-dimensional units or "domes". The number of domes is proportional to the virus released. These appear in densely crowded cultures within 48 hours and persist 6-7 weeks. Estimates of virus harvested was about 1 µg of viral protein per ml of culture fluid. The major protein in the virus band was 56,000 Daltons, and appeared to correspond to Nowinski's S1. By immunofluorescence, dome cells have the highest concentration of viral antigens. Domes come and go in culture but apparently only some of the cells have the capacity to form domes since domes always reappear in the same locations in cultures.

The contractor has developed a radioimmune assay for MMTV which can be used to monitor virus production.

Cultures have been made of over 140 human breast biopsies, benign and malignant. Some are still in culture but not at a high passage level. One culture has produced domes which lasted only several weeks. A method for microdissection of human breast specimens has provided isolated lobules for tissue culture study.

Significance to Biomedical Research and the Program of the Institute: The finding of virus-like particles as well as 70S RNA and reverse transcriptase in human milk and breast cancer tissue cultures by several investigators within the SVCP strongly suggest that a virus may be associated with human breast cancer. If so, it will be necessary to produce such virus in large quantities *in vitro* to further characterize it and determine its relation to the human disease. But this problem, *in vitro* cultivation, has not yet been solved for the mouse MTV.

This contract is for the development of knowledge and technology for *in vitro* cultivation and large scale production of the MTV, with simultaneous application of progress to attempts to cultivate the human candidate virus.

Proposed Course: No change in initial contract.

Date Contract Initiated: 01 February 1972

GEORGETOWN UNIVERSITY SCHOOL OF MEDICINE (PH43-NCI-E-65-53)

Title: Human Breast Cancer Studies

Contractor's Project Director: Dr. William F. Feller

Project Officer (NCI): Dr. Robert H. Depue, Jr.

Objectives:

1. Continuation of studies on the association of virus-like particles in human milk, with human breast cancer.

2. Tissue culture attempts to propagate virus-like particles observed in human milk and breast cancer cells.

3. Attempts to induce mature virus production in established tissue culture lines of human cancers, particularly breast cancers.

Major Findings:

Human Milk Studies

Previous studies had shown that oncorna virus-like particles are found more frequently in the milk of women who have had breast cancer than in normal women of the general population or of high risk populations. A major increase in effort was therefore made during most of this contract year to find, and enlist the participation of milk donors with a history of breast cancer. Only a single donor has been found thus far in spite of the increased effort in four large population centers. This compares with a previous average of about four per year from the Washington metropolitan area, alone and is thought to indicate that as a result of the new abortion laws fewer pregnancies are now allowed to go to term in women with a history of breast cancer.

The simultaneous test for heavy (60-70S) RNA and reverse transcriptase was introduced as a monitoring technique in the search for viruses in milk in place of the less sensitive and more cumbersome method of electron microscopy. In studies of twenty-nine milk specimens from normal women taken at random, 41% were positive for the biochemical markers of oncorna viruses by this more sensitive test. An important finding was that 56% of the women in the lowest age group (15 to 21 years) were positive as compared with 27% of women over 21 years of age. In addition, the strongest positive tests were obtained with milk of the younger donors. However, subsequent studies have confirmed the

presence of an RNAse in human milk which acts as an inhibitor of the reverse transcriptase test. Work is underway to find a method to bypass this difficulty. It is not known if the distribution of positive milks is due to the reverse transcriptase or to the nuclease.

Tissue Culture Studies

Twenty-two biopsies of human breast cancer have been successfully explanted in tissue culture. Of these, 11 have been maintained for 200 days or longer. No virus-like particles have been observed in any of them by EM, and attempts to induce virus by treatment with IDU and DMSO have been negative.

On the other hand, similar attempts to induce covert viruses have been successful in 3 cultures of other types of human cancer (a rhabdomyosarcoma, a lung cancer, and an osteosarcoma). The results on induction of both type C particles budding from cell membranes, and an intracisternal particle budding from the endoplasmic reticulum were published during the year. Collaborative studies with Dr. Werner Schaefer on the first two cultures have shown that the viruses do not cross-react immunologically with known murine or feline oncorna viruses.

Significance to Biomedical Research and the Program of the Institute: The demonstration of particles in human milk resembling the mouse mammary tumor virus is one of the most important leads in breast cancer research. This contractor is attempting to confirm this observation, apply the reverse transcriptase assay to detection of the particles, and recover the particles in infectious form in tissue culture.

Proposed Course:

Work will be concentrated on the problem of inhibitors in human milk so that valid surveys of the incidence of virus-like particles in milk can be validly conducted. Milk particles will be inoculated into appropriate cells in tissue culture in an attempt to replicate them. The virus-like particles produced by the cell lines from other cancers will be studied immunologically to identify the probable species of origin.

Date Contract Initiated: 19 November 1964

HOWARD UNIVERSITY SCHOOL OF MEDICINE (NIH-NCI-E-70-2178)

Title: Immunological Studies on Human Breast Cancers and Other Neoplasms

Contractor's Project Director: Dr. Michael V. Viola

Project Officer (NCI): Dr. Leo Phillips

Objectives:

1. Procurement of human specimens for use by SVCP participants as well as for research conducted in the contractor's institution.

a. Selected specimens of breast and other cancers procured at operation or biopsy.

b. Sera from patients with breast and other cancers, and from patients with non-neoplastic diseases for use as controls.

2. Establishment and characterization of tissue culture lines from selected cancer patients, particularly breast cancer.

3. Immunological studies of established cancer cell lines and of patients with breast cancer and other neoplasms.

Major Findings:

1. Procurement: Sixty (60) serum samples from patients with cancer have been supplied to investigators at the National Institute of Health.

2. Research:

a. Studies with a long term cell line (Welch line) derived from a malignant pleural effusion of a woman with breast cancer have continued. The characteristics of the line are as follows:

(1) monolayer composed of epitheloid and fibroblastoid cells presently in its 20th passage.

(2) generation time of 38 hours (in 12th passage).

(3) contains polyploid human chromosome complement (19th passage).

(4) preliminary evidence indicates that it is a hormonal resistant cell line. It responds to cultivation with insulin, hydrocortisone, estrogens, androgens, and prolactin in the same manner as non-hormonally dependent cell lines (e.g. Hep 2).

Concentrated supernatants from Welch line contain reverse transcriptase. If a brief (10 minutes) reverse transcriptase reaction using an endogenous template is subjected to velocity gradient ultracentrifugation a peak ^{3}H-DNA: RNA hybrids occur in the 70S region which is completely removed by treatment with DNAse-free RNAse. When concentrated supernatants from Welch line were subjected to equilibrium density gradient centrifugation polymerase activity settled between 1.16-1.185 gm/ml.

A second monolayer cell line has been established (Smith line) from a malignant breast effusion. Unlike the Welch line, this line is entirely composed of epithelial cells.

Proposed Course: Terminates April 1973

Date Contract Initiated: 27 April 1970

INSTITUTE FOR MEDICAL RESEARCH (PH43-NCI-E-68-1000)

Title: Studies of Human Milk and Mammary Tumors

Contractor's Project Director: Dr. Dan H. Moore

Project Officer (NCI): Dr. Robert H. Depue, Jr.

Objectives: To explore human milk and breast cancer tissue in search of a virus that might be etiologically related to the disease.

Major Findings: Studies to demonstrate correlation between particles observed in the EM and reverse transcriptase (RT) activity have shown no correlation to date. In general milks containing the MS-1 particle contain RT activity. Reconstruction experiments by adding mouse R-III virus to human milk showed that RT activity was inhibited. In addition some milks effect extensive destruction of MMTV as seen by EM. The latter activity appears to be mainly associated with the cream fraction. Inhibition of the RT activity corresponds to the loss of the 70S RNA template. No correlation was found between the loss of morphologic integrity with the loss of RT activity and infectivity.

Attempts have been made to obviate some of the problems by modifying the assay. These techniques have resulted in 100 to 1000 fold increase in the sensitivity of detection of RT activity of R-III MMTV as compared with the previous method. With the new technique, 74% of human milk specimens were positive for RT activity. However, there is still no correlation between RT activity and particle content in human milk.

It has been found that approximately 25% of human female serum neutralize MMTV by infectivity tests in mice. In this series, no correlation with the cancer status of the patient was shown.

In the past year a total of 110 human breast tumors have been explanted. The life span of breast tumor cells in suspension was never extended beyond the tenth month. A search for 70S RNA and reverse transcriptase in primary cultures and co-cultures of breast tumors did not give clear evidence of an oncogenic virus. One hundred seventy specimens have been searched by EM for the presence of budding virus particles without positive findings; however, many virus-like particles are found in high-speed pellets of culture supernatants. A serum substitute has been developed that appears to stimulate cell multiplication in primary cultures of human breast tumor cells when supplemented with amniotic fluid and prolactin.

Significance to Biomedical Research and the Program of the Institute: This approach to the human breast cancer virus problem is one of using technology developed in the MMTV system to determine whether a comparable viral agent can be demonstrated in human breast cancer. This contractor was selected initially because the Principal Investigator has had long experience with, and has contributed significantly to the development of the mouse model system. Exploratory studies on human materials led to strong evidence for a comparable human virus and resulted in the establishment of this multidisciplinary effort to follow-up these leads.

Proposed Course: This multidisciplinary effort will continue at its current level for further electron microscopic, biochemical, tissue culture, and immunological investigations of the candidate human virus in milk and breast cancer tissue. Emphasis will be placed on confirming the neutralization of MMTV by human sera, the immunological cross-reactivity of the human and mouse particle, the search for tissue culture system, the survey of human milk for virus-like particles and reverse transcriptase activity, search for particles in cultures of human breast cancer.

Date Contract Initiated: 28 June 1968

MASON RESEARCH INSTITUTE (NCI-E-70-2204)

Title: Role of Hormones in Induction of Mammary Cancer in Animals by Viruses

Contractor's Project Directors: Dr. Arthur Bogden, Dr. Marcus Mason

Project Officer (NCI): Dr. Roy Kinard

Objectives: To evaluate the oncogenic potential of two viruses isolated from mammary adenocarcinomas, M-PM from a rhesus monkey and R-35 from a Sprague-Dawley rat, and determine the roles of hormones, especially those which induce mammary hyperplasia and lactation, in pathogenesis of mammary cancer caused by these viruses.

Major Findings:

There are presently inhouse a total of 23 female rhesus, 2 male rhesus, and 3 female cynomologous monkeys that have been inoculated neonatally with M-PMV and being held under observation until maturing to the age of 18 months before being treated with hormones. A total of 30 adult rhesus females are under continuous steroid therapy; 20 have been inoculated with M-PMV directly into the mammae and 10 are serving as controls. Lactation has been induced in all animals and milk samples have been collected and freeze-preserved. Attempts to detect evidence of virus by electronmicroscopy, immunological tests for virus antigen, and reverse transcriptase activity are now in progress in collaboration with other SVCP contractors and NCI scientists. Thus far, tests for reverse transcriptase have produced the only evidence of virus infection. A polymerase positive milk has been detected from a non-virus inoculated control animal.

Lactation and uterine hypertrophy were induced in 5 non-pregnant female rhesus monkeys by exogenous 17β estradiol and progesterone. Hypertrophic uteri were then removed surgically. Following hysterectomy there was a significant increase in milk secretion and in serum levels of estrone and estradiol. The increased mammary secretory activity was correlated with the increased levels of circulating estrogen following hysterectomy indicating a significant competition between the uterus and mammae for estrogen. It would appear that hysterectomy may favor stimulation of steroid responsive neoplastic mammary tissue, and be contraindicated following ovariectomy as a therapeutic procedure.

In a normal rhesus female, received from Bionetics Research Laboratory, lymphadenopathy and lymphocytosis became evident one week after M-PMV injection and persisted for approximately 5 weeks. M-PMV antigen was detected in lymph nodes by fluorescent antibody and electronmicroscopy.

One hundred fifty-six females inoculated neonatally with R-35 virus and a control population of 108 females were used to determine the oncogenicity of the R-35 MTV. There was a total of 15 malignant and 18 benign tumors in the virus inoculated population as compared to 2 malignant and 16 benign tumors in the control population, over a 20-month period.

Significance to Biomedical Research and the Program of the Institute: This project is part of a program to determine if viruses are related to breast cancer in other species besides mice, including monkeys and humans. Animal studies are necessary to develop methods and reagents for the search for viruses in human cancer patients. This will contribute to SVCP objectives by providing substantial support, or lack of it, to the general idea that a type of cancer of great clinical significance in humans is caused by viruses. The leads involved must be investigated and evaluated as soon as possible.

Proposed Course: Continuation as described for one or two more years.

Date Contract Initiated: 09 June 1970

MEDICAL COLLEGE OF WISCONSIN (PH43-NCI-E-68-1010)

Title: Hormone Effects on Virus Particle Activity in Breast Cancer

Contractor's Project Director: Dr. Roland A. Pattillo

Project Officer (NCI): Dr. Robert H. Depue, Jr.

Objectives: To study the effects of human hormones on possible oncogenic virus production in human breast cancers *in vitro*. The hormones to be studied include the lactogenic hormone and biologically active estrogenic and progestational steroids.

Major Findings: The contractor has developed several cell cultures derived from the breast area of perinatal autopsies. This tissue was selected because it undergoes hypertrophy in the last stages of pregnancy and is the closest to normal lactating breast that is available. (Breast biopsy during lactation is generally not possible.) It is hoped that these cells will provide a suitable substrate for replication of the human milk agent.

The effects of progestational hormones on the culture of normal and cancerous human breast tissue is being studied. Several breast cancers have been cultured. No permanent line has resulted. Markers of "dome" formation, casein production, reverse transcriptase, and labeled uridine uptake into virus density bands are monitored *in vitro*.

Significance to Biomedical Research and the Program of the Institute: The ability to manipulate breast cancer and normal breast cells *in vitro* is essential to progress with breast cancer virology. Appropriate target cell must be available. Since breast cancers are a hormone dependent disease, the effect of hormones on these cell cultures will provide the necessary background for work that has proved difficult and unrepeatable so often in the past.

Proposed Course: The contractor will help investigate the effects of hormones on cell line that have been developed in other laboratories and try to place these culture procedures on a firmer practical and theoretical basis.

Date Contract Initiated: 19 September 1963

MEMORIAL HOSPITAL (NIH-NCI-E-71-2194)

Title: Procurement of Human Serum Specimens from Defined Population Groups for Immuno-epidemiological Studies

Contractor's Project Director: Dr. Herbert F. Oettgen

Project Officer (NCI): Mr. John Kvedar

Objectives: Procurement of serum specimens from the following defined population groups as a part of a collaborative effort to determine whether candidate viruses isolated from human or animal sources are related etiologically to human breast cancer.

Basic defined population: Women entering Memorial Hospital, New York City, for first diagnosis of any breast disease.

Test group: Women whose lesions prove to be malignant as determined by biopsy.

Control groups:

a. Women whose lesions are found on biopsy to be benign proliferative reactions or reactions suspected as being pre-neoplastic in nature.

b. Women whose lesions are considered to be unrelated to neoplasia.

Major Findings: From 05/31/72 to 01/19/73 the contractor collected 305 blood samples. The serum was separated and stored frozen in small aliquots. Questionnaires pertaining to family history were completed. Serums collected were from 145 patients with malignant conditions of the breast and from 153 patients with benign conditions of the breast. Specimens continue to be collected at a rate of approximately 50 per month.

Significance to Biomedical Research and the Program of the Institute: Since viral agents suspected of causing cancer in man cannot be tested directly in human subjects, it is necessary to establish etiological relationship indirectly through immuno-epidemiological studies. This contract is for procuring the epidemiologically defined bank of serum specimens essential to the determination of whether antibodies against suspect viruses occur with higher frequency, and in larger amounts, in sera of women with breast cancer as compared with appropriate controls.

Proposed Course: It is anticipated that a sufficiently large bank of sera will have been accumulated in about the next year and a half. At that time the contract will have phased out, except for continued collaboration with users of the collection.

Date Contract Initiated: 23 June 1971

MICHIGAN CANCER FOUNDATION (NIH-NCI-E-71-2421)

Title: Studies in High Breast Cancer Families

Contractor's Project Director: Dr. Michael J. Brennan

Project Officer (NCI): Dr. Robert H. Depue, Jr.

Objectives:

I. Correlate the presence of oncornaviruses in the milk of human donors with familial history of breast cancer.

II. To isolate and purify antigens of a candidate human mammary tumor virus from human milk.

Major Findings: During the starting year (1971-72) of this contract, a comprehensive human milk collection network was established which covers 90 percent of the obstetrical beds in the metropolitan Detroit area (4.5 million population) and at present yields milk samples from 150 women per month. The family histories of these women are obtained by interview and corroborated by rigorous clinical documentation.

In the second year, the contract used the previously developed milk resource, to continue the epidemiological studies and to develop the morphological and biochemical armamentaria necessary to determine the relationship between the presence of virions and family history of breast cancer. The ability to reliably identify milk donors with higher than usual levels of candidate virus would permit them to isolate and characterize viral antigens and to compare them with viruses of known breast cancer inducing activity.

Objective I: The milk procurement activity has built up to 150 specimens a month. Donors so far have fallen into the following groups--

Family history of single breast cancer	57
Family history of multiple breast cancer	3
Family history of breast and other cancer	101
Family history of other cancer	386
No cancer history	506
Total	1053 donors

Confirmation rate of breast cancer records was 93% and 87% of other cancers. Milks from 181 women were screened by EM (Dr. Chopra, NCI) and 18.2% were positive for a Simian-type MTV. The correspondence of enzyme and EM positivity has been absent.

In an effort to evaluate the reliability of the procedures the observations may be summarized as follows:

A. The "simultaneous test" for reverse transcriptase (STRT) as an assay for RNA tumor viruses in saline suspensions is reproducible and is proportional to virus concentration.

B. The STRT is neither reproducible nor reflects virus concentration in human milk or in cell culture supernatants.

C. A specific inhibitor in these biological fluids which interferes with the STRT is ribonuclease.

D. Forty-one of 45 human milk specimens tested exhibited RNAse activity; this RNAse activity migrates with the virus in a centrifugal field; and the ability to detect viral RT by the ST is inversely proportional to the RNAse present in human milk specimens.

Objective II: The contractor determined the quantitative physical distribution of tritium labeled RNA tumor virus in nine human milk specimens subjected to processing protocols currently in use at the NCI and by procedures developed in their laboratory. These studies indicate that the discontinuous gradient step is the source of maximum virus loss (60-70%) in the recovery protocol.

Significance to Biomedical Research and the Program of the Institute: At this time, breast cancer is known to be caused by a virus in only one animal species, the mouse. Therefore, studies based on leads from this animal species have been conducted to determine if evidence might be obtained for an association of viruses with human mammary cancer. The "clustering" of breast cancer within family groups and its similarity to that observed for mouse mammary cancer before the development of inbred mouse strains has suggested that a virus might also be involved in human breast cancer.

Proposed Course: The contractor will pursue the two objectives above and will as the availability of funds allows, pursue studies of human and mouse cell lines established in the contractor's laboratory which show the characteristic "domes" of breast cells *in vitro*. These lines will be examined for evidence of virus production and/or susceptibility to particles from milk.

Date Contract Initiated: 20 June 1971

NETHERLANDS CANCER INSTITUTE (NIH-NCI-E-72-3260)

Title: Immunogenetic Studies on Breast Cancer and Leukemia

Contractor's Project Director: Dr. L. M. Boot

Contractor's Assistant Project Director: Dr. J. H. M. Hilgers

Project Officers (NCI): Dr. Walter E. Heston, Dr. Ernest Plata

Objectives: To study the transmission of mammary tumor virus in the GR strain of mice through segregation experiments to determine whether mammary tumor development is due to a single genetic locus, giving further evidence for the provirus theory.

Major Findings: A rapid test for early mammary tumors has been developed in GR mice employing ethynyl nortestosterone. Bentvelzen's observations suggesting a single gene or two closely linked genes controling MTV expression and mammary tumor development have been confirmed using the rapid test. Linkage experiments have thus far failed to reveal linkage to known mapped characters. Cross breeding of GR to mammary tumor resistant strains of mice are underway to study genetic mechanisms of virus expression, tumor susceptibility, and vertical transmission of MTV. MTV expression is much lower in such F_1 hybrids resembling the situation with leukemia virus expression in similar crosses.

Milk from Dutch women was fractionated on Ficoll/D_2O gradients. Fractions were EM negative for virus-like particle, both by negative staining and thin section. Only smooth particles about the size of B-particles (like Mason-Pfizer virus) were seen in all milks. This observation does not confirm the presence of Moore's MS-1 and MS-2 particles. Reconstruction experiments with MTV and human milk demonstrated recovery of undamaged particles by this technique.

One of 120 sera from breast cancer patients contained an antigen showing partial identity to mouse MTV antigens. None of 423 breast cancer and control sera had antibodies to mouse MTV detectable by immunodiffusion, nor did the sera react with MTV-containing mouse tumors in immunofluorescence tests. A radioimmunoassay is under development.

Significance to Biomedical Research and the Program of the Institute: Epidemiological studies on human breast cancer indicate that in families having a high risk to this disease, the genetic factor associated with the higher risk is transmitted through the paternal as well as the maternal line. The GR strain of mice to be studied under this contract has this type of inherited disease, in contrast to the C_3H strain in which the predominant influence in the high incidence of breast cancer is transmission of the mouse mammary tumor virus (MTV) through the mother's milk. Although MTV is also transmitted through the mother's milk in the GS strain, foster nursing of the

young on low breast cancer strain females does not reduce the excess risk as it does in the C_3H strain. The GR strain may therefore represent a more appropriate animal model for leading studies on the human disease. The GR strain is not now being adequately studied, and more work on it is needed because of the popular question now being raised whether non-nursing of female infants, by mothers belonging to high risk populations, should be practiced. If the human situation is more like that in GR mice, then obviously non-nursing would have little or no effect in reducing risk in the human disease.

Proposed Course: The contractor will develop congenic strains to further study the transmission of the virus. Tests for type specific antigens in back crosses of C_3Hf and GR mice will be correlated with mammary tumor occurrence. Human tumors will be screened with mouse MTV and MuLV antisera for interspecies antigens. EM screening will be continued.

Date Contract Initiated: 28 June 1972

PFIZER INCORPORATION (NO1-CP-3-3239)

Title: Virological Studies of Human and Animal Breast Cancers

Contractor's Project Director: Dr. K. E. Jensen

Project Officer (NCI): Dr. Robert H. Bassin

Objectives:

1. Electron Microscopic Studies—An electron microscopic laboratory with professional and supporting staff is operated for two purposes: (a) the search for viruses in human and animal breast cancers, in collaboration with other SVCP participants not having this capacity; and (b) EM monitoring and research at the contractor's institution associated with virological studies on new candidate viruses isolated from breast cancer (see objective 2).

2. Virological studies directed toward the further biological and biochemical characterization of new candidate breast cancer viruses, and the development of new animal model systems for guiding research on the human problem.

Major Findings: The synthesis of type C virus observed in M-PMV infected cultures was enhanced by propagating the culture at 40° C. The simian type C virus (SCV) was found to share an antigen with the simian sarcoma virus type 1 (SSV) as demonstrated by microimmunodiffusion and immunofluorescence tests.

Polyacrylamide gel electrophoresis of internally or externally labeled M-PMV resolved 8 definite polypeptides. The major polypeptide has a molecular weight of 27,500 daltons and compares well with the group specific (gs) antigen of type C viruses of murine, avian and feline origin. However, this polypeptide is serologically distinct.

A virus resembling M-PMV in ultrastructural morphology has been isolated from the breast tissue of three, normal, lactating rhesus females. Subsequent culturing of the breast biopsies, removed six weeks later, yielded again M-PMV-like virus. The new isolate, X-381 virus, was found antigenically identical to the prototype M-PMV. The virus exhibited many other biophysical properties generally associated with RNA tumor viruses namely; a buoyant density of 1.16-1.18 g/ml in sucrose, 60-70S RNA, RNA directed DNA polymerase. The virus was found infectious for many different cultures of human and subhuman primate origin. Moreover, the breast culture from which X-381 virus was isolated possesses properties that are peculiar to transformed cells, i.e., they proliferate very rapidly, show lack of contact inhibition and are able to grow in 0.45% soft agar.

Budding and extracellular type C virus has been observed in 4/4 rhesus monkey placentas and 2/3 associated embryos examined by electron microscopy prior to culturing. This is the first time that a definite type C virus has been demonstrated in apparently healthy primates. It would appear that the rhesus monkey placental tissue is a rich source for type C virus. A preliminary investigation of human placentas for type C virus has been negative so far.

Significance to Biomedical Research and the Program of the Institute: The contractor's EM laboratory continues to be a key participant in a broad collaborative program in the search for viruses related to human and animal breast cancers.

The further research and development on new candidate animal breast cancer viruses in the contractor's laboratory is essential to the application of these new models to studies of the possible viral etiology of human breast cancer.

Proposed Course: The activities described will be continued at the current level until the determination is made whether the candidate animal viruses are etiological agents of breast cancer of the types from which they were isolated. Electron microscopic examination of human placental tissues will be continued.

Date Contract Initiated: 28 June 1967

TEL AVIV UNIVERSITY (NIH-NCI-E-72-3237)

Title: Isolation, Purification and Propagation of Virus-like Particles from Human Milk in Israel

Contractor's Project Director: Dr. Jafa Keydar

Project Officer (NCI): Dr. Timothy E. O'Connor

Objectives: Purification of candidate virus from various milk samples obtained from women of designated high-breast-cancer-risk populations in Israel and assay for RNA-instructed DNA polymerase as well as attempts to grow the milk "virus" in human tissue cultures.

Major Findings: The contractor has inoculated primary human fetal cultures with material from human milk and from human breast cancers. These cultures reportedly showed evidence of viral proliferation based on the reverse transcriptase test. These studies are being repeated for confirmation. In addition, the contractor has established a cell line from mouse breast tumor that produces *in vitro* virus particles resembling B-particles.

Significance to Biomedical Research and the Program of the Institute: Israel is a particularly favorable setting for an investigation of this type since high-risk donors of milk are easy to identify and line-up for milk studies well in advance of delivery, because of the following reasons: (a) Israel has a mandatory national cancer registry and the occurrence of breast cancers within families can be readily determined, (b) Israel has a national health plan which includes maternity cases who report for care early in pregnancy, allowing ample time for surveys for cancer history, (c) most women deliver in hospitals thus simplifying the logistics of milk procurement, and (d) most importantly, there are several ethnic groups within Israel having breast cancer risks among the highest known.

The evidence of virus-like material in human milk obtained in other SVCP projects makes it essential to propagate this candidate agent for further characterization and determination of its association with human breast cancer. The findings above, if confirmed, could lead rapidly to the *in vitro* propagation of both mouse and human breast cancer viruses. This is the major immediate objective of the Breast Cancer Segment. Its accomplishment would enable sero-epidemiologic studies to determine the significance of this agent in the development of breast cancer.

Date Contract Initiated: 23 March 1972

SUMMARY OF DEVELOPMENTAL RESEARCH SEGMENT CONTRACTS

The mission of the Developmental Research Segment is the elucidation of the association and presumptive etiological relationships of RNA viruses and herpesviruses or of their genetic expression to human neoplasms leading to the development of measures for the prevention or control of virally-induced neoplasia in man. To meet this objective, contract projects include studies on: seroepidemiology; screening of human tumors for virus or virus expression; factors which influence virus genetic expression; molecular processes involved in virus replication and cell transformation; and humoral and cellular immune responses.

Contract research projects within the Developmental Research Segment are administered through the Office of the Chief, Viral Biology Branch. As Chairman of this Segment, the Chief, VBB, arranges for the review of new solicited and unsolicited research proposals pertinent to the mission of the Segment and of proposals for continuation of existing projects. Reviews are conducted for relevance, priority, and need to overall program at the regular meetings of the Program Segment Chairmen and for scientific effectiveness by the Segment Working Group. The voting members of the Working Group include five scientists from non-governmental institutions, two scientists from NCI, and two from government laboratories other than NCI. Whenever possible, the principal investigators responsible for individual projects personally discuss problems and projections with Working Group members at the time of review.

Projects within the Segment are primarily devoted to investigations to detect, define, and control virus activity presumed to cause human neoplasia. Current research projects may be assigned to three major areas of investigation: (1) determination of the association of viruses with, and the presumptive causal relationship of viruses to, human neoplasia; (2) factors influencing virus gene expression in cells; and (3) approaches to the development and evaluation of measures for the prevention or control of virus-induced neoplasia. The viruses under study include members of the herpesvirus group and viruses of the RNA tumor virus group.

Seroepidemiological studies showed a significant association between infection by herpes simplex virus type 2 (HSV-2) and carcinoma of the uterine cervix. Comparisons between cancer cases and controls matched for sexual and reproduction-associated factors suggested that the association of the virus with this carcinoma is not one of covariability with sexual promiscuity. Serological tests to determine the presence of antibodies indicative of HSV-2 have been complicated by the presence of co-existing antibodies to the oral type-1 herpes simplex virus (HSV-1). Preexisting infections by HSV-1 reduce the antibody titers induced by HSV-2 infections. DNA-DNA hybridization studies revealed 60 to 70 percent homology between the genomes of HSV-1 and HSV-2, while about 40 percent of the HSV-1 DNA sequences transcribed in infected cells are common to HSV-2. Cytotoxicity tests using human sera have been developed which appear to be specific in their reactivity with HSV-2 infected cells and may provide more reliable

means for detecting past infections of humans by this virus.

Nonvirion antigens were found to be produced in cells during the early stages of HSV-1 and HSV-2 replication. Antisera specific for the HSV-1 induced antigen failed to react with the antigens induced by HSV-2. However, antibody produced against the HSV-2 induced antigen also reacted with that induced by HSV-1. Preliminary screening of human sera for antibodies to the nonvirion antigens disclosed what appears to be a unique relationship between the presence of antibodies and genito-urinary tract neoplasms. Whereas sera from individuals without these tumors failed to react against nonvirion antigen even though antibodies neutralizing HSV-2 were present, sera from patients with cancer of the uterus, bladder, kidney, and prostate reacted positively. If HSV-2 has an oncogenic potential for human cervical cells, some of its functions must be expressed, particularly those bearing on the cellular transformation. The nonvirion antigen may signal such partial virus gene expression provided its specificity for HSV activity is verified.

Apparently the HSV-2 genome is repressed in cervical tumor cells. Evidence of repressed expression was observed in cells cultured from an intra-epithelial carcinoma of the cervix. After six months of cell cultivation virus production could be induced by elevating the pH of the culture medium. Similar activation may occur intravaginally, for HSV-2 antigens were detected in dyskaryotic exfoliated cells in 90 percent of patients examined, while biopsied tissue from these cases examined by immuno-fluorescence methods applied to impression smears and frozen sections were consistently negative.

To create a better understanding of the factors involved in herpesvirus-cell interactions, temperature sensitive mutants of HSV-1 and HSV-2 were isolated and are being characterized. Progress is being made on the isolation of native individual proteins induced by HSV-1 and HSV-2 and their immunologic identification.

Studies were conducted in experimental systems to determine whether HSV-2 possessed any oncogenic properties. In hamster embryo cell cultures, 7 of 15 UV-irradiated HSV-2 strains and 2 of 12 UV-irradiated HSV-1 strains induced a morphological transformation of cells. Only one, an HSV-2 transformed hamster cell culture, showed neoplastic behavior upon transplantation into hamsters. The results demonstrate strain differences among the viruses isolated with respect to their effect on host cells. Cytomegalovirus treated by UV-irradiation to inactivate its cytolytic property also transformed hamster cells. Repeated trials showed no transformation of human embryo cells when similar procedures were applied.

Since sexual promiscuity contributes to human risk of cervical carcinoma, the Cebus monkey was selected as an experimental primate to determine the effect of combined sexual freedom and repeated HSV-2 infection as factors in the development of cervical tumor. Not all animals responded equally to primary HSV-2 infection. Clinical lesions were observed in some, silent infection in others, and no apparent infection in the remainder. Hormonal levels during the menstrual cycle, as well as the strain of HSV-2 involved,

may affect the infection rate. Spontaneous recurrence of herpetic lesions was observed in one animal. The recovery of virus and development of antiviral antibodies signified positive infection by the virus strain selected. Animals on test will be held for several years of observation.

The high degree of association between Epstein-Barr virus and Burkitt's lymphoma has been established by immunological tests. In immunofluorescence tests the reactivity of patients' sera with antigens induced by the virus in cultured lymphoblasts demonstrated the presence of virus capsid antigens (VCA), "early" restricted (R) and diffuse (D) antigens, early and late cell membrane antigens, and a complement-fixing (CF) antigen. The latter was expressed in replicating virus carrier cells in the absence of the other antigens whose production is usually repressed in most of the carrier cells. Expression of antigens other than the CF antigen results in cell death. By an anticomplementary fluorescence test the EBV-CF antigen now has been visualized in the form of finely granular intranuclear material present in 80 to 90 percent of non-producing cell lines. The negative fraction probably consisted of dead or dying cells. The variations of the antibody levels in patients' sera to EBV induced "early" antigens and cell membrane antigens during the course of induced remission and relapse in Burkitt's lymphoma biopsy tissues were shown to contain EBV DNA with genome equivalent amounts ranging from 3 to 113 per cell on the average. The method has a sensitivity of two genome equivalents per cell, and the negative specimen may have contained EBV DNA below detectable limits. In contrast, the number of genome equivalents in African nasopharyngeal carcinoma tissue specimens was lower, ranging from 5 to 85 per cell, and 5 of 23 of the specimens were negative for EBV DNA by this test procedure.

Despite the high degree of association of EBV with Burkitt's lymphoma, the wide distribution of EBV infection in world populations, contrasted with the concentration of lymphoma cases in specific regions of Africa, has suggested the possibility that a co-factor is involved. The survey of biopsied tumors from African patients by nucleic acid hybridization techniques disclosed both the presence of nucleotide sequences homologous to sequences in the 70S RNA of Rauscher murine leukemia virus and of RNA-dependent DNA polymerase activity in a particulate fraction extracted from the tumor cells. While the reactive components have not yet been shown to be of viral origin, the observation does suggest the possibility that RNA tumor virus-like information is being transcribed in the tumor cells. Studies in avian systems demonstrating an apparent necessity for interaction between Marek's disease herpesvirus and an RNA leukosis virus to produce classical Marek's disease suggests by analogy that EB virus could be an indispensible but not the sole factor in the etiology of Burkitt's lymphoma.

Whereas the herpesviruses have long been studied with respect to non-cancerous disease, the observation that infection by certain members of this group induce lymphoma and leukemia in different animal species dictated the need for intensive study of virus-cell relationships. Infection of human lymphoid cells with EBV confers a capability for their unlimited growth in vitro. By cRNA-DNA hybridization, 21 lymphoblastoid cell lines were shown to contain between 29 and 235 virus genome equivalents per cell. Such lines exhibited differences in susceptibility to superinfection by EBV

and to the activation of the endogenous, repressed virus genome by BUdR or IUdR. Studies on these cell systems suggested the existence of restrictive mechanisms in cells that recognize the exogenous, superinfecting virus genome and the endogenous, activated viral genome in a similar way. The endogenous genome is associated with cellular chromosomes, but it is not yet clear whether it is covalently bound to cellular DNA.

Investigations on cell-mediated immune responses to EBV-infected cells showed that peripheral lymphocytes exposed to autologous infectious mononucleosis (IM) derived cells turned into killer cells that very efficiently lysed cells of Burkitt's lymphoma and IM derived lines. Lymphoblastoid lines derived from normal human donors were either not or much less affected.

Herpesvirus saimiri (HVS) produces lymphoma or leukemia in marmosets and in owl monkeys, but not in its natural host, the squirrel monkey. In Vero cells, the virus produces two kinds of early antigens, a "punctate" and a "trabecular" type as visualized intracellularly by immunofluorescence methods. In this respect its activity resembles that of EB virus in human cells and provides a useful animal model. In the seronegative squirrel monkey, infection is followed by the production of antibodies in 14 to 17 days, whereas in owl monkeys and marmosets, antibodies did not appear until 30 to 80 days at which time their tumors already were well established suggesting that immunological factors may play a role in resistance to oncogenesis. Infection of the squirrel monkey induces a disease-free carrier state, and infection may be transmitted horizontally from animal to animal. Horizontal transmission was not observed in marmosets.

The presence of endogenous virus of the RNA C-type observed in small laboratory animals has been recognized in monkeys and in the cat. Particular attention is being given to the cat viruses to determine their possible threat to children. Budding particles of the endogenous cat virus were detected in several placentas from cats in a specific pathogen-free colony free of the known A, B and C type feline leukemia viruses. The endogenous virus differed antigenically from the known viruses and was infectious for human cells. Cat embryo cells were refractory to infection and in some instances could be induced to produce endogenous virus following activation by halogenated uridine.

Attempts are being made to determine whether RNA viruses are associated with human tumors. One approach is to search for evidence of viral expression within tumor cells. Earlier studies had detected the presence of nucleotide sequences in the polysome fractions separated from human mammary tumor cells that corresponded to sequences in the 70S RNA extracted from virions of the mouse mammary tumor virus. RNA-dependent DNA polymerase (RDDP) activity was also demonstrated in the human mammary tumor cells. A simple assay procedure, developed for the simultaneous detection of 70S RNA and RDDP activity, was applied to particles from human milk banding at densities of 1.17 to 1.20 g per ml. The results showed that these particles contained 70S RNA and RDDP, which are properties of known RNA viruses of the C-type.

To further assess the nature of a heavy RNA and RDDP obtained in particulate fractions extracted from human mammary carcinoma cells, the product of the endogenous reaction was analyzed and found to hybridize with nucleotide sequences in the 70S RNA of mouse mammary tumor virus (MMTV) but not with 70S RNA of Rauscher murine leukemia virus (RLV) or avian myeloblastosis virus (AMV).

Similar studies on fresh human leukemia cells and sarcoma cells obtained from patients showed evidence of RDDP activity and 70S RNA in extracted particulate fractions which hybridized with labeled DNA probes prepared from RLV 70S RNA but not with MMTV or AMV 70S RNA. The product of the endogenous reaction hybridized only with RLV 70S RNA.

The RDDP obtained in the particulate fraction of human leukemic cells banding at 1.16 g per ml in a sucrose gradient was purified and characterized. The enzyme was not found in phytohemagglutinin stimulated lymphoid cells obtained from non-leukemic donors. It could be distinguished from the major DNA polymerases of normal cells by its properties, including acceptance of an RNA heteropolymer as template. Four RDDP preparations were purified from human leukemic cells. An antiserum prepared against the Gibbon leukemia virus RDDP was tested against the four human RDDP preparations. One showed complete cross reactivity with the Gibbon enzyme, two showed partial crossing, and one was negative.

The RNA of all the known RNA tumor viruses contains long stretches of poly A sequences. A technique involving hybridization with labeled poly U was developed which permits quantitative estimates of the number of virus particles in virus preparations. The use of cordycepin to inhibit poly A formation yielded data suggesting that poly A is formed and attached to the viral RNA 12 to 16 hours after the RNA is transcribed.

Nucleic acid hybridization methods were developed to determine the species of origin of an RNA tumor virus. Determinations were correct even though the virus examined was recovered from tumors induced in species other than the natural host. By this method, the RD 114 virus was undeniably shown to be of feline origin.

To demonstrate that unique genetic information was contained in the human leukemic cell, DNA products of the endogenous reaction between the leukemic cell RNA and the RDDP were exhaustively hybridized with normal human lymphocyte DNA to remove any nucleotide sequences found in normal cells. The residual probe retained sequences that did hybridize with leukemia cell DNA but not with normal cell DNA, indicating the presence of nucleotide sequences in leukemic cells that differed from sequences present in the normal cells used in this experiment.

Experience with the C-type RNA viruses of animals demonstrates that endogenous virus genomes are carried in host cells in a partially or completely repressed state and that their expression is sometimes induced by treatment with halogenated uridine. When the endogenous virus genome is defective for expression leading to virion production, genetic complementation by the genome of a related virus may permit complete expression. Once

activated, the continued replication of an endogenous virus may not be sustained within the cells of the natural host, but infection and reproduction may occur in cells of another species. Thus, the endogenous feline virus infects and replicates in human cells. Attempts to recover viruses from human tumors were based upon the experience with animal viruses. Human sarcoma and leukemia cells were mixed in cultures, and the culture fluids were tested for the presence of a cell-transforming agent using different human embryo fibroblast cultures as indicators. Suggestive results were obtained with fluids from co-cultures of osteosarcoma cells and leukemia cells. Cell-free fluids produced small focal areas of altered cell morphology in one of four different indicator cultures. No virus was detected by electron microscopy, but sera from 17 of 32 patients with osteosarcoma, 11 of 21 patients with leukemia, and 2 of 41 apparently normal donors reacted with a cytoplasmic antigen contained in these cells. Suggestive evidence was obtained for the presence of RDDP and heavy RNA in particulate fractions of density 1.19 to 1.21 g per ml separated from these cultures.

Intensive study of the ESP-1 virus, produced in cells cultured from a human lymphoma, demonstrated the presence of mouse gs-2 and interspecies gs-3 antigens in virions and of mouse gs-1 antigen in the virus-producing cells. The gs-3 interspecies antigen has been detected in all the known RNA C-type viruses with one exception; no gs-3 antigen has been demonstrated in the virions or virus-producing cells from leukemic cattle. Therefore, this antigen may not necessarily be a criterion for the demonstration of C-type virus expression in human cells.

The immune reaction of patients to antigens of their neoplasms was studied by the chromium-51 release test for serum cytotoxicity and by the mixed lymphocyte-target cell interaction test. Some children with leukemia were shown to produce antibodies cytotoxic for their peripheral blood leukocytes. The parents and siblings of these cases also contained antibodies cytotoxic for the patient's leukocytes, suggesting that an antigen on the leukemic cells might have been introduced by an exogenous agent. Normal persons unrelated to patients with leukemia were free of reactive antibody. The mixed lymphocyte-target cell interaction test was applied to determine whether neoplastic cells stimulated the peripheral blood lymphocytes of the patient. Significant stimulation was obtained with cells of biopsies taken from 20 of 44 sarcomas, 6 of 29 brain tumors, 5 of 9 carcinomas, and none of 3 myelomas. Blocking of stimulatory tumor cell-lymphocyte combinations was demonstrated by about half of the serum specimens taken from other patients with similar tumors and with much reduced or equal frequency by sera from patients without tumors or with unrelated tumors. Non-specific killing of target cells by lymphocytes was observed from recently operated patients and from patients with non-neoplastic neurological disorders.

Investigations were pursued to explore host factors and viral interactions that influence tumor virus expression as these might apply in humans. Lymphocyte-lymphocyte interactions in the graft versus host (GVH) reaction were found to activate expression of endogenous leukemia virus in mice.

The degree of virus activation was shown to be much less when minor histocompatability antigens were involved than when interaction between major H2 antigens took place. The failure of the blastogenic response of sensitized lymphocytes to activate endogenous virus projection upon exposure to the specific sensitizing H2 antigen borne by fibroblasts suggested an apparent uniqueness of the lymphocyte-lymphocyte interaction in the virus activation process. Immunosuppression alone is also apparently insufficient to explain virus activation in the GVH reactions studied. In the study of responses of different strains of mice, it was evident that oncogenesis was not always a sequel to virus activation.

Modification of host response to interaction of viruses associated with oncogenesis was demonstrated in avian systems. Marek's disease (MD) of chickens is induced by infection with a herpesvirus (MDHV). No disease results if MDHV is neutralized by a specific antiserum, and attenuated virus strains protect birds from disease. Therefore, MDHV is an essential factor in the induction of classical MD. However, cell-free MDHV did not produce classical MD in a specific pathogen-free flock of chickens after exposure to infection unless there was coinfection by RAV-2 avian leukosis virus. In the dual infections, the production of RAV-2 antigens and RAV-2 viral RNA was found to be considerably enhanced by MDHV infection when compared to infection by RAV-2 alone. Experiments with Japanese quail showed this species did not develop overt disease when exposed either to MDHV or to RAV-2 alone, whereas typical MD lesions were evident in some birds following coinfection. Although further development of these avian systems is required, these data are valuable to an understanding of the potential of herpesviruses in contributing to disease processes in humans that may involve endogenous RNA viruses and activation of RNA virus gene expression by exogenous factors acting on the host cells.

Conceivably, host cell immune processes and specific inhibition of intracellular, virus-specified events leading to neoplasia may provide the means to prevent or control virus-induced cancer. To provide a basis for progress in this area, information was sought to elucidate the mechanisms involved in the integration of RNA tumor virus genetic information into host cells and the relationship of virus genetic expression to the process of cell transformation.

Virions of avian myeloblastosis virus (AMV) can be produced in sufficient quantity to permit studies on the nature of the RNAs encapsulated in the virus particles. A small 4S RNA component derived from the 70S viral RNA was compared with "free" 4S RNA contained in the virion. Both had a similar minor base composition and satisfied criteria for transfer RNA (tRNA), showing a high acceptance for lysine. Their minor base content differed considerably from that in tRNA from host myeloblasts or chicken liver cells. A molecular weight of about 2.3×10^6 daltons was determined for the 35S component of 70S RNA, and adenosine was found as the major 3'-OH terminal nucleosides of the 70S and the 35S RNA.

The RNA tumor viruses contain a protein kinase also found in other viruses that mature at the cell membrane or intracytoplasmically. The kinase differs from cellular kinase, is independent of cyclic AMP, and catalyzes

phosphorylation of virion structural proteins. The yield of DNA transcribed by the RDDP of AMV is substantially increased by a stimulatory viral protein. Stimulation by this protein is specific for AMV RDDP. The hybridase (RNAase-H) of AMV associated with the RDDP of AMV is a processive exonuclease active only on the RNA of free ends of a DNA-RNA hybrid structure. Its activity may provide single strand DNA ends on a duplex viral RNA-DNA hybrid structure, resulting from the action of RDDP. This may be the form in which the molecule is integrated into nuclear DNA where it would then be subjected to the activity of nuclear enzymes effecting DNA repair to yield a DNA-DNA structure.

Attention is being given to the protein products of virus gene expression in relation to the transformation of cells. For comparative purposes, four representative avian and mammalian viruses having sarcomagenic and leukemogenic properties were selected. The major immunogenic polypeptides of each virus are being separated, purified, and used for the preparation of monospecific antisera to permit identification in virus-infected cells. Five polypeptides obtained from AMV were shown to be immunologically distinguishable, but three contained some determinants in common. Antibody to the common determinants was removed from the antisera to yield sera with monospecificity. A close interrelationship was found between the polypeptides from AMV and those recovered from the Prague strain of Rous sarcoma virus (RSV). Four distinct polypeptides, two containing common determinants, were obtained from feline sarcoma virus and Friend mouse leukemia virus. All were antigenically and immunogenically active. End group analyses and amino acid composition of individual polypeptides and glycoproteins are under study. The localization of these components in the virions was determined. The capability to detect and identify these polypeptides within cells should provide a powerful tool for following virus gene expression in the transformation process in defined systems leading to investigations in human cells.

Measures for inducing immunological resistance to the development of virus-induced neoplasia are being explored for effectiveness. Trials in experimental animals on a practical basis showed SV40 tumor cell ghost vaccines and fetal hamster cell vaccines to be ineffective against SV40 tumor cell challenge. No protection was provided by the synthetic cell surface antigen of Shier against methylcholanthrene-induced tumors or myeloma cell tumors. Preparations are being made to determine the value of antiviral vaccine preparations that would be acceptable for human trials.

During the year, three projects were phased out.

Dr. Robert A. Manaker, VBB, Division of Cancer Cause and Prevention, Chairman
Dr. Michael A. Chirigos, Associate Chief, VBB, Division of Cancer Cause and Prevention, Vice Chairman

BAYLOR COLLEGE OF MEDICINE (PH43-68-678)

Title: Studies on Viruses as Related to Cancer

Contractor's Project Director: Dr. Joseph L. Melnick

Project Officers (NCI): Dr. Robert A. Manaker
Dr. Michael A. Chirigos

Objectives: To determine the relationship of viruses to selected neoplasias and their significance in the neoplastic process.

Major Findings: Sera from children with leukemia and from patients with melanoma were tested for the presence of antibodies reactive with cells of their neoplasms. The sera of the parents and/or siblings of children with leukemia who possessed cytotoxic antibodies reacting with their autologous peripheral blood leukocytes also reacted with the patients' leukocytes. In contrast, normal persons unrelated to patients with leukemia were free of such antibodies nor did their leukocytes react with positive patients' sera. The melanoma patients studied showed no evidence of antibodies detectable by immunofluorescence that react with tumor cell membranes. Attempts to induce release of a virus from four melanoma cell lines were unproductive.

Studies were continued on the relationship between herpes simplex virus type 2 (HSV-2) and cervical carcinoma. Analysis of sera of 85 women with this cancer and 367 normal controls collected in Houston showed a greater occurrence of type 2 antibodies in cases than in controls matched for sexual and reproduction-associated factors, suggesting that the association between virus and the cancer is not one of covariability with sexual promiscuity. Preliminary data from 87 cases and 188 controls suggest the same relationship between HSV-2 and tumor among Caucasian women in West Virginia. Breast cancer is more frequent among women who marry later in life and have fewer pregnancies than among women at risk of developing cervical carcinoma. Women with breast cancer were found to have a much lower occurrence of HSV-2 antibodies than those with the cervical tumor. The Cr-51 release cytotoxicity test is valuable for the seroepidemiological surveys conducted because antibodies reacting only to HSV-2 infected cells can be detected.

Comparative characterization of HSV type 1 and HSV-2 was continued to acquire data useful in determining properties related to oncogenesis. DNA-DNA hybridization studies revealed 60-70% homology between the genomes of HSV-1 and HSV-2. DNA-RNA hybridization experiments indicated that about 40% of HSV-1 DNA sequences transcribed in infected cells are common to HSV-2. In continuing hybridization studies, the kinetics of appearance of viral mRNA in polysomes of cells infected with HSV-1 was established. Progress is being made on the isolation of native individual proteins induced by HSV-1 and HSV-2 and their immunologic identification.

Twenty-two temperature sensitive (ts) mutants of HSV-1 were found to fall into 15 non-overlapping complementation groups. Based upon complementation data and recombination frequencies, a partial additive linkage map of HSV-1 has been constructed. Through the use of TK^- mutants, it has been possible to distinguish between cellular and viral TK activity at 34° by analytical polyacrylamide gel electrophoresis (PAGE). PAGE analysis of polypeptides and glycopeptides synthesized by HSV-1 mutants at non-permissive temperature indicated the existence of mutants with different lesions.

Seventeen ts-mutants of HSV-2 were isolated and are being placed into complementation groups. Efficient complementation occurs between some ts-mutants of HSV-1 and HSV-2; the results permit identification of genes common to both.

Through the disturbance of normal carbohydrate metabolism with various concentrations of 2-deoxy-D-glucose, an attempt was initiated to further define the role of virally-induced glycoproteins within HSV-1 and HSV-2 infected cells. Although only slight differences were observed in the production of complement-fixing and immunofluorescence antigens, the SDS-PAGE profile of the three major virally-induced glycopeptides was shifted to lower molecular weight components. The degree of shift was proportional to the concentration of deoxyglucose and could be reversed by removal of the deoxysugar. Incorporation of radioactive 2-deoxyglucose into the glycolipids and glycopeptides of normal versus SV40 transformed cells demonstrated a greater percentage of high molecular weight glycopeptides in the transformed cells. Some glycopeptides appeared only in normal cells and others only in transformed cells. Transformed cells contained a 3-fold higher percentage of labeled gangliosides than did normal cells and about two-thirds the amount of phosphatidylethanolamine found in normal cells at confluence.

Significance to Biomedical Research and the Program of the Institute:
The occurrence of antibodies in close contacts of leukemic children which react with antigens on the patients' leukemic cells suggests a virus association with this form of neoplasia. Serological evidence obtained by controlled studies on patients with cervical carcinoma implicate genital strains of herpesvirus as a factor in the genesis of this malignancy. Intensive study of the herpesvirus associated with this neoplasm provides fundamental information which may be invaluable as the role of the herpesviruses in oncogenesis is further developed.

Proposed Course: The studies relating to childhood leukemia and intensive studies on herpesviruses as agents of apparent importance in oncogenesis will continue.

Date Contract Initiated: June 27, 1963

Current Annual Level: $623,000

COLUMBIA UNIVERSITY (NIH-70-2049)

Title: Replication of Oncogenic RNA Viruses and its Relation to Human Cancer

Contractor's Project Director: Dr. Sol Spiegelman

Project Officers (NCI): Dr. Timothy O'Connor
Dr. Robert A. Manaker

Objectives: This project is directed toward the elucidation of the molecular biology of RNA tumor viruses and the mechanisms by which they induce oncogenic transformation of infected cells. This knowledge is applied to the determination of the viral etiology of human neoplasms and ultimately to development of effective control measures for human cancers.

Major Findings: Both reverse transcriptase and its DNA product can be used to detect evidence of oncogenic viral information in human neoplasias. Homologous RNA in human breast tumors has been detected by labeled DNA produced from mouse mammary tumor virus, but no positive reactions occurred with RNA's from normal breast tissue, fibroadenomas, non-malignant fibrocystic and gynecomastia tissues, and human leukemias and sarcomas. Similar specificities of reaction were observed in studies with human leukemic cell RNA using leukemia virus DNA as a probe and with human sarcoma RNA using sarcoma virus DNA as a probe.

RNA homologous to mouse mammary tumor virus was detected by hybridization studies in 19 of 29 polysome fractions of human mammary carcinomas. No homology was observed with RNA of viruses causing leukemia in mice or chickens.

White blood cells of 24 out of 27 leukemia patients contained RNA homologous to that of mouse leukemia virus (RLV) but not to that of mouse mammary tumor virus or avian myeloblastosis virus (AMV).

The parallelism in leukemias of the mouse and man was strengthened by RNA homology exhibited by 18 of 25 polysome fractions of human sarcomas to the RNA of RLV but not to RNA of MMTV or AMV.

Twenty-two of 32 human lymphoma specimens contained RNA sequences to RLV RNA but not to RNA's of MMTV or AMV.

Nucleotide sequences present in the polysomal RNA from fresh human leukemia, Burkitt's lymphoma and sarcoma tissues were found to hybridize with DNA probes synthesized from Rauscher murine leukemia virus (RLV) RNA template but not with probes from AMV or MMTV RNA templates. The products of the endogenous reaction of leukemia cell RNA and RT in particulate fractions hybridized specifically with RLV RNA.

In collaboration with Gillespie and Gallo, a method was perfected whereby virus content in biological materials could be quantitated. This was based upon knowledge of the amount of poly-A present in a virion to which labeled poly-U may be found.

In collaboration with Deinhardt, a method was proven which permitted determination of the natural host of an RNA tumor virus even when the virus is recovered from tumors induced in another species. This technique is based upon the number of copies of the genome of the virus present in cells of the natural host as compared with infection in a heterologous species.

The presence of DNA unique to human leukemic cells was demonstrated in eight patients by DNA probes produced by the endogenous RT reaction with leukemic cell heavy RNA template. The DNA product of the endogenous reaction was exhaustively hybridized with normal lymphocyte DNA and the residual DNA was shown to hybridize with leukemic cell DNA but not with normal cell DNA. The sensitivity of the procedure was considered to be sufficiently high to eliminate the possibility that the normal cells could have contained more than one hundredth of the number of sequences contained in the leukemic cells signifying that this genetic information could not have been evenly distributed in the normal cells.

Significance to Biomedical Research and the Program of the Institute: A systematic molecular biological study is being pursued to determine the role of viruses in the genesis of human cancer. Studies demonstrated the presence in human cancers of particulate materials possessing characteristics unique to the known animal RNA tumor viruses. New approaches to the study of virus-cell relationships have been devised. The contractor has developed data which are highly provocative and his pioneering advances provide a foundation for future investigation leading to an understanding of the virus-cell relationship as it applies to human cancer.

Proposed Course: Molecular probing of human neoplastic tissue for evidence of the presence of oncogenic RNA viruses will continue. Similar procedures will be applied to "normal" human tissues. The reverse transcriptases of oncogenic RNA viruses and of neoplastic tissues will be purified and characterized. The existence of RNA nucleotide sequences in particulate fractions of human cells which are homologous to the RNA of animal viruses requires further investigation to determine the significance of the observations made.

Date Contract Initiated: October 29, 1969

Current Annual Level: $1,000,000

CORNELL UNIVERSITY (NIH-71-2508)

Title: Leukemia Studies in the Cat

Contractor's Project Director: Dr. Charles Rickard

Project Officers (NCI): Dr. Michael A. Chirigos
Dr. Robert A. Manaker

Objectives: To determine mechanisms for transmission of oncogenic viruses in cats and to investigate the possibility of natural infection of humans by feline viruses.

Major Findings: C-type virus particles were detected as particles and buds in three placentas among 19 examined from cats in the specific pathogen free (SPF) colony. Release of this virus was also induced by treatment of cat embryo cell cultures with IUdR. The virus differs from the feline leukemia viruses of sub-types A, B and C. It is not infectious for cultured cat embryo cells but infects and grows in human cells. It is interpreted to be an endogenous cat virus derepressed during embryonic development.

C-type viruses resembling the known feline leukemia virus and infectious for cat embryo cultures were detected in spontaneous hypoplastic anemias in cats. These will be compared with known agents.

Ten cats with mammary tumors showed no evidence of the presence of particles morphologically resembling the mouse mammary tumor virus. C-type particles were detected in four of the tumor tissues examined. Seven mammary tumors have been implanted into 76 kittens. No tumor expression has been observed over the one to nine months that these animals have been under observation.

Significance to Biomedical Research and the Program of the Institute: The cat offers several advantages for the study of viral relationships to oncogenesis. It is susceptible to horizontal infection by three sub-types of RNA leukemia virus, it provides a natural population for study, cat viruses reproduce in cells of species other than the cat, including humans, and the cat possesses an endogenous C-type virus differing from the cat viruses already characterized. Since the cat is a common household pet and its C-type viruses are infectious for human tissue, intensive study to determine whether its tumor viruses pose a threat to humans, particularly young children, is necessary.

Proposed Course: The experimental systems developed in the cat will be applied to search for and attempt recovery of human oncogenic viruses. Studies will be conducted to determine whether any relationship exists between the endogenous and the exogenous C-type viruses of the cat and neoplasia in children. At present a mammary tumor virus is known only in the mouse. Investigations will be made to determine whether a mammary tumor virus can be recovered from the cat. To assist in immunological approaches to cancer control, attempts will be made to obtain carcinogen-induced tumors in the cat and to determine whether viruses are a co-factor in genesis of such tumors.

Date Contract Initiated: June 23, 1965

Current Annual Level: $300,000

DUKE UNIVERSITY MEDICAL CENTER (NIH-71-2132)

Title: Expression of the RNA Tumor Virus Genome in Animal and Human Malignant Cells

Contractor's Project Director: Dr. Dani P. Bolognesi

Project Officer (NCI): Dr. Charles W. Boone

Objectives: To characterize the structural components of animal RNA tumor virus particles and develop serological probes to detect virus gene products in animal and human malignancies.

Major Findings: Four viruses were selected for study, the Prague strain of Rous sarcoma virus, avian myeloblastosis virus, Friend mouse leukemia virus, and feline sarcoma virus. Principal attention was centered on the immunological and biochemical characterization of the major avian and mammalian virus polypeptides. Five avian myeloblastosis virus (AMV) polypeptides are immunologically distinguishable, but three of these contain determinants in common. Polypeptides of RSV (Prague), a helper-free member of a distinct subgroup, behave similarly, and a close interrelationship between AMV and RSV (Prague) polypeptides shows the existence of multiple group specific antigens.

Four distinct polypeptides, two containing common determinants, were isolated from the feline and murine agents and all were antigenically and immunogenically active. Two antigenic determinants (inter - and intra-species) could be demonstrated in the major component -30000 daltons - as shown by others. Comparable studies of RD114 indicated a relationship of this agent to feline virus.

End group analyses and amino acid composition of individual polypeptides, including the avian glycoproteins are being studied, and tryptic peptide maps have been obtained for the virus proteins. The glycoproteins are being analyzed for relative protein and carbohydrate content as well as for amino acid composition. The results are being correlated with the biological and immunological properties of these materials.

Studies on the localization of polypeptides in the virus indicated that mammalian virus cores are very similar to cores of avian agents in terms of structure, physical properties, and polypeptide composition. Studies of the biological activity of the virus core substructure is continuing.

Significance to Biomedical Research and the Program of the Institute: The virus genome is expressed by translation of message into protein products. This project was initiated to characterize the major protein antigens of selected RNA tumor viruses, prepare immune sera to identify

these antigens in infected cells, and to utilize these materials to determine basic molecular events in the process of cellular transformation. The knowledge gained may be used to develop sensitive methods for detection of virus activity in human cells and to provide a basis for therapy by blocking translational events at the sub-cellular level to prevent cell transformation.

Proposed Course: The characterization of the structural protein components of the mammalian RNA tumor viruses will be continued. A bank of highly specific immune sera against the intraspecies specific determinants of the mammalian viruses will be developed for use in the study of translational events related to the process of cell transformation and in the search for protein products of viral activity in human tumors.

Date Contract Initiated: April 19, 1971

Current Annual Level: $220,000

ALBERT EINSTEIN COLLEGE OF MEDICINE (NIH-71-2251)

Title: Research Studies of the Molecular Biology of Oncogenic Viruses and Malignant Transformation

Contractor's Project Director: Dr. J. Thomas August

Project Officers (NCI): Dr. Timothy O'Connor
Dr. Robert A. Manaker

Objectives: To determine the molecular events involved in the adsorption and penetration of RNA tumor viruses into host cells, in viral nucleic acid replication, and in malignant transformation of cells.

Major Findings: A protein kinase has been described for purified preparations of several enveloped viruses, both membrane maturing and intracytoplasmic maturing species. The kinases were not activated by cAMP and phosphorylate certain polypeptides of the virion or added basic proteins. Frog virus 3 contained the highest specific activity of protein kinase, 50 to 100-fold greater, among the viruses tested. The high level of protein phosphorylation was related to the activity of the protein kinase rather than to the effectiveness of frog virus proteins as substrate. The kinase was shown to be incorporated as an integral component of the virus. The phosphorylated polypeptide species were estimated to comprise less than 5% of the total protein. The major capsid protein was not labeled by the *in vitro* reaction. The enzyme incorporated phosphate most effectively into the arginine-rich histone fraction 3 whereas cAMP-dependent kinases most readily phosphorylated the lysine-rich histone fraction 2b. The enzyme was unlike other kinases in that it catalyzed phosphorylation of the virion structural proteins of R-MLV and reovirus.

Reverse transcriptase (RT) from Rauscher mouse leukemia virus (R-MLV) and avian myeloblastosis virus (AMV) behave identically in their priming activity with DNA. All incorporation of deoxynucleotide occurs at the 3'-OH ends with resultant covalent attachment of primer DNA and product DNA. Short single stranded regions of the DNA duplex are efficiently repaired while long single stranded regions are poorly used. However the 2RT's behave differently when primed by viral 70S RNA in that no RNA synthesis of DNA occurs when R-MLV RT is primed by R-MLV, AMV, and Rous sarcoma virus (RSV) 70S RNA's. The AMV RT has a molecular weight of 160,000, whereas the R-MLV RT molecular weight is 85,000, indicating perhaps a loss of a specific subunit during purification. With purification of AMV RT, the yield of DNA decreased from 30% to 5% due to the removal of a stimulatory protein. The presence of the stimulatory protein resulted in free DNA products and RNA-DNA hybrids. The protein has no effect on the RT of R-MLV, E. coli DNA polymerase II and III, and HeLa cell DNA polymerase. RNAase H of AMV is a processive exonuclease and cannot attack RNA in hybrid structure if both ends of the RNA are blocked. A model has been devised which predicts that after infection integration of oncornaviral RNA into the host chromosome occurs. The RT may convert viral RNA into RNA-DNA hybrids rather than catalyze transcription of the entire genome into DNA. Further, the model predicts that synthesis of oncornal RNA is catalyzed by the DNA-dependent RNA polymerase of the nucleus.

1300 plaques of cloned B-tropic Friend leukemia virus were collected from plaques of mouse embryo cells (B-tropic) developed by the XC assay. Of these five clones were temperature sensitive but were too leaky for practical use. Clones have recently been isolated from plaques of mutagenized stocks. The rate of adsorption of Friend virus by N or B cells is the same for both. Biophysical studies indicated that five subunits exist in the parent 70S genome, however the characterization of the subunits is still incomplete.

Both Friend leukemia virus and R-MLV can be precipitated at low centrifugal force from supernatant fluids of infected cultures with Concanavalin-A with rapid concentration and purification and little change in ionic osmolar conditions.

Significance to Biomedical Research and the Program of the Institute:
The protein kinase detected in the virions of enveloped viruses differs from cellular kinase. Its role in the cycle of viral infection and reproduction is unknown. The studies underway are designed to determine the effects of viral infection on the phosphorylation of cellular and viral proteins and their significance in cellular response to infection. Investigations on the role of the reverse transcriptase and hybridase in the cycle of reproduction of oncogenic RNA viruses lead to an understanding of the mechanisms underlying the integration of viral information into cellular DNA. The information obtained from experimental systems suitable for intensive laboratory investigation should apply to human neoplasia where evidence has been found suggestive of C-type RNA virus infection.

Proposed Course: The analysis of the effect of virus infection on the phosphorylation of cellular and viral proteins will continue. The transformation of avian cells by RSV will be studied biochemically and purified virus structural proteins will be used as probes to analyze viral gene expression in animal tissue. Enzymes active in the synthesis and function of viral-specific DNA and RNA will be purified and characterized, and termini of the AMV and RLV RNA will be analyzed.

Date Contract Initiated: April 26, 1971

Current Annual Level: $357,500

HAZLETON LABORATORIES (NIH-69-2079)

Title: Etiology of Cancer

Contractor's Project Director: Dr. Robert C. Good

Project Officer (NCI): Dr. Stuart Aaronson

Objectives: To investigate mechanisms of action of oncogenic viruses and examine human neoplasms for evidence of viral etiology. To provide a holding facility for dogs used in cancer control studies.

Major Findings: A large number of temperature sensitive (ts) mutants of murine leukemia virus (MuLV) were isolated. These are being examined to define viral genes involved in replication. Several mutants of murine sarcoma virus (MSV) were isolated which are temperature sensitive for the transformed state. Cells non-productively transformed by MSV were shown to possess weak, virus-related cell surface antigens which are under investigation for their importance in expression of the transformed state. Morphologically normal mammalian cells containing sarcoma virus were isolated and are being examined to determine cellular genetic requirements for cell transformation.

Endogenous RNA tumor virus genetic information was demonstrated in high and moderate leukemia incidence mouse strains and a number of genetic loci involved in expression of endogenous viral information were demonstrated. To extend studies on endogenous virus expression to human tissues, cloned lines of human tumor cells were established in culture for study by immunological, biochemical and biological methods to detect evidence of the presence of virus.

Three dogs bearing spontaneous tumors were obtained for immunotherapy trials. Sixty-one dogs, that had been inoculated with tumor extracts, are being examined for sensitization to tumor-specific antigens.

Significance to Biomedical Research and the Program of the Institute: Virus mutants defective for different properties provide valuable tools to determine virus genetic factors and products of virus replication and cellular transformation as well as to investigate the contribution by the cell in the events leading to the transformation process. Dogs bearing tumors provide opportunity to determine the effectiveness of immunotherapy regimens developed in small laboratory animals.

Proposed Course: The mechanism of action of MSV will be investigated in infected non-virus-producing cells wherein virus nucleic acids and virus-specific proteins will be identified and characterized as measures leading to development of methods for interference with the expression of the transformed state. Conditional lethal mutants of MuLV will be used in attempts to elucidate mechanisms of replication and functional relationships between leukemia and sarcoma viruses and host range mutants of MuLV will be used for complementation and recombination studies with ts-mutants of MuLV. Cultured human tumor cells will be examined biologically and biochemically for evidence of virus expression.

Date Contract Initiated: May 26, 1969

Current Annual Level: $740,720

HOPITAL ST. LOUIS (NIH-72-3263)

Title: Molecular Virological Studies on Human Leukemia

Contractor's Project Director: Dr. M. Boiron

Project Officers (NCI): Dr. George Todaro
Dr. Robert A. Manaker

Objectives: To search for viral DNA sequences in human leukemic cells and for expression of the RNA expression of an integrated genome in these cells using murine leukemia and sarcoma viruses grown in human and in mouse cells as a source of the synthetic DNA probe.

Major Findings: Preliminary experiments and original approaches were undertaken to characterize the virus-specific nucleic acids in murine and human leukemic cells. Single-stranded DNA was synthesized which contained complete and almost uniform sequences of the entire viral 70S genome. RNA tumor virus RNA present in cellular RNA was detected by two methods: competition hybridization between ^{32}P-labeled 70S viral RNA and unlabeled cellular RNA to a single-stranded DNA product and direct hybridization of single-stranded product DNA to "poly A-rich RNA". To detect virus-specific nucleotide sequences integrated into cell DNA, the complementary product DNA was annealed to sheared and denatured cell DNA. Murine sarcoma virus with its helper leukemia virus was adapted to growth in the J III human cell line for production of template for synthetic DNA preparation. Testing of human leukemia specimens by the complementation-hybridization method was begun.

Significance to Biomedical Research and the Program of the Institute: Data have been reported demonstrating the presence of nucleotide sequences in human leukemia cells homologous to sequences in RNA tumor virus RNA. An intensive study of human leukemia and Hodgkin's disease cells under this project is expected to provide information expanding on these observations to determine the nature of the common nucleotide sequences, their distribution in patients' cells, and the relationship between the presence of the common sequences in virions and the species and nature of the cells in which the virus is grown.

Proposed Course: The original and reproducible techniques developed will be applied to the investigation of as many human leukemias as possible.

Date Contract Initiated: June 28, 1972

Current Annual Level: $94,700

JOHNS HOPKINS UNIVERSITY (NIH-71-2121)

Title: Herpesvirus Antigens and Virions in Neoplastic Cells from Squamous Carcinoma of the Human Cervix

Contractor's Project Director: Dr. Laure Aurelian

Project Officers (NCI): Dr. Charles W. Boone
Dr. Robert A. Manaker

Objectives: The immediate objective of this project is the identification of herpes simplex virus type 2 (HSV-2) antigens and virions in neoplastic cells. The ultimate objective is development of evidence for or against HSV-2 as a factor in the etiology of carcinoma of the human uterine cervix.

Major Findings: The prevalence of antibodies to HSV-2 in patients, whether at the atypia, in situ, or invasive stages of cervical carcinoma, was found to be about 100 percent as compared to 55 percent obtained in a control population matched for age, race, and socio-economic class. The prevalence of antibody in carcinoma patients is independent of socio-economic class. The frequency of two other venereal diseases, trichomoniasis and syphilis, was not significantly different in the carcinoma and control populations.

Virus-specified proteins were detected in exfoliated dyskaryotic cells by indirect immunofluorescence in 90 percent of 59 cases. Normal cells did not stain. Impressions and frozen sections from carcinomas of 23 patients whose dyskaryotic cells stained for HSV-2 failed to show evidence of viral antigens in all cases and no virus was detected by electron microscopy, suggesting that the virus genome might be harbored in a repressed state, but is derepressed in exfoliated cells in the vagina. Evidence of repression of the virus genome in cells was obtained by recovery of virus from cells of an intraepithelial carcinoma of the cervix after six months of cultivation *in vitro*.

If HSV-2 has an oncogenic potential for human cervical cells, some of its functions must be expressed, particularly those bearing on the transformation of cells from normal to neoplastic behavior. Sera from patients were tested for antibody to an antigen induced in HEp-2 cells at an early stage in the replicative cycle of HSV-2. Sera positive for virus neutralizing antibody did not react with this antigen, suggesting a non-virion nature. Preliminary studies appear to indicate that this "early" HSV-2 induced antigen is tumor specific. Reactive antibodies were present in sera of patients with cervical carcinoma and absent in sera of patients following therapy. The observations suggest that the "early" HSV-2 induced antigen(s) may be of prognostic significance in cervical carcinoma and show a relationship existing between this virus and the disease. Apparently this antigen is the product of the expression of the virus genome in the tumor.

Significance to Biomedical Research and the Program of the Institute: Considerable data has been acquired demonstrating an association between HSV-2 infection and carcinoma of the uterine cervix. In humans, it is extremely difficult to conclusively show that virus associated with neoplasms is a factor in oncogenesis and not simply a passenger. Studies conducted under this project are expected to provide some of the data required to reach a decision regarding the role of this virus in oncogenesis.

Proposed Course: Further studies will be made to determine the presence of "early" HSV-2 antigens in cervical tumors, the prevalence of antibodies to this antigen in patients, and the prognostic significance of these antibodies. Cervical tumor cell cultures will be analyzed for expression of persisting HSV-2 genome or of a fraction of a genome. A search will be made for HSV-2 specific antigens on the surface of tumor cells, biopsied, exfoliated, or cultured in vitro.

Date Contract Initiated: May 5, 1971

Current Annual Level: $79,780

KAROLINSKA INSTITUTE (NIH-69-2005)

Title: Studies on the Significance of Herpes-type Viruses and RNA Viruses in the Etiology of Some Human Cancers

Contractor's Project Director: Dr. George Klein

Project Officers (NCI): Dr. Charles W. Boone
Dr. Gary R. Pearson

Objectives: To elucidate EB virus-cell-host interactions, mechanisms of cell-mediated anti-tumor immune reactions, and regulation of C-type virus expression in defined systems.

Major Findings: All lymphoblastoid lines of human origin so far tested have been found to carry the genome of EBV, by nucleic acid hybridization and by the presence of the EBV-determined CF antigen. The virus can convert

lymphoid cells with a limited life span into established non-permissive lymphoblastoid cell lines (LCL) with an unlimited proliferative capacity in vitro. An anticomplementary fluorescence test that permits the demonstration of EBV-CFA at the cell level was developed. EBV-CFA is present in most cells (80-90%) of nonproducer lines, in the form of finely granular, intranuclear material, and is superficially similar to the T-antigen systems. The minority fraction lacking this antigen may represent dead or dying cells.

Receptor positive, superinfection resistant LCL could not be activated at all or only to a very limited extent by BUDR or IUDR, in contrast to the superinfection-sensitive lines that were readily activable. This suggests the existence of restrictive mechanisms that recognize the exogenous, superinfecting viral genome and the endogenous, activated viral genome in a similar way. Receptor negative, superinfection resistant lines could be activated to a variable degree. The P3HR-1 derived virus showed no transforming ability for cord blood cells, but a clear cytopathic effect on cord blood cells and, to an even larger extent, on the Raji line. Neither 833L nor WIL-derived virus had any cytopathic effect for Raji or for cord blood cells, but readily transformed cord blood cells. It is possible that different viral particles are responsible for the cytopathic and the transforming effects, respectively.

A fixation procedure that preserves the specificity of the membrane antigen has been developed. The membrane antigen complex (MA) was found to consist of early MA (EMA) and late (LMA) components. EMA is present on the Burkitt biopsy cell, and appears on EBV-superinfected Raji cells in the presence of DNA inhibitors such as Ara C. The late membrane antigen (LMA) is present on the surface of virus producing (VCA+) cells and also on a small number of VCA-cells that are presumably on their way towards VCA production. EMA+ cells were compared with MA- cells with regard to their relative positions in the cell cycle. EMA appeared preferentially during the later part of G1, was fully expressed during the S phase and stayed on during G2. Early G1 phase cells were negative, as a rule. This reaffirms the compatibility of EMA expression with cell division. A series of hybrid cell clones, produced by fusing the mouse fibroblast line A9 with the EBV-carrying, membrane-IgM positive, BL-derived Daudi cell, did not exhibit MA, EA or VCA, were not susceptible to activation or superinfection with EBV, and did not produce human IgM.

Only the EBV-associated antibodies showed reproducible changes that could be related to the disease in serological studies of BL patients. The relationship of EA titer to prognosis was particularly clear for antibody directed against the R ("restricted") type of EA specificity. The anti-MA titers showed very different patterns than the EA titers. When tested by the BT (blocking titration) method anti-MA levels remained fairly steady in patients whose tumors have gone to long lasting, total regression, with only a very slow, continuous decrease. Another reproducible feature was the relatively sudden fall of anti-MA level prior to recurrence, often several months before tumor regrowth became clinically obvious. While irradiation of the tumor was followed by a parallel increase of anti-MA and anti-EA, BCG therapy has led to a selective increase of MA titers, without any major effect

on anti-EA or VCA levels.

In studies of tumor-associated antigen-antibody complexes, there was an apparent relationship between the anticomplementary activity (ACA) and the presence of tumor in certain series of sera. The ACA may have been due to an antigen-antibody complex, but indicates nothing about the nature of the complex, or its possible relationship to the tumor. Peripheral lymphocytes exposed to an autologous infectous mononucleosis derived cell line, turned into killer cells that lysed BL and IM derived lines very efficiently, whereas the lines derived from normal donors were not or much less affected. The methodology involved in these studies has been greatly simplified by the use of the more rapid and precise isotope release microcytotoxicity test. Preliminary experiments suggest that IM lymphocytes, stimulated by exposure to the autologous LCL, are efficiently adsorbed by IM-LCL but not by HVS-transformed marmoset LCL.

The first BL patient has been described where the recurring tumor was not of the same, but of a different clonal type than the original, as judged by the G6PD marker.

Serially heterotransplanted tumor lines, derived from a Burkitt lymphoma biopsy and two nasopharyngeal carcinoma biopsies, have been established in thymusless (nude) mice. The BL derived tumor has the human isozyme and IgM markers characteristic for the original in vivo tumor, a normal human female chromosome complement, and a typical BL histology. It contains EBV-DNA, and the EBV-CFA can be demonstrated in virtually all tumor cells. The nasopharyngeal carcinoma-derived nude mouse passaged tumors are typical squamous cell carcinomas with human isozymes and chromosomes. The represent excellent materials for study of EBV association with the lymphoid or the carcinomatous elements in the human tumor.

Two kinds of early antigens in Herpesvirus saimiri (HVS) infected Vero cells could be distinguished, a "punctate" and a "trabecular" type. Antibody positive, healthy adult squirrel monkeys had antibodies to the late but not to the early viral antigens. Acutely infected squirrel monkeys developed antibodies to both early and late antigens. The same was true for the acutely infected marmosets and owl monkeys that also developed tumors. There was a considerable difference in the timing of antibody appearance between the natural squirrel monkey host, resistant to the oncogenic effect of the virus, where positive antibody titers could be registered 14-17 days after virus inoculation, and the two susceptible species. In the latter, antibodies did not appear until 30-80 days; by then their tumors were already well established.

Using inhibition of DNA synthesis by Ara C in cytomegalovirus (CMV) infected human cells, early antigens (EA) could be distinguished from late antigens (LA). In the course of acute CMV infection, patients developed antibodies to both EA and LA, whereas normal, antibody positive adult cells had antibodies against LA only.

In studies on human neoplasms significant stimulation of peripheral blood lymphocytes was obtained in the mixed lymphocyte-target cell interaction

(MLTI)-test with 20 of 44 sarcoma specimens, 6 of 29 brain tumors, 5 of 9 carcinomas and none of three myelomas. In several cases where biopsies did not stimulate, lymph node cells draining the tumor area did stimulate the peripheral lymphocytes of the same patient. In 37 of 46 cases, there was a significant blocking of stimulatory tumor-lymphocyte combinations by autologous serum, in 25 of 40 cases by allogeneic serum taken from patients with similar tumors as the donor, in 3 of 17 cases by allogeneic serum taken from donors with unrelated tumors, and in 7 of 44 cases by the sera of healthy controls. An increased killing of the glioma lines was frequently noticed with glioma patient lymphocytes. Recently operated patients often showed a certain non-specific killing of all the lines, however. This may or may not be related to the increased background of DNA synthesis (i.e. blast transformation) found in the lymphocytes of recently operated patients. Control lymphocytes from patients with certain nonneoplastic neurological disorders were also frequently cytotoxic, however, and more so against glioma than against normal glia lines.

Immunological studies in mice showed the H-2 system to be exclusively restricted to the outer cell membrane. In addition, the Moloney virus determined surface antigen, studied on the same cell and by the same techniques as H-2, shows a different distribution and is present on the plasma membrane and in internal fractions as well. The cell cycle-related antigenic changes were shown to depend on cycle-related differences in the availability of surface antigen receptors. Genetically determined H-2 and virally determined Moloney type surface antigens showed parallel changes, with maximum expression during the latter part of the G1 phase, decrease during the S phase, and minimum expression during the latter part of S, G2 and M.

Immunoresistant tumor cell sublines are usually characterized by a quantitative reduction in the number of relevant antigenic sites exposed on the outer cell surface. The emergence of such lines may be an important factor in frustrating the immune rejection of the host. TA3/Ha (derived from the TA3 mammary adenocarcinoma in strain A mice), has, compared to TA3/St, a 30-50 fold lower $H\text{-}2^a$ antigen expression, has no agglutinability by concanavalin A, and has a smooth cell surface in the scanning electron microscope. TA3/St has a high antigen expression, can be readily agglutinated by concanavalin A and has a rough surface. TA3/Ha shows approximately one third of the antibody binding capacity characteristic for TA3/St. It is therefore possible that the difference in antigen expression is due to steric factors that only operate in viable cells. Upon hybridization of TA3/Ha with normal ACA fibroblasts that do not carry the genetic information for the $H\text{-}2^a$ antigens characteristic for the TA3 lines, the antigen suppression that characterizes the living TA3/Ha cell is released and its anti-$H\text{-}2^a$ antibody binding capacity becomes equal to and, in some cases, even higher than the value characteristic for the TA3/St cell. When the hybrid cells are reselected *in vivo* to grow as high malignant ascites tumors in A x ACA F_1 hybrids, they maintain their high $H\text{-}2^a$ level, and remain strain specific. They also develop a rough surface membrane. In all these properties, the hybrid thus resembles the original TA3/St line and not the TA3/Ha parent. There are two properties, however, which do not change in

parallel. One is agglutinability by concanavalin A that remains negative. It can be therefore concluded that the cellular mechanism that renders TA3/Ha non-agglutinable by con A is not linked to the low antigen expression or the smooth surface membrane characteristic for this cell. Even more important, the hybrid-derived ascites lines (representing four independent fusions), although invariably highly antigenic, are not uniformly sensitive to the cytotoxic action of anti-H-2^a antibodies. Whereas a high antigen expression is obviously a prerequisite for sensitivity to humoral antibodies, it is not sufficient per se, and other membrane characteristics may render a high-antigenic cell resistant to antibody mediated killing. However, both the sensitive and the resistant hybrid cells are rejected in H-2-incompatible strains and it is conceivable that the postulated repair mechanisms works only in relation to humoral, not cell mediated killing.

T-cells were found to play a dominating role in cell mediated killing in the mouse H-2 system. It was shown that the killer T-cells carry specific receptors on their surface and can be selectively adsorbed to appropriate, antigen carrying, viable or glutaraldehyde fixed cell monolayers, and that the T-cells can kill in an autonomous fashion without the cooperation of macrophages or B-cells. The relative T- and B-cell contribution to killing in lymphoid cell populations from rats immunized in vivo against (MLV) induced tumor cells or against the virus was determined. Lymphocytes of tumor bearing animals had a much lower killing efficiency than the lymphocytes of virus immunized or regressor animals. Non-T cells acted maximally at relatively long times after virus inoculation or tumor regression. In contrast to the H-2 system, preliminary evidence of an essentially similar kind, showing a predominant target cell killing by non-T cells, is available for tumor specific antigens in the methylcholanthrene sarcoma system. In the MSV (MLV)-associated antigen system, killing action was abolished by lymphocyte passage over an anti-Ig coated column, suggesting a high surface Ig concentration in the active cell population.

A correlation has been found between the presence of humoral antibody to the feline oncornavirus cell membrane antigen (FOCMA) and the regression of tumors induced by FeSV. Cats with progressing tumors failed to develop high titers of antibody. Passively transmitted humoral antibody at birth protected neonatal kittens against the oncogenic effect of even high FeSV doses. In the course of these experiments, evidence was obtained that horizontal transmission of the virus does occur under the laboratory conditions. A number of EBV-carrying human lymphoblastoid lines could be superinfected with FeLV in vitro, with the expression of the FOCMA antigen. Specific anti-FOCMA antibodies developed in marmoset monkeys injected with FeSV, or transplanted with FeSV induced tumor cells from the corresponding species. A significant number of normal control street cats had low or moderate titers of the FOCMA antibody, suggesting horizontal transmission of the virus under natural conditions.

In a study concerning the surface antigen specificity of MSV-induced mouse sarcoma cells, no sarcoma-distinctive surface antigen could be detected. Virus released by the parental Moloney lymphoma sublines (YAC and YACIR) and their hybrid derivatives were compared by a number of independent tests and showed a remarkably independent assortment of infectivity characteristics.

The pattern was stable for any given line and did not change over a period of seven months. The virus released by the A9 and A9HT sublines of mouse L cells was shown to infect C3H (N-type), but not C57B1 (B-type) mouse embryo fibroblasts. Infection was indicated by distinct single giant cell formation in the XC monolayer, used to overlay the mouse embryo fibroblasts. On the basis of these results it was concluded that the L cell virus is N-tropic. The major genetic locus affecting resistance to infection with leukemia viruses seem to regulate infectious virus production within somatic cell hybrids. The same genetic locus did not seem to affect the expression of all virus-related function, since the virus controlled membrane antigen was demonstrated in many of the N x B-type hybrids.

Significance to Biomedical Research and the Program of the Institute: Investigations under this project are directed to two areas of importance to overall program. First, the recognition that certain herpesviruses induce neoplasms in animals and that EB virus and herpes simplex virus type 2 are associated with human neoplasms requires intensive study to provide a better understanding of the host-virus relationship for this group of agents. Data acquired under this project contributes to assessment of the role of herpesviruses in the causation of human neoplasms. Second, the analysis of the immunological responses of the host to tumor cell surface antigens provides basic information important in approaches to control of tumor development. The project is strongly oriented to human neoplasia, utilizing defined animal systems as required for progress in understanding the fundamental mechanisms involved.

Proposed Course: This project will continue without change.

Date Contract Initiated: April 9, 1968

Current Annual Level: $95,000

LIFE SCIENCES, INC. (NIH-73-3205)

Title: Studies on Marek's Disease as a Model for Herpesvirus Associated Oncogenesis

Contractor's Project Director: Dr. Jack Frankel

Project Officers (NCI): Dr. Gary R. Pearson
Dr. Michael A. Chirigos

Objectives: To determine the exact nature of the role of the herpesvirus associated with Marek's disease in the etiology of this disease using specific pathogen free avian hosts and to operate an avian virus testing laboratory for monitoring both hosts and virus for freedom from extraneous agents including infectious leukosis virus.

Major Findings: Two lines of chickens were used in these studies: S-line conventional birds maintained at Cornell University and LSI-line birds originated from Connecticut-A stock and maintained as a specific pathogen free SPF colony at the contractor's facility. LSI-line birds are free of infectious leukosis virus. Two strains of Marek's disease herpesvirus (MDHV) were used: the Georgia (GA) strain of Itsen and the cloned GA strain of Burmester. GS-Itsen strain virus produced small plaques on chicken embryo cultures; the cloned Burmester strain produced large plaques on these cultures. Pre-infection of cultures with RAV-2 leukosis virus inhibited plaque production. Methods were developed to recover cell-free MDHV from the feather follicles of infected birds. Infection of birds was accomplished by direct inoculation or by exposure to a contaminated environment.

S-line birds developed classical Marek's disease (MD) with visceral and neural pathology following infection by the Itsen and the Burmester strains of virus. LSI-line birds developed a chronic infection with low mortality when infected by MDHV/Burmester, but succumbed to acute infection by MDHV/Itsen without developing classical MD. This acute disease was accompanied by lytic neural lesions and destruction of lymphoid cells with replacement by reticular elements. The birds showed no immune response to the virus. Co-infection of LSI-birds by contact with either strain of MDHV and the RAV-2 strain of avian leukosis virus resulted in classical Marek's disease and an immune response to the MDHV. C-type particles predominated in feather follicles and an apparent enhancement of RAV-2 virus occurred. Molecular hybridization studies in collaboration with Dr. Spiegelman showed co-infection with RAV-2 and MDHV resulted in a significantly greater amount of RAV-specific RNA in involved organs than did infection by RAV-2 alone.

The combined activity of RAV-2 and MDHV was also apparent in tests made in SPF Japanese quail. Exposure to infection by either virus did not produce overt disease. Combined infection brought quail down with visceral and neural tumors. It appears that interaction between an RNA avian leukosis virus and the MDHV is required for development of the classical picture of MD.

Significance to Biomedical Research and the Program of the Institute: In comparison to the RNA tumor viruses, comparatively little is known concerning the role of herpesviruses in oncogenesis. Certain herpesviruses have been implicated in the etiology of carcinoma, lymphoma and leukemia in different species of animal and other viruses of this group have been shown to be strongly associated with neoplasia in man. This project provides opportunity to acquire information on one herpesvirus in relation to a malignant disease which may aid in understanding the role of herpesviruses in oncogenic processes in man.

Proposed Course: Studies on the interaction between MDHV and other viruses as this relates to the disease process will be continued.

Date Contract Intitiated: November 1, 1968

Current Annual Level: $534,060

LITTON-BIONETICS, INC. (NIH-73-3211)

Title: Studies on Molecular Events Leading to Transformation by RNA Oncogenic Viruses and Relationships to Human Neoplasms

Contractor's Project Director: Dr. Robert C. Y. Ting

Project Officer (NCI): Dr. Robert Gallo

Objectives: To elucidate the molecular mechanisms by which RNA tumor viruses transform normal cells into malignant cells.

Major Findings: The RNA dependent DNA polymerase (RDDP) activity in human acute leukemia cells was isolated in a particulate fraction banding at 1.16 gm/ml in a sucrose gradient. The purified enzyme possessed the essential biochemical properties of RNA tumor virus reverse transcriptase (RT) and catalyzed an endogenous ribonuclease-sensitive DNA synthesis in the disrupted pellet using a native endogenous RNA molecule present in the human cells. The RDDP was distinguished from the major DNA polymerases of normal human blood lymphocytes by its properties, which included its capability to use heteropolymers such as 70S RNA of AMV and RLV as templates for synthesis of partial DNA copies.

Immunological studies on four purified RDDPs isolated from human leukemic cells were conducted in collaboration with Dr. George Todaro. Two showed partial antigenic cross reactivity with antiserum to the enzyme from the virus isolated from Gibbon leukemic cells, one was negative, and one showed complete cross reactivity.

The major DNA polymerases present in fresh normal human blood lymphocytes were purified and characterized. The viral RT differs from the normal cell enzymes by accepting viral 70S RNA as template and showing a greater response to the synthetic templates: oligo dT · poly rA compared to oligo dT · poly rA in the presence of magnesium, an absolute response to oligo dG · poly rC, and the absolute response to oligo dC · poly rI.

The RNAs of all the RNA tumor viruses examined contain long poly A stretches, distinguishing the poly A of tumor viruses from poly A from other sources, and provides a biochemical criterion for the probably viral nature of RNA molecules detected in human cancer. The observation suggests that viral RNA may act as messenger RNA and not simply as a template since mRNA also contains poly A.

It was assumed that poly A is added to viral RNA after transcription from a provirus DNA. Cordycepin, an inhibitor of poly A formation, was tested and found to block production of virus induced by iododeoxyuridine. Inhibitory action was limited to a 12-16 hour period following iododeoxyuridine. The observation suggests that this is the period, following transcription of viral RNA, during which poly A is formed and attached to the viral RNA

transcript.

The RTs of Mason-Pfizer monkey virus (MPMV) and of simian sarcoma virus (SSV-1) were purified to homogeneity. Comparison of biochemical characteristics of these enzymes with the RTs from viruses of lower forms of animals showed that biochemical properties differed from virus to virus. The RDDP from human cells resembles most closely the enzyme of the MPMV.

Investigations were conducted to determine the natural host of a virus by a molecular hybridization technique. Present results have been completely accurate. Examination of RD-114 virus left no doubt of its feline origin. Relationships between serologically related viruses were found to be greater by molecular hybridization than between those not so closely related serologically.

It was shown that inhibition of RT activity, accomplished by treatment with different rifampicin derivatives, resulted in the inhibition of RNA tumor virus infection in vitro. These observations were recently extended to experiments with murine leukemia in vivo.

Significance to Biomedical Research and the Program of the Institute: It is known that RNA tumor virus genomes within cells may be repressed and in some instances may be defective. Molecular biological methods are valuable to probe cells for evidences of virus expression. Under this project, criteria were defined to differentiate between purified tumor virus RDDP activity and the activity of the purified major DNA polymerases of normal cells. The RDDP activity in particulate fractions separated from leukemic human cells was purified, characterized, and found to resemble the virus enzyme. This work contributes to the search for evidence of viral expression in tumor cells and provides a basis for studies on the inhibition of viral function in the neoplastic process.

Proposed Course: Studies will continue to further define the nature of the virus-like activity expressed in human leukemic cells.

Date Contract Initiated: September 1, 1972

Current Annual Level: $323,650

MASSACHUSETTS GENERAL HOSPITAL (NIH-71-2174)

Title: Characterization of Nucleic Acids of the Avian Myeloblastosis Virus

Contractor's Project Director: Dr. Paul C. Zamecnik

Project Officers (NCI): Dr. Timothy O'Connor
Dr. Robert A. Manaker

Objectives: To analyze the end groups and acromolecular sequencing of the large molecular weight RNA of avain myeloblastosis virus (AMV) and to analyze the minor base composition of the transfer RNA (t-RNA) of AMV.

Major Findings: The "free" 4S RNA of AMV and the 4S RNA derived from the 70S viral RNA were compared. Studies on the minor base composition showed they resembled each other and were significantly different from host myeloblast or chick liver 4S t-RNA in the levels of minor bases present. The 70S-associated 4S RNA accepted amino acids and resembled the "free" 4S RNA of the virus in having a high acceptance for lysine. Both 4S RNAs satisfied criteria for t-RNA. The significance of the "70S-associated" 4S RNA is unclear.

Virus RNA is being extracted under ribonuclease-free conditions using sucrose gradients containing Me_2SO, and provides a very sharp 35S peak. The 35S component obtained had a molecular weight of about 2.3×10^6.

A terminal adenosine was found as the 3'-OH terminal nucleoside of the 70S and the 35S RNA. Experiments to determine the penultimate nucleotide in 35S RNA from AMV have not yet been successful. The 35S RNA fractions apparently contain some cryptic breaks with termini ending in a 3'-phosphate. Control experiments with phosphomonoesterase uncovered additional C and U residues on the terminal position of the RNA in addition to the high percentage of A usually found. Efforts must be made to isolate 35S RNA containing no molecules of a similar size range terminating in a 3' phosphate.

Significance to Biomedical Research and the Program of the Institute: This project was initiated in the expectation that elucidation of the nucleotide sequences of the 70S RNA of AMV and possibly other oncogenic RNA viruses might reveal segments active in specific functions and as binding sites of viral polymerases or inhibitors, thereby increasing our knowledge of transcription processes. Elucidation of the differences in t-RNA encapsulated in virions and those present in normal and infected cells might show how virus infection dominates translation processes.

Proposed Course: Improved procedures for isolation of 35S RNA free of contaminating 3'-terminal phosphate molecules will be investigated and the sequence studies on these purified fractions will be continued. The viral 4S RNA fraction will be fractionated into a few individual tRNA species and compared with their normal counterpart to ascertain whether these species are unique to the virus or are of host cell origin. Minor base patterns of the RNAs will be compared.

Date Contract Initiated: June 29, 1971

Current Annual Level: $104,010

MASSACHUSETTS GENERAL HOSPITAL (NIH-70-2012)

Title: Activation of Oncogenic Viruses and Induction of Cancer by Immunologic and Non-immunologic Methods

Contractor's Project Director: Dr. Paul H. Black

Project Officers (NCI): Dr. Michael A. Chirigos
Dr. Adi Gazdar

Objectives: To determine the relationships between chronic allogeneic disease, immunosuppression, and interferon inducers on the activation of covert infections by oncogenic RNA viruses.

Major Findings: Continuation of studies on activation of covert infections by oncorna viruses in mice in the graft versus host (GVH) reaction demonstrated that leukemia viruses were activated when minor histocompatability antigens were involved, but to a much lower degree than when certain major H2 antigens were involved. To determine whether reactions against antigens other than lymphocyte H-2 antigens would induce leukemia virus release in vitro, lymphocytes from BALB/c mice, either previously sensitized against A/J mouse histocompatability antigens or unsensitized, were cultured for a week with fibroblasts from (BALB/c x A/J)F_1 embryos. Considerably more blastogenesis was observed in the sensitized lymphocyte cultures, but no virus production was detected. Thus the apparent uniqueness of leukocyte-leukocyte interaction in viral activation is further suggested, probably because lymphocytes have more H-2 antigen expression on their surfaces than do fibroblasts.

The GVH combination DBA/2 and (C57B16 x DBA/2)F_1 has a high incidence of glomerulonephritis but a low incidence of tumors. F_1 hybrids not inoculated with DBA/2 lymphocytes showed only 2 of a group of 17 animals to be positive for virus in contrast to 13 positive animals in 34 that were inoculated. This result reinforces the proposition that virus activation alone cannot be equated to oncogenesis.

To determine whether immunosuppressive agents that are blastogenic in certain concentrations activate leukemia viruses, cultures of (BALB/c x A/J)F_1 lymphocytes were cultured with stimulatory and non-stimulatory concentrations of anti-lymphocyte serum without significant effect. Repeated immunosuppressive doses of cyclohexamide given to mice of this cross also failed to increase the frequency with which leukemia virus was detected, thus supporting the concept that immunosuppression alone is insufficient explanation for virus activation in GVH reactions.

Differences in interferon production were not observed in the blastogenic reactions induced by phytohemagglutinin as compared to mixed lymphocyte culture. Therefore, differences in interferon production do not account for the differences in virus activation observed in these two systems.

Significance to Biomedical Research and the Program of the Institute: This project contributes new information concerning immunological factors involved in the activation of covert virus and its influence on the development of virally-induced cancer.

Proposed Course: The effect of immunosuppression will be evaluated in *in vivo* systems in which there is slight virus expression and negligible oncogenesis. The effect of cell surface active agents that alter cellular antigenicity on virus activation will be determined. The leukemogenic viruses activated in the GVH reaction and mixed lymphocyte culture systems will be characterized and studies are to be conducted to determine whether anti-viral agents can prevent virus activation.

Date Contract Initiated: September 15, 1971

Current Annual Level: $93,350

MELOY LABORATORIES (NIH-72-2020)

Title: Cell Biology Facility: Mechanisms of the Immune Response to Squamous Cell Carcinoma, Adenocarcinoma, and Fibrosarcoma in the Mouse and Experimental Immunotherpay

Contractor's Project Director: Dr. Kenneth Blackman

Project Officer (NCI): Dr. Charles W. Boone

Objectives: To elucidate the mechanisms of specific tolerance to tumor exhibited by the cellular immune system in a tumor-bearing animal, to develop improved *in vitro* and *in vivo* assays for detecting tumor specific antigens and antibodies, and to develop systems of immunotherapy.

Major Findings: The augmentation of tumor transplantation antigens by influenza virus was further studied. Tests are being conducted with mouse SV40-induced fibrosarcomas, methylcholonthrene-induced fibrosarcomas, mammary adenocarcinomas, squamous cell carcinomas, and lymphoma to determine the effect of influenza virus in augmenting the immunogenicity of the tumor transplantation antigens. The plasma membrane fraction from influenza virus infected SV40 tumor cells was shown to possess the major portion of the tumor-inhibiting effect. Peritoneal cells, lymph node cells, and spleen cells from tumor immune animals completely inhibited the growth of tumor cells when mixed directly with the challenge inoculum. Intravenous administration of lymphoid cells from tumor immune animals effectively prevented growth of tumor cells inoculated one day earlier.

The phytohemagglutinin reactivity of lymphoid cells in tumor-bearing animals was shown to be markedly decreased in a number of cases. Splenectomized animals exhibited greater resistance to the growth of a number of immunogenic tumors. Mammary adenocarcinoma, paradoxically, was enhanced by prior splenectomy.

Significance to Biomedical Research and the Program of the Institute: Prior infection of tumor cells with influenza virus to augment the immunogenicity of specific tumor transplantation antigens in cell-free fractions of tumor cell homogenates provides a method whereby a patient could be immunized without the risk associated with X-irradiated viable cells. Research on the mechanisms of the immune response to tumor could provide methods for manipulation of the immune system to benefit the cancer patient both in diagnosis and in therapy.

Proposed Course: The relationship of cell surface structure to host response to tumor will be continued.

Date Contract Inititated: August 20, 1971

Current Annual Level: $358,740

MERCK AND COMPANY, INC. (NIH-71-2059)

Title: Research on Oncogenic and Potentially Oncogenic Viruses, Virus Production and Vaccine Development

Contractor's Project Director: Dr. Maurice Hilleman

Project Officers (NCI): Dr. Robert A. Manaker
Dr. Michael A. Chirigos
Mr. J. Thomas Lewin

Objectives: To conduct investigations designed to develop vaccines or other agents for the prophylaxis and therapy for human neoplasia of suspected viral etiology.

Major Findings: SV40 tumor ghost cell vaccines were found to be ineffective against homologous tumor cell challenge. Such vaccines did not interfere with the ability of the host to develop resistance to tumor growth following subsequent vaccination with a gamma irradiated whole cell vaccine. Irradiated fetal hamster cell vaccines did not show a protective effect against SV40 tumor cell challenge.

The synthetic cell surface antigen of Shier, di-N-acetylchitobiose poly-L-aspartate, was tested in mice for protective efficacy against tumor induction by 2 myeloma cell lines and by methylcholanthrene. The tests were clearly negative.

The first phases of studies to evaluate the effectiveness of vaccines against RNA tumor virus-induced disease and against human herpesvirus type 2 were continued.

Significance to Biomedical Research and the Program of the Institute: If viruses are essential in the genesis of some human cancers, prophylaxis by vaccines to prevent or minimize infection must be evaluated. Similarly, the practical value of vaccination by tumor cell or fetal antigens must be determined. Although greatest benefit would be expected by prevention of viral infections transmitted horizontally, vaccines could be effective in control of tumor development where vertically transmitted virus genetic information is not expressed in the production of proteins until later stages in the life of the host. Viruses may act as essential co-factors in the development of neoplasms, and immunization against such secondary agents could prevent the disease. This project provides for the evaluation of immunological approaches to cancer control to determine feasibility for use in humans.

Proposed Course: The contractor will devote two-thirds of his effort to the evaluation of viral vaccines and the remainder to the tumor cell antigen approach. Studies on immunological control of RNA virus-induced tumors will use the feline sarcoma/leukemia system and the L2C guinea pig system. Herpesvirus type 2, which is associated with human cervical carcinoma, and Herpesvirus saimiri, which induced lymphoma and leukemia in lower primates, are systems selected for investigation of measures for control of DNA virus-related oncogenesis.

Date Contract Initiated: March 1, 1971

Current Annual Level: $845,800

UNIVERSITY OF NAPLES (NIH-71-2056)

Title: Studies of Non-Virion Antigens of Herpes Simplex Virus

Contractor's Project Director: Dr. Giulio Tarro

Project Officers (NCI): Dr. Charles W. Boone
Dr. Michael A. Chirigos

Objectives: The determination of the presence of non-virion antigens of herpes simplex virus in tumors of the urogenital tract and of antibody to nonvirion antigens in the sera of cancer patients. These studies should provide more evidence concerning the association of herpesvirus type 2 with tumors of the genitourinary system.

Major Findings: Not all strains of herpes simplex virus type 1 (HSV-1) produce sufficient non-virion antigens in guinea pig cell cultures to permit their detection by complement-fixation (CF) tests, but antibodies to non-virion antigens are produced in guinea pigs immunized with the infected cells. Antibodies to HSV-1 non-virion antigens do not react with HSV-2 induced non-virion antigen, but antibodies to HSV-2 non-virion antigen also cross-react with the HSV-1 antigen. The peak concentration of type 2 non-virion antigen was found at three hours after infection in guinea-pig cells

and at 24 hours in rabbit kidney and human HEp2 cells. The type 2 nonvirion antigen, like the type 1, was sedimented at 33,360 g for one hour and disappeared after storage at +4C for 15 days, whereas activity was retained, although at a reduced titer, when stored at -80C for 48 days.

It was previously demonstrated that in sera obtained at different times following human infection with HSV-1 there was no demonstrable antibody for nonvirion antigen. Recently, the absence of CF antibody was observed for HSV-1 and HSV-2 nonvirion antigens in HSV-2 positive human sera, after absorption of virion antibody. Therefore, it then became possible to determine whether sera from humans with carcinomas of the cervix, nasopharynx, prostate, bladder, or kidney contained antibodies for the nonvirion antigens. Preliminary tests for nonvirion antibody on such sera and appropriate controls were carried out after absorbing the virion antibody with herpes virion antigen stored at +4°C for 15 days and testing with type 1 and type 2 HSV preparations containing the maximum concentration of nonvirion antigens as measured with guinea pig sera. Eleven sera from patients with carcinoma of the cervix have been tested for antibody against HSV nonvirion antigens. Seven cancer sera were positive for the HSV coded antigens, two were anticomplementary and two were incompletely absorbed for virion antibody; six matched control sera yielded negative results. Presence of CF antibody for HSV-1 and HSV-2 nonvirion antigens was also shown in each of five tumors of the nasopharynx, prostate, bladder and in four malignancies of the kidney.

With the use of antibody for HSV nonvirion antigens CF reactivity has been shown for separated soluble cell membrane antigens extracted from lip and cervical carcinomas but not for similar extracts from normal vaginal tissue or intestinal carcinoma. Neither the serum obtained from the guinea pig before hyperimmunization with the HSV nonvirion antigen nor the antisera of guinea pigs immunized with comparable uninfected cell extracts reacted with these tumor soluble membrane antigens.

Trypsin-treatment of normal human diploid cells (WI-38 and MRC 5) resulted in CF reactivity with antibody prepared against purified HeLa cell antigen. That reactive antigenic groups preexist in untreated normal cells was shown by absorption of the antibody with large quantities of packed human cells but not with similar quantities of rabbit and guinea pig kidney tissue culture cells. The change produced by trypsin treatment is similar to the previously demonstrated appearance of comparable reactive groups with the same antiserum at different times after infection with herpes and vaccinia viruses.

Significance to Biomedical Research and the Program of the Institute:
Present evidence indicates that the HSV-2 genome is largely repressed in cervical carcinoma cells. Some virus gene expression is expected if this virus is a necessary factor in the development of this neoplasm. Preliminary studies showed that the sera from patients with cervical carcinoma contained antibodies to HSV-2 induced non-virion antigen whereas in cancer-free persons with HSV-2 neutralizing antibodies in their sera, no antibodies to non-virion antigens were detected. This suggests continuing

stimulation of the cancer patient's immune system with virus induced non-virion antigen produced in the tumor cells, a situation apparently different from the virus-cell relationship in cancer-free persons. Further studies are required to determine the significance of these observations with respect to the virus-tumor relationship.

Proposed Course: The investigation of the immune status of cancer and control populations with respect to the non-virion antigens induced by HSV-1 and HSV-2 will be expanded to better define the virus-tumor relationship.

Date Contract Initiated: April 9, 1971

Current Annual Level: $30,000

NINDS/NCI COLLABORATIVE RESEARCH PROJECT
EMORY UNIVERSITY (NIH-72-2301); MELOY LABORATORIES (NIH-72-2306)

Title: Collaborative Project on the Oncogenic Potential of Herpesvirus in Primates

Contractor's Project Directors: Dr. Andre Nahmius, Emory University
Dr. John Verna, Meloy Laboratories

Project Officers (NINDS): Dr. John Sever
Dr. William T. London

Project Officers (NCI): Dr. Gary R. Pearson
Dr. Robert A. Manaker

Objectives: To determine whether intra-vaginal infection of the Cebus monkey by herpes simplex virus type 2 (HSV-2) will induce neoplasia of the uterine cervix.

Major Findings: This collaborative project with the National Institute of Neurological Diseases and Stroke involves two contracts, providing animal care at Meloy Laboratories and laboratory monitoring at Emory University.

To date, 64 male and 138 female Cebus monkeys have been received, conditioned and paired. The Benefield strain of HSV-2 was inoculated into 26 females, and 8 females were inoculated with control materials. Ten of the 26 animals showed evidence of infection by virological or serological determinations following primary exposure. Clincial herpetic lesions were seen in 6 of these animals. The effect of hormonal levels during the menstrual cycle on the infection rate is under study.

Because differences probably exist in the oncogenic potential of HSV-2 strains, criteria were established for strain selection. These include isolation from women with cervical carcinoma, transformation of hamster cells *in vitro* or induction of sarcomas in hamsters *in vivo*, and the ability

to infect the genitalia of the Cebus monkey. On these bases, the Benefield strain of HSV-2 was selected for this project.

During three months of observation, one of the six animals with herpetic lesions showed a spontaneous recurrence of lesions. Antibody responses to HSV-2 antigens could be detected in the immunoglobulin G, A, and M serum fractions of the infected animals and may become important parameters if cervical carcinoma can be established in the Cebus monkeys.

Cytological data obtained in 185 female monkeys showed no evidence of cervical anaplasia, indicating thus far the lack of spontaneous cervical neoplasia in the Cebus monkey.

Significance to Biomedical Research and the Program of the Institute: This project utilizes an experimental lower primate to contribute data to establish a causal relationship between a herpesvirus infecting human genitalia and the induction of cervical carcinoma. The demonstration of an etiological relationship to carcinoma in the primate could provide basic information leading to control measures for humans.

Proposed Course: This project is expected to continue for at least three years following primary infection of animals.

Date Contract Initiated: March 1, 1972

Current Annual Level: $193,813

UNIVERSITY OF NORTH CAROLINA (NIH-72-3228)

Title: Molecular Studies on Herpes-type Viruses of Potential Oncogenicity

Contractor's Project Director: Dr. Joseph Pagano

Project Officers (NCI): Dr. Timothy O'Connor
Dr. Robert A. Manaker

Objectives: To define at the molecular level the virus-cell relationships for herpes simplex virus type-2 (HSV-2) and human cervical carcinoma and for Epstein-Barr virus (EBV) and Burkitt's lymphoma (BL) and nasopharyngeal carcinoma (NPC).

Major Findings: By cRNA-DNA hybridization, 21 established lines of human lymphocytes were shown to contain between 29 to 235 EBV genome equivalents per cell on the average. EBV DNA is found regardless of whether the cells display EBV viral capsid antigen. Twenty-three of 24 BL biopsy specimens contained readily detectable EBV DNA with genome equivalent amounts ranging from 3 to 113 per cell, on the average, with a mean value of 38 per cell.

Eighteen of 23 NPC tissue specimens from Kenya contained EBV DNA ranging in amounts from 5 to 85 genome equivalents per cell. Five specimens were

negative (<2 genome equivalents). The mean number of genome equivalents in the positive NPC specimens is distinctly lower than in BL, and 6 of 18 specimens contained less than 10 genome equivalents in contrast to only 2 of 23 BL biopsies with less than 10 genome equivalents. Nineteen of the 22 control tumors obtained from the same geographical region (Kenya) did not contain detectable EBV DNA by RNA-DNA hybridization. The three positive biopsies contained relatively small amounts of EBV DNA (6 to 12 genome equivalents). Two of the tumors were located in the nasopharynx; the site of the third lymphoma is unknown. None of the 11 American tumors so far tested by RNA-DNA hybridization have contained detectable EBV DNA. The tumors included Hodgkin's disease, melanoma and myelogenous leukemia.

Most of the cellular EBV DNA which is synthesized before superinfection of Raji cells with EBV and the small amount of cellular EBV DNA synthesized after infection is fragmented into 10-12S pieces. In alkaline gradients approximately unit length viral DNA can be separated from the majority of the Raji cell chromosomal DNA. This result suggests that the viral DNA is not covalently bonded to the cellular DNA unless there are nicks in the cellular DNA so that small pieces are covalently bonded to the viral DNA. Preliminary data suggest that the EBV DNA remains associated with the chromosomal DNA after extraction with detergents. However, if a proteolytic enzyme is added to the detergent extraction, then up to 50% of the viral DNA separates from the chromosomal DNA and sediments with a coefficient of approximately 55S.

In studies on the quantitation of virus particles by electron microscopy, comparison of the spray droplet technique with agar pseudoreplica counting of AMV confirmed the reliability of the pseduoreplica technique for assaying down to the level of 10^{11} virions per ml and lends confidence in results obtained with blood plasmas as low in virus as 10^{8} per ml, where only the pseudoreplica method can operate.

Significance to Biomedical Research and the Program of the Institute: The studies accomplished verify the close relationship between EB virus and cells in BL biopsies. The same consistency of association was not apparent in biopsy specimens from African NPC patients. The molecular methods applied provide an important tool in the study of viral associations with tumor cells where repression of virus genetic expression masks the presence of infection.

Proposed Course: The molecular aspects of the relationship between HSV-2 and cervical carcinoma will be emphasized.

Date Contract Initiated: April 25, 1972

Current Annual Level: $102,960

OREGON STATE UNIVERSITY (NIH-71-2175)

Title: Studies on the Replication and Function of Nucleic Acids Isolated from Oncogenic Viruses

Contractor's Project Director: Dr. George S. Beaudreau

Project Officers (NCI): Dr. Albert J. Dalton
Dr. Ursula Heine

Objectives: To study enzymatic and biochemical changes occurring during cellular transformation by oncogenic virus.

Major Findings: The avian virus MC 29, which transforms fibroblasts, was studied. The reverse transcriptase of this virus appeared in cells late in the cycle of virus replication. The sedimentation rate of the polymerase from cells was slightly greater (8.5 $\pm$ 0.2S) than the polymerase from the virion (7.7 $\pm$ 0.1S). The enzyme activity induced by virus infection was clearly different from that of DNA polymerases in uninfected cells. Similar viral polymerase activity was detected in fibroblasts infected with avian myeloblastosis virus (AMV).

The DNA associated with purified virus was retained in viral cores prepared from mature virus, providing strong evidence that this DNA is a component of the virus nucleoid. The priming activity of viral DNA for transcription of RNA by the reverse transcriptase was destroyed by nuclease S-1, which is specific for single strand DNA. This suggests that the priming activity of the viral DNA is dependent on a single-stranded region.

There was cross-hybridization between excess AMV RNA and the labeled DNA product synthesized from MC 29 RNA template. Annealing experiments showed not all DNA sequences copied from MC 29 viral RNA were complemntary to AMV RNA under conditions where complete hybridization was demonstrated with the labeled DNA product copied from AMV RNA.

Significance to Biomedical Research and the Program of the Institute: The observations made suggest that the MC 29 virus not only carries the nucleotide sequences of the AMV but also contains additional sequences probably associated with its fibroblast-transforming properties.

Proposed Course: This project terminated on June 27, 1973.

Date Contract Initiated: June 28, 1971

Current Contract Level: $34,440

PENNSYLVANIA STATE UNIVERSITY (NIH-70-2024)

Title: Studies on the Oncogenic Potential of Defective Human Viruses

Contractor's Project Director: Dr. Fred Rapp

Project Officers (NCI): Dr. Robert A. Manaker
Dr. Michael A. Chirigos

Objectives: To conduct a systematic study of the oncogenic potential of defective human viruses.

Major Findings: Herpes simplex viruses types 1 and 2 (HSV-1 and HSV-2) were exposed to UV-irradiation to induce defects preventing a lytic cycle of reproduction. The defective viruses were tested for transforming activity on hamster embryo fibroblasts in culture, and transformed cells were implanted into hamsters to determine their neoplastic nature. Seven of 15 type 2 strains and 2 of 12 type 1 strains induced morphological transformation of cells, but such transformed cells were not necessarily neoplastic. Most of the transformed cell lines carry HSV-specific antigens but none showed evidence of the gs-1 or gs-3 antigens of RNA tumor virus.

Hamster cells transformed by irradiated cytomegalovirus contain virus-specific antigens but their neoplastic nature has not been ascertained. Attempts to transform human cells were unsuccessful.

Forssman antigen present in the HSV-transformed cells renders them susceptible to the cytotoxic effect of hemolysin.

Significance to Biomedical Research and the Program of the Institute: This project was established to determine whether viruses producing common diseases in man might, under appropriate conditions, establish a chronic cellular infection culminating in neoplastic transformation. The results obtained with herpesviruses commonly afflicting humans suggest that these viruses have oncogenic properties that may be expressed in humans. The failure to find all strains of HSV-1 or HSV-2 to possess similar transforming properties in addition to information reported by other laboratories suggests that strain differences among these two herpesviruses do exist and must be considered in studies to determine their relationship to oncogenesis in man.

Proposed Course: Studies will continue to determine the nature of the relationship established between virus and the transformed cells.

Date Contract Initiated: October 27, 1969

Current Annual Level: $452,140

UNIVERSITY OF PENNSYLVANIA (PH43-65-1013)

Title: Research on Experimental and Natural Transmission of Bovine Leukemia

Contractor's Project Director: Dr. Robert Marshak

Project Officers (NCI): Dr. Michael A. Chirigos
Dr. Robert A. Manaker

Objectives: To investigate the possible viral etiology of bovine leukemia, attempt cell-free transmission of this disease, and investigate natural transmission through reciprocal foster nursing experiments.

Major Findings: The C-type virus associated with bovine leukemia was further characterized. The interspecies gs-3 antigen detected in the known leukemia viruses could not be demonstrated in the bovine virus or infected cells by repeated testing in several laboratories. The same precipitating antigen was present in ether-treated virus, in virus-producing cultured cells, and in phytohemagglutinin-treated buffy coat cell cultures from leukemic cattle. This antigen is analogous to the gs antigens of other viruses of the C-type in that it appears to be an internal, soluble, ether-resistant component of the bovine C-type virus.

Immunofluorescence tests against viral antigens in cultured cells showed the presence of reacting antibodies in most cattle with leukemia or persistent lymphocytosis in two multiple case herds. No antibodies were demonstrable in 20 cattle in a leukemia-free herd, less than 10 percent of animals in three other leukemia-free while 17 percent of animals in a fourth leukemia-free herd had precipitating antibodies and 33 percent had antibodies reacting by immunofluorescence with the bovine C-type virus antigens. The results show a wide distribution of the virus in cattle and a close association of the infection with leukemia or risk of leukemia.

Despite many attempts to increase the yield of virus from bovine cell cultures, yields are not consistent. Attempts to infect bovine monolayer cultures with the virus were not successful. The low yield of virus has made recovery and purification of particulates extremely difficult. Attempts are being made to demonstrate reverse transcriptase activity and 70S RNA in viral preparations.

Two of six chimpanzees fed from birth on raw milk from a virus-producing cow died at 35 and at 45 weeks of age respectively from Pneumocystic carinii pneumonia and erythroleukosis, two diseases not previously reported in chimpanzees. A heifer from a low-leukemia family that had been inoculated at birth with cells of a culture producing C-type particles died of generalized lymphosarcoma at two years of age. Even in high-incidence families, leukemia rarely occurs before five years of age.

Significance to Biomedical Research and the Program of the Institute: The studies conducted under this project extend the association of virus of the C-type with leukemia from the mouse, hamster, guinea pig, cat, rat, and monkey to include cattle. The virus is apparently highly repressed in bovine cells, and it appears to carry no antigen of the interspecies gs-3 type found in other animal tumor viruses studied. The broad distribution of RNA viruses of the leukemia-inducing type among different animal species make it highly unlikely that humans are free of similar infection.

Proposed Course: This project was terminated on April 15, 1973.

Date Contract Initiated: June 18, 1965

Current Annual Cost: $450,000

PUBLIC HEALTH RESEARCH INSTITUTE OF THE CITY OF NEW YORK, INC (NIH-71-2129)

Title: Evaluation of Methods for Isolation of Virus from Human Neoplasia

Contractor's Project Director: Dr. Hidesaburo Hanafusa

Project Officers (NCI): Dr. Charles W. Boone
Dr. Robert A. Manaker

Objectives: To apply methods used successfully to isolate viruses from covert infections in animals in an attempt to isolate viruses from human cancers and to characterize viruses so recovered.

Major Findings: Thirty-seven tumors, predominately sarcomas, and 18 bone marrows from acute leukemia patients were received and examined as cell cultures. Assay for transforming virus on feline or human embryo cells showed no evidence of the presence of a transforming virus. The effect of added helper viruses has not yet been studied. Examination for physical particles by electron microscopy, labeling with a radioactive precursor or assay for reverse transcriptase activity failed to demonstrate virus with the characteristics of the C-type viruses. Myxo- or paramyxo-like virus particles were observed in several preparations. Problems were encountered by the difficulty to obtain sufficient amounts of tumor cell outgrowth in cultures to permit extensive studies. The composition of media strongly affects cellular morphology. A stable line of osteosarcoma cells was established and is under study. Experience with this culture aided in establishing methods whereby seven other tumors are now growing well and the effects of exogenous viral infection can now be undertaken.

Significance to Biomedical Research and the Program of the Institute: Since virally-induced sarcomas occur in mice, rats, chickens, and some nonhuman primates, the human may be no exception. Methods developed in animal systems which permit recovery of RNA tumor virus from covert infections are being applied systematically to human sarcoma and leukemia tissues in attempts to demonstrate similar viral associations in the human neoplasma.

Proposed Course: The research effort will continue as planned using the culture techniques successfully applied to maintain neoplastic cells in continuous culture for intensive study.

Date Contract Initiated: April 27, 1971

Current Annual Level: $125,000

RUSH-PRESBYTERIAN-ST. LUKE'S MEDICAL CENTER (NIH-73-3219)

Title: Studies of Tumor Viruses in Small Primates

Contractor's Project Director: Dr. Friedrich Deinhardt

Project Officers (NCI): Dr. Robert A. Manaker
Dr. Michael A. Chirigos

Objectives: To study selected viruses and virus-induced neoplasia in marmosets.

Major Findings: The simian sarcoma virus (SSV-1) isolated from a Woolly monkey produced tumors in marmosets. An associated helper virus, SSAV-1, was found in SSV-1 preparations and was separated from the sarcoma virus. The RNA dependent DNA polymerase of the complex SSV-1/SSAV-1 was characterized and the base ratio of the RNA of the viruses was established. Purified major proteins of the viruses are in preparation for use in producing monospecific antisera.

The lymphoma-producing Herpesvirus saimiri (HVS) recovered from squirrel monkeys was tested in antibody-free squirrel monkeys. A carrier state accompanied by antibody production developed in infected animals without induction of any clinical or hematological disease. Infection was horizontally transmitted in squirrel monkeys but not in marmosets. In collaboration with Dr. George Klein and investigators at NCI, early and late antigens induced by HVS were recognized and the antibody distribution to these antigens with respect to disease was studied in owl monkeys, marmosets, and the squirrel monkeys.

Several marmoset and squirrel monkey lymphoblastoid lines of cells carrying and producing Epstein-Barr virus were established and their oncogenicity in autologous and homologous hosts is under study.

The Gibbon lymphoma virus (GLV) was infectious for a fetal marmoset lung cell line. These cells could be used to show infection by this virus by the XC cell mixed culture cytopathogenicity reaction wherein large syncytia were observed in 24 to 48 hours after XC cells were added to infected marmoset cells.

Significance to Biomedical Research and the Program of the Institute: Restrictions in the host range of viruses require that a primate animal be available for study of viruses isolated from human tumors. The marmoset has been proven to be responsive to the oncogenic activity of viruses of lower animals and of other primates. This project has contributed considerable information, not only on oncogenic RNA viruses, but has been contributing substantially to understanding of the role of herpesviruses in oncogenic processes.

Proposed Course: Emphasis will be placed on the RNA-and herpes-viruses associated with neoplasia in non-human primates and viruses recovered from human neoplasms as these become available.

Date Contract Initiated: March 15, 1962

Current Annual Level: $579,370

RUTGERS UNIVERISTY (NIH-71-2077)

Title: Studies on Genetic Acquisition of Oncogenic Potential and Cell-transforming Capacity by RNA Animal Viruses

Contractor's Project Director: Dr. Robert W. Simpson

Project Officers (NCI): Dr. Michael A. Chirigos
Dr. John W. Pearson

Objectives: To determine whether a non-oncogenic RNA animal virus can acquire tumor-producing or cell-transforming capability as a consequence of host-induced genetic modification of the viral RNA, by intracellular persistence of incomplete but functionally active viral genetic material, or by induced mutation.

Major Findings: Research was continued to determine whether the genomes or portions of the genomes of chemically-inactivated RNA animal viruses can enter and persist in cells with the expression of certain viral functions and whether such resident genomes might incorporate host genetic information and be activated or rescued by appropriate means. Approaches to inactivate viral reproductive capacity without abolishing all gene functions included treatment with special classics of alkylating agents, treatment with water soluble polyene antibiotics, and dye-mediated photodynamic inactivation followed by examination of the ability of treated virus to synthesize virus-specific proteins in guinea pig embryo or human HEp-2 cells. Vesicular stomatitis virus (VSV) inactivated by these procedures can still induce synthesis of virus specific proteins detected in cells by immunofluorescence microscopy with antisera against whole virions, but the appearance of these proteins is transient. Inactivation of virus by the alkylating agent, Trenimon, and the polyene antibiotic, amphoteracin B methyl ester, appears to involve partial inhibition of the virion polymerase. Treatment with a variety of virus-inducing agents has not activated virus genomes in cells originally infected with chemically-modified virus.

Certain conditional lethal mutants of VSV persist in cells under restrictive conditions, producing serologically-reactive proteins, while other mutants give only "silent infections". Attempts are being made to activate these infections after many cell generations.

The MSC tumor produces both mouse sarcoma and leukemia virus particles. An attempt is being made to determine whether superinfection by genetically distinct, nononcogenic viruses can result in formation of hybrids with cell-transforming and oncogenic capacity. A temperature sensitive mutant of VSV caused strong cytopathology in MSC cells incubated at the restrictive temperature. Efforts are being made to determine whether ts-positive VSV revertants or a ts-positive hybrid virus with traits of the mouse virus and VSV is responsible.

Significance to Biomedical Research and the Program of the Institute: Comparatively little is known of viral interactions or the long-term effects of genetically-modified viruses with respect to oncogenic processes. This project was initiated to acquire information in this area.

Proposed Course: This project will terminate on August 13, 1973.

Date Contract Initiated: February 15, 1971

Current Annual Level: $99,000

ST. JUDE'S HOSPITAL (NIH-71-2134)

Title: Studies on the Etiology of Selected Amphibian Tumors

Contractor's Project Director: Dr. Allan Granoff

Project Officers (NCI): Dr. Gary R. Pearson
Dr. Wilna Woods

Objectives: To determine the role of viruses, particularly herpes-type virus, in the etiology of renal carcinoma of Rana pipiens (Lucke' tumor).

Major Findings: Efforts to propagate the Lucke' herpesvirus (LHV) in cell culture were not successful, although LHV replication in virus-free tumor fragments was induced by low temperature, confirming a previously published report. The tumor inducing agent is ether sensitive. Inhibition of tumor induction by normal rabbit serum is complicating interpretation of virus neutralization tests.

Antilymphocyte sera are being tested and may be useful in establishing a transplantable Lucke' tumor.

Significance to Biomedical Research and the Program of the Institute: Herpesviruses have been associated with the induction of lymphoproliferative diseases in animals and man. The Lucke' virus differs in its association with a carcinoma. It is not known whether herpesviruses are sole factors in inducing certain tumors or whether they act as co-factors to activate oncogenic expression by other agents. This project was instituted to contribute data for more intensive analysis of the role of herpes viruses in neoplastic transformation.

Proposed Course: This project terminated on May 12, 1973, due to difficulties in acquiring sufficient viral and tumor material for broader study.

Date Contract Initiated: May 13, 1971

Current Annual Level: $60,900

UNIVERSITY OF TEXAS (PH43-65-604)

Title: Studies on the Relationship of Viruses to Neoplasia

Contractor's Project Director: Dr. Leon Dmochowski

Project Officers (NCI): Dr. Gary R. Pearson
Dr. Robert A. Manaker

Objectives: To pursue a systematic study of selected human patients with neoplastic disease to establish the association of viruses with their cancers.

Major Findings: Cells from human osteosarcoma tissue and human leukemia bone marrow were co-cultivated. Cell-free fluids from these co-cultures, transferred to normal whole human embryo cell cultures, induced small focal areas of altered cell morphology. Cultures of cells cloned from foci of transformation were fixed and used for immunofluorescence tests to determine the presence of antibodies in selected human sera reactive with cellular antigens. Sera from 17 of 32 patients with osteosarcoma, 11 of 21 patients with leukemia, and 2 of 41 apparently normal donors reacted with a cytoplasmic antigen contained in these cells. Suggestive evidence for the presence of reverse transcriptase activity and heavy RNA was obtained by tests made on particulates, released in these cultures, which banded in gradients at a density of 1.19 to 1.21 gms per ml.

Studies on the ESP-1 virus showed the presence of a murine gs-2 antigen and the interspecies gs-3 antigen in purified virions. Extracts of cells of the culture carrying this virus contained the murine gs-1 antigen.

A nucleolar antigen, previously detected in the tumor cells of advanced melanoma cases by immunofluorescence tests with patients' sera, apparently is a protein-RNA complex. Similar nucleolar antigen was detected in the cells of other types of tumor. These nucleolar antigens are not found consistently; their presence appears to reflect the stage of disease.

The sera from mice with spontaneous mammary tumors were positive by immunofluorescence tests for antibodies reacting with antigens in mouse mammary tumor cells. Some breast cancer patient sera also reacted in similar tests. Antibodies to intracellular antigens were of the heterophile type, some were tumor specific, and others virus specific, while antibodies reacting with cell surface antigens were only of the heterophile type.

Biochemical studies on murine sarcoma virus showed that a structural rearrangement occurred within released virions. After a two hour labeling period, 43S RNA was observed while after 24 hours labeling, a 53S RNA was detected.

A technique was devised to quantitate virus electron microscopically. Particles are sedimented on membranes and counted directly after thin sectioning.

Significance to Biomedical Research and the Program of the Institute: Current program requires intensive, systematic investigations on selected human neoplasms to detect viral associations with the disease process.

Proposed Course: Greater emphasis will be given to the search for viral expression and to attempts to recover viruses from human neoplasms.

Date Contract Initiated: March 19, 1965

Current Funding Level: $579,580

SUMMARY REPORT

IMMUNOLOGY-EPIDEMIOLOGY SEGMENT
July 1, 1972 - June 30, 1973

During this fiscal year, the Immunology-Epidemiology Segment continued to develop programs with four major goals: 1) the immunological detection of oncogenic viruses; 2) the detection of tumor-specific and virus-related antigens and immune reactivity to these antigens in humans and animals; 3) studies on immunogenetics and immuno-epidemiology relevant to the etiology of cancer and 4) stimulation of anti-viral and anti-tumor immunity.

In addition to the twelve contracts that were in operation throughout most of FY'72, three contracts (with Makerere University, the University of Washington, and the Atomic Energy Commission) were transferred to the Immunology-Epidemiology Segment and four new contracts (George Washington University, New York Medical College, Scripps Clinic, and University of Miami) were initiated.

The objectives of the first category, the immunological detection of oncogenic viruses, are : 1) to develop sensitive and specific immunologic assays for the detection of antigens and immune reactivity associated with potential oncogenic viruses; 2) to apply useful immunological assays to well controlled clinical studies relevant to the etiology and pathogenesis of cancer; and 3) to develop suitable model systems relevant to the control of probable virus-induced tumors in man. Five I-E Segment contracts emphasizing one or more of these aspects were: Children's Hospital, Univ. of Pennsylvania; Scripps Clinic and Research Foundation; University of Miami; the University of Texas, M.D. Anderson (72-3262), and TRW.

At TRW, studies were initiated to purify distinct antigenic components of the Epstein-Barr virus (EBV) while at the Children's Hospital in Philadelphia, the importance of antibodies to the EBV induced early antigens (EA) were emphasized by serological studies in patients with Burkitt's lymphoma (BL) and nasopharyngeal carcinoma (NPC). The antibody to the restricted or "R" early antigen was prominent in patients with BL in relapse and in some cases appeared to be predictive of relapse. Antibody to the diffuse or "D" early antigen was more commonly found in patients with NPC in advanced stages of disease rather than patients with Stage I NPC. This laboratory also continued to develop new methodology in the EBV field by initiating a neutralization test which is applicable to large scale screening and an in vitro method of measuring cell-mediated immunity using a soft-agar technique. The importance of serology to monitoring the treatment of patients with Burkitt's lymphoma and NPC was therefore emphasized and indicates the possible development of a useful clinical test for monitoring therapy of human cancer patients. The development of new tests to follow the immune response to EBV provides additional parameters to demonstrate the significance of this potentially oncogenic virus in human cancer. Animal studies using Gibbons demonstrated that biologically active EBV can produce tonsillitis in an experimental animal system thus

providing the first in vivo test system to study the pathogenesis of EBV induced diseases.

The other three contracts in this area were involved in developmental studies on the immunology of RNA viruses. The newly initiated contract at the Scripps Clinic focused on the development of techniques to demonstrate the specificity of immune complexes associated with RNA viruses in the kidneys of leukemic mice with the goal of using similar techniques to isolate antigens of oncogenic RNA viruses from human leukemic kidneys. A second contract initiated this year by the I-E Segment, a study at the University of Miami evaluating virus and tumor associated immunity in the mouse mammary tumor system as a model for human breast cancer, developed a lymphocyte stimulation assay which appeared to detect immunity to tumor associated antigens in mice with breast cancer. Animals with early disease had higher immune reactions than those with advanced disease, of particular interest since these results parallel the findings by an I-E Segment contract studying immunity to tumor-associated antigens in breast cancer patients at New York Medical College (see below). The findings on these animal projects are highly relevant to the counterpart studied in humans in that their success will enhance the possible detection of oncogenic viruses in humans and lead to better control of the disease. One study that is providing important reagents for characterization of the immune response to possible oncogenic human RNA viruses is a project at the University of Texas, immunizing cancer patients with Rauscher Leukemia Virus (see details below).

Since animals model systems indicate that virus-induced tumors tend to have common antigens whereas chemically-induced tumors tend to have distinct antigens in each tumor, the detection of common tumor specific antigens in human cancer is a major area of effort within the I-E Segment. At the present time, the thrust of this portion of the Segment effort is: 1) to detect tumor specific antigens in patients with tumors of suspected viral etiology; 2) to relate the tumor specific or tumor associated antigens to virus associated antigens relevant to these tumors, and 3) to study the immune response to tumor and virus associated antigens with the purpose of determining the relevant methods of controlling the disease. Six I-E Segment contracts working on human cancer are involved in this effort and four contracts are pursuing relevant studies in animals. (Although the segment plans to pursue leads in any form of human cancer where epidemiological and laboratory evidence provides strong evidence for a viral etiology, the major focus at the present time is on human lymphoma, leukemia, breast cancer and sarcoma because of the opportunities to compare the human studies with well defined animal systems of virus induced neoplasia). Three segment contracts (New York Medical College, George Washington University, and Robert Bent Brigham Hospital) focused on applying different methods to the identification of common antigens in breast cancer with significant results. At New York Medical College, studies on breast cancer patients indicated 1) that common antigens were most likely to be found in breast lesions that were in a very early stage (in-situ carcinoma) and 2) that the more advanced the lesion the more likely there was to be

antigenic diversity as measured by immune reactivity of autologous cells against breast cancer biopsies. These studies indicate that it is the biopsies of the early cases that are most likely to reveal viral antigens and the contractor thus can select those biopsies most relevant for virological, biochemical, and immunological detection of oncogenic viruses. In addition, the contractor demonstrated that immune response to these antigens was strongest in patients with early disease and diminished in patients with advanced disease. Thus the use of two immunological tests, the Rebuck skin window and the leukocyte migration assay, were utilized in conjunction with pathology to demonstrate leads relevant to the etiology and prognosis of human breast cancer. In its first year of operation, a contract at George Washington University obtained additional evidence for common tumor-associated antigens in breast cancer patients when membrane preparations of tissue biopsies from breast cancer patients caused delayed hypersensitivity in breast cancer patients but not in colon cancer patients; the converse was also found, with colon cancer patients only reacting against antigens prepared from colon cancers but not breast tumors. Characterization of the specific antigenic components will lead to clinical studies demonstrating which antigens are relevant to the etiology of the disease and which antigens can be used in better control of the disease. Investigators at the Robert Bent Brigham Hospital better defined the conditions under which the migration inhibition assay could be used, including the further development of a direct migration assay rather than the indirect assay using guinea pig macrophages.

Evidence for tumor specific antigens in a cell line derived from sarcoma patients was demonstrated in a second University of Texas contract dealing with an in vitro lymphocyte cytotoxicity test. Lymphocytes from car patients attacked carcinoma cell lines but not sarcoma cell lines, while lymphocytes from sarcoma patients only attacked sarcoma cell lines. Depletion of blocking antibodies by chemotherapy was not associated with an improvement in the clinical course, and there are only partial correlations between the in vitro tests and the prognosis of the patients. For the most part, however, the correlation between in vitro immunity and survival was a good one, thus providing the segment with an assay usable for the detection of virus induced surface antigens relevant to etiology and for monitoring the effect of therapy on the outcome of the disease. The reactivity of certain normal individuals to selected target cell lines apparently unassociated with HL-A type differences has provided impetus for expanding the epidemiological aspects of the contract to look at the possible role of environmental factors such as viruses in causing these immune responses. Further development of similar tests have continued at the University of Minnesota where tumor immunity was shown to correlate with the stage of disease in cancer patients and where experimental studies demonstrated that anti-plasma cell serum could be an effective weapon by abrogating the production of blocking antibodies.

In the leukemia system, immunity against leukemia associated antigens was demonstrated by a variety of techniques at Johns Hopkins University. A particularly intriguing finding was the presence of antibody cytotoxic for leukemic cells in untransfused males as well as other individuals, suggesting

the possibility that some individuals may have immunity against an oncogenic agent. This cytotoxic antibody is now being closely investigated for its relevance to etiology, virus detection, immunodiagnosis, and immunotherapy.

The meaning of these common antigens is being investigated in depth in an animal system at the Atomic Energy Commission and its subcontract at the University of Tennessee where workers successfully demonstrated that oncodnavirus tumors of syngeneic hamsters contained fetal embryonic or phase specific antigen in the plasma membrane. Soluble antigen which mimics the membrane bound antigen has been isolated and characterized. Masking antibody associated with multiple pregnancy was described which augments the antigenicity of fetal antigen. Conditions for achieving fetal immunization against tumors in hamsters, rats and guinea pigs were described. The other three contracts evaluating the role of immunity to tumor and virus associated antigens in animal systems (University of Miami - MTV and breast cancer; University of Minnesota - MSV and sarcoma; and Roswell Park Memorial Institute - Gross virus and leukemia) are described elsewhere in this report.

Three contracts (International Agency for Research on Cancer, National Center for Disease Control, and University of California, Los Angeles) worked in the areas of immunogenetics and immunoepidemiology. The objective of this aspect is to apply specific immunological tests to controlled studies in order to determine whether immune reactivity against a tumor associated antigen follows an epidemiologic pattern suggesting a viral etiology for the tumor. In addition, immunologic tests can be used to evaluate susceptibility to cancer. The International Agency for Research on Cancer provided laboratory evidence for a genetic contribution to the etiology of nasopharyngeal cancer by demonstrating that NPC patients lacked two HL-A antigens on their leukocytes in comparison to normal controls and patients with other head and neck tumors. Seroepidemiological studies performed by the contract showed clear differences in incidence and titer of EBV antibody by geographic location and by ethnic group, providing baseline information of great importance to determining the role of EBV in BL, NPC, and other EBV-associated tumors. The prospective study in Africa passed the half way mark in its plan to collect sera from 30,000 children at high risk of developing Burkitt's lymphoma. In a companion study with Makerere University investigating other factors associated with the etiology of BL, there was evidence that sickle cell disease protected against Burkitt's tumor, which is in line with the expected data since it is presumed that malaria, which is far less virulent in patients with sickle cell disease, is an important co-factor in the etiology of Burkitt's lymphoma.

Studies on Burkitt's lymphoma in the United States were assisted by the National Center for Disease Control which, in its interagency agreement with the I-E Segment, studied two clusters of Burkitt's lymphoma in the United States. Identification and analysis of clustering in a number of lymphoproliferative diseases indicated that time-space clustering of childhood leukemia could be demonstrated using two different

statistical analyses of data obtained in Houston and Atlanta.

At the University of California in Los Angeles, an important challenge to the concept of tumor specificity resulted from the findings on non-HLA cell mediated cytotoxocity found in the analysis of more than 5,000 assays. Lymphocytes from normal individuals were active as often as those of the specific cancer patients against the target cell lines, and there was great cross-reactivity among different types of cancer patients. These results, supporting those of the University of Texas contract (71-2178),will provide a basis for launching an immuno-epidemiologic study on the possible role of viruses in stimulating the immune response as well as leading to more fundamental work on the nature of the antigens being detected. In another large study, the linkage of HL-A type to cancer susceptibility was shown in studies of multiple case cancer families revealing a laboratory handle on the genetic association with cancer not apparent in a study of random cancer cases.

In the fourth area of research effort within the segment, that of evaluating methods of stimulating anti-viral and anti-tumor immunity, studies at the University of Texas indicate that it is possible to immunize patients with an oncogenic RNA virus (Rauscher leukemia virus). Evidence for humoral and/or cell-mediated immunity against RLV was found in more than half of the immunized patients and investigators were able to utilize the sera to improve several assays testing for RLV antibodies. A cure for spontaneous AKR leukemia was indicated by studies at Roswell Park Memorial Institute where investigators concentrated on defining the best conditions for chemoimmunotherapy and related in vitro studies of immunity to the in vivo finding of increased survival . Neuraminidase-treated leukemic cells were also found to be far more effective immunogens when combined with live BCG and MER. Also at Roswell Park Memorial Institute, a contract that has successfully demonstrated effective immunotherapeutic methods for skin cancer has successfully applied these techniques to some patients with breast cancer and a patient with hemangiosarcoma. Prevention of skin tumors in patients with xeroderma pigmentosum were also shown to occur with application of skin sensitizers. Progress in definition of tumor-specific antigens in dogs developed at the University of Washington, Seattle, has led to important models for human cancer. This contractor has also found that certain forms of therapy result in evidence of viral activity in the plasma of lymphomatous dogs. Studies are now under way to investigate biochemically and immunologically the possible role of these particles in the etiology of canine tumor. These studies on the etiology and control of lymphosarcoma and other tumors in dogs appears to be providing important leads relevant to their human tumor counterparts.

Other activities of the segment: Preceedings of a workshop held by the Immunology-Epidemiology Segment on the use of in vitro assays and in vivo assays relevant to cell-mediated immunity in humans was published as a National Cancer Institute Monograph. Two subsequent SVCP workshops, one on the application of laboratory techniques to epidemiological studies of breast cancer and lymphoma in humans and an international workshop on

human tumors associated with Herpesviruses, resulted from the efforts of the Immunology-Epidemiology Segment.

Dr. Paul H. Levine, Chairman, VLLB, VO, DCCP
Dr. Ronald B. Herberman, Vice-Chairman, LCBGY, DCCP
Dr. Gary R. Pearson, Executive Secretary, VBB, DCCP

UNIVERSITY OF CALIFORNIA, LOS ANGELES (NIH 72-2008)

Title: Studies on the Interrelationship of Viruses, Genetics and Immunity in the Etiology of Human Cancer

Contractor's Project Director: Dr. Paul Terasaki

Project Officer (NCI): Dr. Ernest Plata

Objective: The overall objective is to determine whether virus-associated antigens can be detected on tumor cells by immunological tests and whether HL-A antigens can act as genetic markers for linkage to tumor susceptibility.

Major Findings: The most important accomplishment of this past year has been the re-examination of an important basic concept regarding specificity of tumor immunity. According to the theory advocated by the Hellstroms and others, tumors at each site have specific cancer antigens against which lymphocytes from cancer patients react. The cell-mediated immunity is, however, blocked by antibodies in these patients. Three critical observations have been made: (a) Lymphocytes from normal persons are often as active as cells from specific cancer patients in producing cell-mediated cytotoxicity (a report of this finding has been submitted for publication). (1,126 tests); (b) Lymphocytes from other cancer patients are also often as cytotoxic as are lymphocytes from patients with the same cancer type as the targets. (4,454 tests); and (c) Blocking of target cell reduction can be demonstrated by conditions which do not involve antibodies from cancer patients.

From these findings then, it appears that tumors which originate at each site do not have a site-specific tumor antigen, or at least not on the basis of evidence from cell-mediated immunity (CMI) tests in vitro. If cell-mediated killing of tumor cells occurs because of a tumor virus on the cells, the virus antigen is not specific to each tumor type. Healthy normal persons often have immunity to these antigens.

In a large study, HL-A antigens have been shown not to have a genetic linkage disequilibrium with many cancer types (Cancer Research, in press, April, 1973). However, by studies of cancer families, a marked degree of linkage was found. It is possible that genes controlling susceptibility to cancer are linked with HL-A as shown by family studies, though not evident by the population survey.

Antigens shown by direct antibody reactions with cytotoxic plating inhibi-

tion tests have been further characterized. Specificities which are detectable are being identified. (22,699 tests)

Significance to Biomedical Research and the Program of the Institute: This contract allows the application of findings in tumor immunology and genetics to large scale populations studies. As evidence for tumor-specific antigens develops, population studies allow the identification of diseases which give an epidemiological pattern suggestive of an environmental agent. By determining the immune status of unaffected individuals and family members of cancer patients to tumor-specific antigens, it is possible to utilize epidemiology to select tumors which have the greatest (and poorest) chances of being caused by a virus. Studies on HL-A antigens are particularly significant because of the ability to separate diseases which have a genetic predisposition from those which do not. The identification of people who are at high risk of developing cancer will allow specific actions to be taken in regard to diagnosis, prevention and treatment on a more manageable level.

Proposed Course: During the next fiscal year, the major effort will be to determine whether viral tumor antigens can be detected on tumor cells by cell-mediated and humoral antibody tests. This will be done by narrowing the scope to fewer human tumor cell systems which are believed to be most likely associated with viruses, i.e. cervical carcinoma, Hodgkin's disease, sarcoma, and breast cancer. The HL-A frequency in Hodgkin's disease and lymphomas will be studied in depth since some aberration is undoubtedly present. Close collaboration with other SVCP contractors will allow careful evaluation of distinct population of lymphoma patients and controls.

Date Contract Initiated: July 12, 1971

Current Funding Level: $273,640

CENTER FOR DISEASE CONTROL (VCL-42)

Title: Etiologic Studies of Leukemia and Related Diseases Occurring in Unusual Epidemiologic or Genetic Situations

Contractor's Project Director: Dr. Clark W. Heath, Jr.

Project Officer (NCI): Dr. Adi F. Gazdar

Objectives: (1) To perform epidemiologic and virologic studies of leukemia and related illnesses (cancer, congenital defects) occurring in unusual epidemiologic or genetic circumstances. (2) To maintain leukemia case surveillance programs in selected population areas in the United States.

Major Findings: Out of approximately 40 cases of malignancy brought to the attention of the CDC, 21 cases associated with unusual circumstances were selected for epidemiological study. The studied cases consisted of 11 community clusters, 8 multiple case families, and 2 human-animal case associations.

A series of analyses have been conducted using different statistical approaches on data concerning acute leukemia in the metropolitan Atlanta and Houston areas and on data concerning Hodgkin's disease in Atlanta. Analyses on acute leukemia have shown evidence of time-space clustering in childhood disease in both Atlanta and Houston, using either the Knox-census tract approach or the Chi-square approach. Partial analysis of data concerning childhood leukemia in Houston shows the same pattern.

The idea that prenatal influenza may be a risk factor for later childhood leukemia/lymphoma has been tested on data from Atlanta and Houston. A significant association could not be demonstrated.

Detailed plans for a large followup study of infectious mononucleosis in relation to later development of lymphoid malignancy have been formulated. A total of 7,000 cases and 7,000 age-matched controls will be identified from college and alumni files at selected college health services. Followup data will be sought through questionnaires.

A total of 260 serum specimens were collected in connection with various field investigations described above. An inventory of all serum specimens collected since the start of the contract was carried out, so that record keeping of the total collection could be maintained at the central NCI computer facilities.

Several cell cultures, mainly from leukemia cases, have been established and characterized for the presence of adventitious viruses.

Serum specimens and culture materials have been supplied to 3 NCI investigators and 10 SVCP investigators outside NIH.

Significance to Biomedical Research and the Program of the Institute: This contract deals with epidemiologic and genetic studies on cases of human leukemia and attempts to ascertain and clarify the etiology of human leukemia through intensive investigations of selected cases. Since clustering and genetic defects are associated with the easier detection of virus in animal systems, this contract provides specimens of particular importance to SVCP investigators because of the greater chance of identifying a human tumor virus.

Proposed Course: Two areas of ongoing work will be continued: (1) identification, field investigations and appropriate laboratory work of leukemia, lymphoma and related diseases occurring in unusual epidemiological or genetic circumstances, and (2) statistical assessment of clustering tendencies of lymphoid malignancies and related disorders in selected populations. The study of the relationship between infectious mononucleosis and lymphoma will be initiated as outlined above.

Date Contract Initiated: July 1, 1967

Current Funding Level: $101,640

TRW SYSTEMS GROUP (NIH NO1-CP-3-3252 formerly NIH 70-2200)

Title: Viral Antigens and Anti-Viral Antibodies

Contractor's Project Director: Dr. Norman Weliky

Project Officer (NCI): Dr. Vincent Hollis, Jr.

Objectives: To isolate those antigens from EBV infected or transformed cells which react with human sera found to be positive for Epstein-Barr virus by fluorescent antibody test.

Major Findings: Current objectives are to isolate antigens from EBV infected cells which react with EBV-FA positive human sera, and use these to produce specific antisera. Because of low antigen and antisera titers, CF positive antigens are the first target. Sources of antigen are homogenates of EBV infected P3J HR-1 and Nk-Ly-28 cells. The homogenate can be fractionated in 7% polyacrylamide gel. Before proceeding, they have determined the CF stability of the antigen and homogenate to various conditions required for further separations. The antigen can be left at room temperature for several hours and loses little activity overnight. At 37° activity is lost in 30 min. The antigen is relatively unstable to sonication. Dialysis vs. saline inactivates the antigen but the antigen is stable to mild acid and base in buffers. Part of the cell homogenate precipitates on exposure to acid. A number of reagents which dissociate proteins and other aggregates do not irreversibly inactivate the antigen. A human serum immunoadsorbent has been prepared for isolating the antigen. The antigen they are working with is not the same as the antigen

responsible for cytotoxicity of tumor cells in the laboratories of Drs. P. Terasaki and M. Takasugi.

Significance to Biomedical Research and the Program of the Institute: EBV continues to be the one documented human virus that shows evidence of having both oncogenic properties in vitro and an association with human tumors. Most of the evidence suggesting an association between EBV and certain human malignancies has come from sero-epidemiological studies using a complex of EBV related antigens. Recent evidence suggests that antibodies directed against at least one of these complexes (EA) correlated well with the clinical stage of disease. The studies on EBV, however, have been hampered by the lack of mono-specific reagents for the herpesviruses similar to those available for investigation of C-type virus particles. Reagents of this type will be invaluable to other members of the SVCP investigating the role of herpesviruses in the etiology of human tumors. Specific reagents are particularly needed for sero-epidemiological investigations attempting to identify disease-related EBV antigens. They are also important to investigators attempting to identify common antigens between herpesviruses associated with neoplastic conditions in humans and animals. This contract is the sole contract in the SVCP attempting to fulfill this need.

Proposed Course: They expect to proceed with their attempts to isolate CF positive antigen with immunoadsorbents. When this is accomplished they expect to examine its purity, homogeneity, number of components, characterize the antigen, and compare antigens isolated with different human sera and cell preparations. They hope at that time to be able to obtain some high titer Burkitt's lymphoma and/or nasopharyngeal cancer serum and compare antigens isolated qualitatively and quantitatively with antigens isolated with "normal" sera and sera from IM patients.

Date Contract Initiated: June 15, 1970

Current Funding Level: $118,812

NEW YORK MEDICAL COLLEGE (NIH 72-3289)

Title: Immunologic Measurements as a Guide to Behavior and Etiology of Breast Cancer

Contractor's Project Director: Dr. Maurice M. Black

Project Officer (NCI): Dr. Clarice E. Gaylord

Objectives: To measure the cellular immunologic response of breast cancer patients to autologous and homologous breast tissue by means of the Rebuck skin window and migration inhibition assays; to compare the in vitro data with the patient's pathological evidence of immune reactivity and the clinical and biological behavior of the disease; to use cell-mediated

immunity assays to evaluate susceptibility to breast cancer and to detect specimens most likely to harbor human oncogenic viruses.

Major Findings: In 40% of the breast cancer patients studied, Rebuck skin window responses correlated directly with sinus histiocytosis (SH) in axillary lymph nodes and inversely with the clinical stage of the disease at the time of mastectomy. The per cent positive response decreased progressively with the progression of the disease. The relationship between SH and skin window responses as well as between stage and skin window was maintained 1-2 years postoperatively. Although the migration indices of breast cancer patients against autologous tissue did not correlate directly with skin window or SH responses, a positive test ($<.75$) did inversely correlate with the progressive stages of the disease, viz. maximal cellular response in Stage 0 (in situ carcinoma) and minimal response in Stage 2 (invasive carcinoma). In the migration inhibition studies with homologous breast tissue, a low level of cross reactivity was found against invasive breast cancer tissue (9%) and a high level (50%) was seen when in situ cancer tissue was used as the antigenic source.

Significance to Biomedical Research and the Program of the Institute: Since the findings at this laboratory strongly suggest that the Rebuck skin window and migration inhibition procedures provide biologically significant indices of maximal tumor-host interaction in the initial stages of the disease, both techniques may be useful tools in the possible early detection of breast cancer in women.

Proposed Course: In the next contract year the contractor proposes several courses: (1) to test cancer free women in terms of their in vitro cellular response to in situ and invasive breast cancer tissue and to determine whether there is any correlation between hypersensitivity responses and women grouped according to risk factors such as ethnic groups, parity, family history of breast cancer and endocrine status; (2) to serve as a central pathological reference service for grading of breast lesions and structural lymph node responses in I-E Segment international studies; (3) to supply breast cancer tissue and serum for immunological, virological and biochemical analysis by other SVCP laboratories.

Date Contract Initiated: June 26, 1972

Current Funding Level: $86,003

M.D. ANDERSON HOSPITAL AND TUMOR INSTITUTE (NIH-71-2178)

Title: Immunity to Sarcoma Related Antigens in Patients and Controls

Contractor's Project Director: Dr. Joseph Sinkovics

Project Officer (NCI): Dr. Paul Levine

Objectives: (1) To develop methods for detecting tumor and virus associated antigens in human sarcoma using a series of sarcoma cell lines. (2) To determine whether tests of immunity to these sarcoma associated antigens can be used to identify a virus related to the etiology of the disease. (3) To determine whether assays for the immune response to sarcoma associated antigens can assist in the control of the disease in patients suffering from soft-tissue sarcomas.

Major Findings: In the past year, lymphocytes from 150 blood samples taken from sarcoma patients and 38 samples from carcinoma patients were tested against the sarcoma and carcinoma cell lines. Ninety percent of the lymphocytes from sarcoma patients reacted against the sarcoma cell lines but not against the carcinoma cells, and none of the lymphocyte preparations from carcinoma patients reacted with the sarcoma cell line. Measurements of blocking antibody were also made and an attempt was made to suppress the blocking antibody by the use of cytosine arabinoside. Studies of cancer patients and normal controls indicate that the assay is able to detect sarcoma specific antigens.

Significance to Biomedical Research and the Program of the Institute: Virological, immunological and experimental animal studies suggest that human sarcomas are caused by an RNA virus. Other investigators have noted C type particles in a cell line derived from a sarcoma patient and a higher frequency of sarcoma related humoral antibody is found in family members of sarcoma patients as compared to random normal controls. The identification of tumor specific antigens in sarcoma cell lines would provide evidence for the presence of a sarcoma virus within the cell line. In addition, it would be possible to do immuno-epidemiological studies to determine whether there is cell-mediated immunity against a possible sarcoma virus and whether there is an assay which can be applied to clinical studies attempting to control the disease in sarcoma patients. In addition to studies on the etiology of sarcoma being performed by this contractor, the cell lines derived as a by-product of this investigator's research provide an important resource to other cancer research projects within the Special Virus Cancer Program.

Proposed Course: The investigator will expand the number of normal individuals tested in the assay to provide further assurance of tumor specificity. In addition, he will exchange tissue culture target cells and human lymphocytes with other SVCP investigators working on similar assays to determine if these in vitro assays of cell-mediated immunity are measuring the same antigen. Other tests, such as skin testing, will be utilized in parallel with the in vitro cell-mediated immunity tests to determine which are the assays most relevant to control of the disease.

Date Contract Initiated: June 29, 1971.

Current Funding Level: $106,000

ROBERT B. BRIGHAM HOSPITAL (NIH 71-2172)

Title: Cell Mediated Tumor Antigens as Measured by Macrophage Migration Inhibition

Contractor's Project Director: Dr. W.H. Churchill and Dr. John David

Project Officer (NCI): Dr. Gary Pearson

Objectives: To search for and characterize tumor-specific antigens in human tumors using the macrophage migration inhibition assays.

Major Findings: Initial investigations over the past contract year concentrated on attempts to demonstrate cellular immunity to breast cancer antigens using the indirect macrophage migration inhibition assay. Autologous and allogeneic tumor cell suspensions served as the sources of antigen in these experiments. In the past year, they initiated indirect MIF assays in 13 patients with breast cancer and obtained results that could be evaluated in 9 of these patients. The migration indices from breast cancer patients was in general lower than the controls. However, the degree of overlap between the patient and control groups was too great and the magnitude of differences detected in individual experiments was not sufficiently large to indicate that this assay could successfully be used to monitor cellular immunity in individual patients. This assay was therefore discontinued and replaced by the direct leukocyte migration assay. Saline or KCL extracts of allogeneic and autologous breast cancer were used as antigen in the direct assay. Positive reactions were noted in 2/9 patients. No differences were observed in reactions elicited by saline or KCL extracts. Preliminary data suggested that human peripheral leukocytes might well be a better indicator for human MIF than guinea pig macrophages. Experiments continued on the mechanisms of cellular cytotoxicity in vitro utilizing the guinea pig hepatoma system. Results were obtained that demonstrated that cell free supernatants containing lymphocyte mediators were cytotoxic. Also, cytotoxic activity of splenic lymphocytes or purified peritoneal exudate lymphocytes could be augmented up to 5X by the addition of non-immune peritoneal exudate cells. Evidence was also presented that activated macrophages play an important part in the effector mechanism of tumor cell kill. Specific accumulation of basophils at the site of tumor rejection was demonstrated by light microscopy. Electron microscopy of line 1 tumor rejection in peritoneal cavities of specifically sensitized guinea pigs demonstrated aggregates of "activated" macrophages, lymphocytes, basophils and damaged or dead tumor cells. These observations support the view that activated macrophages are present at the site of tumor rejection in vivo.

Significance to Biomedical Research and the Program of the Institute: In vitro assays for the degree of cellular immunity in human cancer patients are urgently needed so that (1) anti-tumor immune responses may be followed during the course of the disease and (2) large numbers of human tumors can be screened for tumor distinctive antigens. Successful application of the MIF, blocking antibody, and cell cytotoxicity tests

in this laboratory has shown that in vitro assays of the guinea pig system can be useful in correlating cytotoxic and morphologic parameters in human studies. Extracts made by methods that were useful in human skin testing also work in KCL-macrophage migration test in guinea pigs. It is expected that improvements of the MIF assay and other tests with the same extracts will improve comparative studies in human and animal tumors. Better defined cross-reacting tumor antigens will provide leads for the morphological type of tumor that is most likely to have a virus causation.

Proposed Course: (1) To continue evaluating the direct migration assay as a method for investigating cellular immunity to breast cancer antigens; (2) To continue studies on the mechanism of tumor cell kill in vitro.

Date Contract Initiated: March 12, 1969.

Current Funding Level: $85,600

CHILDREN'S HOSPITAL OF PHILADELPHIA PH 43-66-477

Title: "The Propagation and Seroepidemiology of EB virus"

Contractor's Project Director: Dr. Gertrude Henle

Project Officer (NCI): Dr. Paul Levine

Objectives: To determine whether the Epstein-Barr virus is etiologically related to Burkitt's Lymphoma and other human malignancies using the following techniques: a.) the evaluation of antibody patterns to EBV related antigens with the aim of relating the antibody response to clinical aspects of the disease; b.) the development of additional test procedures for humoral and cell-mediated immune responses to EBV; c.) evaluation of methods to improve the yield of infectious virus from present sources and to search for new sources of infectious EBV preparations; and d.) the transmission of EBV to non-human primates with the aim of demonstrating the oncogenicity of EBV in vivo.

Major Findings: As in the past, the contractor has continued to be a leader in the field of research evaluating the role of EBV in the etiology of human cancer. Twenty publications have been produced since October 1971 dealing with various aspects of the investigators' work. In addition to first describing the early antigen and then distinguishing at least two of its components, the D and the R, the investigator has shown that the R antibody is of the prognostic value in Burkitt's tumor. The relationship of anti-D antibody to disease stage in nasopharyngeal cancer was also observed in a study of a series of patients in Hong Kong. Patients with minimal disease and long term survivors had lower antibody levels than those with advanced disease and short survival. In addition, a new neutralization test has been developed which is applicable to large scale seroepidemiological

studies of EBV. Characterization of 23 cell lines in regard to their ability to be super-infected showed a remarkable difference among the cell lines. Transmission studies indicated that EBV could infect gibbons and produce an infectious mono-like disease.

Significance to Biomedical Research and the Program of the Institute: Demonstration of a correlation between EBV titers and clinical parameters in Burkitt's lymphoma and nasopharyngeal cancer are of great importance because of the possible use of these laboratory tests in both immuno-diagnosis and monitoring of therapy. The R antibody appears to be of particular importance because of its general restriction to patients with Burkitt's lymphoma and Hodgkin's disease, and its relationship to disease state further implicates EBV in the etiology of this tumor. The development of tests for measuring neutralizing antibody on a large scale will facilitate the attempt to determine whether there is more than one strain of EBV and the utilization of tests for cell-mediated immunity will be of great benefit to determine whether or not the titers to EBV are elevated because of abnormal cell-mediated immunity or whether they are elevated because of the specific response to EBV as an oncogenic agent. The ability to transmit EBV to non-human primates is of great importance because the disease apparently produced, acute tonsillitis, is a disease that is frequently seen in young children first infected with EBV. These studies could lead to the further development of important animal model systems for determination of the pathogenesis of EBV in humans and the possible factors leading to the development of lymphoma as an alternative disease effect to acute tonsillitis or infectious mononucleosis.

Proposed Course: Continuation of the above studies with particular attention to applying the newly developed tests to immuno-epidemiologic studies of lymphoma patients and controls.

Date Contract Initiated: January 1, 1966

Current Funding Level: $136,544

MAKERERE UNIVERSITY (NIH-67-47)

Title: Epidemiological Study of Burkitt's Lymphoma

Contractor's Project Director: George Kafuko, M.D.

Project Officer: Robert H. Depue, Jr., Ph.D.

Objectives: The overall objective was to conduct studies on the natural history, occurrence, and transmission of Burkitt's lymphoma (BL), with special reference to the etiologic role of Epstein-Barr virus (EBV). Since malaria has been considered as a contributing factor in the etiology of BL, the following specific objective has been a target during the current

contract year: To study the relationship between malaria and BL; to investigate the immunological effects of malaria in the susceptible childhood population of the West Nile District, and to ascertain immune response to malaria and measles immunization as compared with a well-selected control population.

Major Findings: Since the malaria investigations are supplementary to the prospective seroepidemiologic survey (IARC contract), the pace of the work was geared to that of the main survey.

Over 18,000 smears have been taken and analyzed for malaria species and count. The counties of the West Nile Region surveyed have continued to fluctuate between hyper- and holoendemic by parasite rates in children. Spleen examinations were not as reliable as counts and have been discontinued. Malaria slides from each child in the prospective study have been preserved for reference when cases of Burkitt's lymphoma begin to be found in the study population. This will result in a determination of the malaria status of each patient before and after onset of BL. The highest parasite rates occur in children 3-5 years of age, the age period just prior to the peak age of onset of BL. The species incidence is falciparum--83% and malariae--17%. There was no difference between BL patients and controls in distribution of malaria species.

Malaria antibody determinations have shown that essentially all children above 2 years of age have high anti-malaria titers.

Nearest neighbor studies of BL ptients showed that there were no sickle hemoglobin among patients, while 15% of controls had this abnormal hemoglobin. This is in sharp contrast to other studies which detected no difference. This new data supports the idea that if sickle trait protects against severe malaria it should also protect against BL.

Significance to Biomedical Research and the Program of the Institute: Malaria has long been postulated to be a cofactor of BL. This study will attempt to provide evidence relating to this hypothesis by making available in conjunction with a prospective study of that disease the malaria status of patients before onset and also provide population background data on malaria to interpret this data. In addition the project will measure the response of children to live measles vaccine in order to determine if the presence of high parasite density index suppresses the response. This would bear on the hypothesis that the reason that malaria is a cofactor for BL is that it suppresses the normal cell-mediated response to viral infection, such as measles and EBV.

Proposed Course: This project will continue with objectives above throughout the course of the prospective survey directed by IARC.

Date Contract Initiated: 26 September 1966

Current Funding Level: $12,500

M.D. ANDERSON HOSPITAL AND TUMOR INSTITUTE (NIH 72-3262)

Title: Human Immunity and Immune Response to Rauscher Leukemia Virus

Contractor's Project Director: Dr. Evan Hersh

Project Officer (NCI): Dr. Clarice E. Gaylord

Objectives: To determine the ability of human subjects to mount a primary and secondary immune response to Rauscher Leukemia Virus (RLV) and to establish the kinetics of that response; to determine the optimal dose and schedule of immunization to produce a vigorous humoral and/or cell mediated immune response to RLV; to search for virus or virus associated antigens in leukemic patients with immunological methods; and to collect and distribute serum and cells from patients with leukemia to investigators within the SVCP for laboratory studies.

Major Findings: Twenty-five patients have been entered into the study and data is relatively complete on all of them. In the majority of patients immunized with RLV, evidence of specific cell mediated and humoral immunity to the virus was observed. Specific immunization to RLV was demonstrated by *in vitro* lymphocyte stimulation techniques when data showed that RLV immunization augmented the immunological reactivity to Rauscher virus or to Rauscher transformed or infected cell antigens but did not augment reactivity to non-specific mitogens or antigens unrelated to RLV. Evidence of delayed hypersensitivity to RLV immunization was observed in 18 out of 25 patients. Three of these patients had positive skin test reactivity prior to immunization but one of these three developed marked augmentation of responsiveness upon successive inoculations. These data indicate that both *in vitro* cell mediated immunity and *in vivo* cell associated immunity develop after immunization with a killed Rauscher leukemia virus preparation. In addition, specific membrane antigen preparations derived by KCL extraction of JLSV9 Rauscher infected cells and Rauscher virus transformed ascites tumor cells also stimulated *in vitro* lymphocyte blastogenesis. Serological studies to demonstrate antibodies to virus associated antigens are being performed by collaborating SVCP scientists.

Significance to Biomedical Research and the Program of the Institute: The goal of the Special Virus Cancer Program is to determine the role of viruses in human cancer and to establish methods of control for the disease using the information gained from the etiological studies. The evidence from a number of laboratories suggesting a cross-reactivity between Rauscher leukemia virus and antigens in human leukemic cells has been supported by biochemical studies showing similar polymerases. It is expected that these studies on the kinetics of the immune response to an animal leukemia virus will provide critical information on the human immune response to the hypothetical human leukemia virus. The studies in this contract may clarify the question as to whether humans are tolerant to leukemia

viruses. Tolerance has been clearly shown to be incomplete in naturally occurring animal leukemia and it will be important to document how complete it is to suspected common antigens in human leukemia, so that rational procedures for immuno-therapy can be developed.

Proposed Course: The contractor proposes: (1) to continue studies involving the immunization of leukemia and solid tumor patients with RLV in order to determine a humoral and cellular response; (2) to use antisera and other reagents for the detection of common antigens in pretreatment leukemic cells; (3) to evaluate the use of human adapted RLV for in vitro and in vivo studies.

Date Contract Initiated: May 24, 1972

Current Funding Level: $102,058

SCRIPPS CLINIC AND RESEARCH FOUNDATION (NIH 73-3204)

Title: Immunopathologic Studies of Leukemia

Contractor's Project Director: Dr. Michael Oldstone

Project Officer (NCI): Dr. Tadao Aoki

Objectives: 1. To determine whether antibody to C-Type particles can be found in AKR mice; 2. To establish direct immunological techniques for the isolation of virus antigen-antibody complexes from the renal glomeruli of AKR mice and quantitate the eluted immunoglobulins as to specific antibody to Gross virus structural and/or coded antigens (i.e., gs 1, gs 3, and GSA) using adsorption and radioimmunoassays; 3. To apply the techniques developed in the animal models to studies on the etiology of human cancer.

Major Findings: Since initiation of this contract six months ago, the contractor has begun collecting and sectioning renal tissue containing glomeruli from approximately 200 AKR mice so that the tissue can be assayed directly for evidence of immune complex disease. Monospecific antisera against mouse IgG and C3 has been prepared. Eluates from mouse and human kidneys are being tested for a variety of RNA-virus antigens in collaboration with other SVCP investigators.

Significance to Biomedical Research and the Program of the Institute: If human leukemia is similar to animal leukemia, then one would expect that in man, too, a low grade immune response is made against an oncogenic agent, and viral antigen-antibody complexes can be recovered from the kidneys. This contract is important to the SVCP since the development of specific reagents and methods may be able to clarify the issues on the possible role of RNA viruses in the etiology of human leukemia.

Proposed Course: The contractor will collect kidneys from AKR mice containing heavy deposits of IgG. This material will be pooled and immunochemically treated to elute off glomerular bound IgG. Studies on the mechanisms of suppression or activation of Gross murine leukemia virus budding and virus-associated antigens will be carried out in collaboration with other SVCP scientists as a model for human oncogenic virus detection.

Eluates from kidneys of cancer patients will be made and evaluated in collaboration with other SVCP investigators in an attempt to detect specific antigens cross-reacting with oncogenic animal viruses.

Date Contract Initiated: May 24, 1972

Current Funding Level: $50,000

THE UNIVERSITY OF MINNESOTA (NIH 69-2061)

Title: Evaluation of the Immune Response to Tumor Associated Antigens in Solid Tumors

Contractor's Project Director: Dr. Charles R. McKhann

Project Officer (NCI): Dr. Ronald B. Herberman

Objectives: To study events in the immune response to tumor-associated antigens by assays for lymphocyte stimulation, cytotoxicity by immune cells, blocking antibody, and cytotoxic antibody. To study the effect of clinical state on the immune response. To perform studies on immunological manipulation of experimental tumors.

Major Findings: Lymphocyte stimulation studies with syngeneic mammary tumors and methylcholanthrene-induced tumors have been performed. Normal lymphocytes were stimulated by intact tumor cells and by soluble extracts. During tumor growth, reactivity disappeared, but returned after removal of the tumor.

A serial study of cellular immune reactivity of patients with cancer is now in progress.

Studies with heterologous antisera to mouse plasma cell tumors have continued, with efforts directed toward getting sera with higher and more specific activity. An anti-human plasma cell serum has also been developed. These reagents appear promising in inhibiting the growth of MSV-induced tumors and other tumors.

Significance to Biomedical Research and the Program of the Institute: Study of immune reactions to tumor-associated and possible virus-associated

antigens on human tumors is an important part of the SVCP. Separation of virus and non-virus associated tumor antigens in animals may lead to information on the etiology of human cancer. Studies of immunological manipulation in animal model systems also may provide information leading to successful immunotherapy in man.

Proposed Course: The contractor will continue to define the immune response to tumor and virus-associated antigens in animal model systems. Longitudinal studies of cellular immune reactivity in cancer patients and individuals at increased risk of developing cancer will be pursued.

Date Contract Initiated: April 14, 1969

Current Funding Level: $112,216

UNIVERSITY OF WASHINGTON (NIH-NCI- 72-2037) SEATTLE, WASHINGTON

Title: Immunological and Transplantation Studies on Dogs with Cancer for the Detection of an Oncogenic Virus-Carrier State

Contractor's Project Director: Dr. Rainer Storb

Project Officer (NCI): Dr. Gary Pearson

Objectives: The primary emphasis of this contract is to develop immunological and virological studies in canine tumors as a model for studying similar disease in the human. The investigations are carried out on dogs with lymphosarcoma and solid tumors. The immediate objectives are: (1) To develop procedures for studies of immune competence in dogs with lymphosarcoma and other tumors before, during and after therapy; (2) To monitor closely those dogs under-going relapse for biochemical or other expression of an oncogenic virus; (3) To set up immunological assays for investigation of tumor-specific antigens of canine tumors as a model for similar types of human cancers; (4) To carry out allogenic marrow grafts in dogs with tumors as a means of studying the mechanisms of reinduction of leukemia in donor cells in humans.

Major Findings: Seventy-nine dogs or biopsy specimens were received over the last contract year. All dogs that were received were subjected to a variety of immune function tests including skin grafting, antibody protection, PHA stimulation, MLC, and cytotoxicity assays. The data analyzed thus far indicates that lymphomatous dogs generally have abnormal immune functions while dogs with solid tumors generally show immune functions within the normal range. The micro-cytotoxicity assay has been used for the demonstration of cellular immunity against dog histocompatibility antigens (DLA) using lymphocytes from dog chimeras and from dogs immunized with skin grafts. Blocking factors have been demonstrated in the sera of chimeric dogs. A variety of dog cell lines have now been established from

lymphosarcomas, osteogenic sarcoma, breast cancer and melanoma. Reverse transcriptase activity has been detected in the plasma of lymphosarcomatous dogs following whole body X-irradiation. However, election microscopy examination of plasma concentrate containing this activity and gs antigen studies have failed thus far to provide convincing evidence that the irradiation induced plasma particle is a C-type virus. Attempt to rescue C-type virus from cultured canine lymphoma cells are in progress.
The canine histocompatibility typing serum panel was increased to 23 antigenic groups which seem to segregate independently. Segregation analysis yielded information about the allelism of two sets of specificities thus confirming earlier suggestions of a two-locus histocompatibility system in the dog. Therapy studies using autologous or allogeneic bone marrow grafts in dogs with solid tumors and lymphomas following supralethal whole body irradiation have been initiated.

Significance to Biomedical Research and the Program of the Institute: The major goal of the SVCP is the detection, prevention and/or control of human neoplasia. The major objectives of this contract are (1) to develop and improve immunological techniques relevant to the determination of the etiology of neoplasia and its detection, prevention and treatment utilizing the canine as a model system for similar human disease and (2) to apply the information gained from these studies to human cancer, with specific reference to the detection, prevention and treatment of human cancer, especially those of possible viral etiology.

Proposed Course: Immunological and virological studies on canine tumors will continue. Immunological investigations will be directed toward establishing the necessary laboratory tests for searching for virus-specific and tumor-specific antigens on canine tumor cells. Biochemical studies on the significance and nature of the DNA polymerase-containing particulates found in the plasma of irradiated dogs will be continued.

Date Contract Initiated: November 1, 1971

Current Funding Level: $145,000

THE JOHNS HOPKINS UNIVERSITY SCHOOL OF MEDICINE (NIH 71-2109)

Title: Anti-Tumor Reactivity in Leukemia Families and Controls

Contractor's Project Director: Dr. George W. Santos

Project Officer (NCI): Dr. Ronald B. Herberman

Objectives: 1) To perform assays for cellular and humoral immunity against tumor-associated antigens in patients with acute leukemia and lymphoma; 2) to search for common antigens in these tumors and to look for immunological reactivity of family members and unrelated individuals to the tumor antigens; 3) to correlate the results of these assays with each other, with the clinical state of the patients, and with epidemiological information to obtain further information relevant to the cause and control of these tumors.

Major Findings: The contractor has found: (1)Patients with acute leukemia on first presentation tend to be anergic as revealed by skin testing. (2) There is a low definite degree of reactivity by some family members to acute leukemic blasts, as measured by serologic reactivity or by cell-mediated immunity (^{51}Cr release test or MIF). (3) HL-A identical normal siblings as a rule (10 of 13 in this population) are stimulated by acute leukemia cells from an affected sibling in mixed lymphocyte culture. (4) There is an incidence (probably low) of otherwise normal individuals who show cytotoxic antibody to acute leukemia cells, but not to otherwise normal cells. (5) In at least 1 instance, a course of passive immunotherapy with sera derived from 1 normal individual was without therapeutic effect in an individual who demonstrated acute lymphocytic leukemia only in the marrow. (6) Patients with acute leukemia in remission may show cell-mediated reactivity to cells previously stored in liquid nitrogen by MLC, 51Cr, MIF, and skin tests, and serologic cytotoxic reactivity employing 51Cr labelled target cells. (7) Skin testing with 3 molar KCL extracts gives specific results which can be correlated with MIF and 51Cr release testing in rodent models of acute leukemia and solid tumors.

Significance to Biomedical Research and the Program of the Institute: Clinical, epidemiological and laboratory studies suggest that acute leukemia in man is a virus induced disease. Study of immune reactions to tumor-associated antigens in patients with leukemia and their family members should be useful in evaluating the role of possible oncogenic viruses in human tumors and in providing assays for monitoring attempts at immunotherapy.

Proposed Course: In the coming year the contractor will concentrate his efforts on demonstrating the specificity of the antibody detected in normal individuals that reacts against leukemic cells. He also plans to expand his studies to the pediatric acute leukemia population and study reactivity of family members to the patients' tumor employing 51Cr release and MIF

testing for cell-mediated immunity.

Date Contract Initiated: May 1, 1971.

Current Funding Level: $110,000

ROSWELL PARK MEMORIAL INSTITUTE (NIH 72-2014)

Title: "Stimulation of Immunity to Virus and Tumor Antigens by Enzymatically Treated Autologous Cells"

Contractor's Project Director: Dr. James F. Holland and Dr. J. George Bekesi

Project Officer (NCI): Dr. Paul Levine

Objectives: To develop a vaccine that is effective in the prevention and cure of murine leukemia and to apply these findings to the treatment of human leukemia.

Major Findings: Studies in this contract year continued to concentrate on the best dose and type of chemotherapy and immune stimulation effective in the control of murine leukemia. Measurements of the immune response to virus and tumor associated antigens were performed in collaboration with SVCP investigators with immunoelectron microscopy and cytotoxicity tests failing to reveal evidence for antibody against Gross-virus associated antigens in the AKR studies. In the DBA/2 mice immunized with neuraminidase treated L-1210 cells, however, a high degree of humoral and cell-mediated immunity measured by in vitro tests was found.

Correlations between in vitro measurements and resistance to challenge were also found in the DBA/2 mice injected with L-1210 cells. As expected, cytoxan was found to suppress the immune response but other carcinostatic agents (such as methyl CCNU or BCNU) did not. A significant adjuvant effect was found with live BCG and MER while monovalent con A abrogated the immunogenic effect of neuraminidase-treated L-1210 cells.

Significance to Biomedical Research and the Program of the Institute: In spite of the increasing effectiveness of chemotherapy and the treatment of acute leukemia in humans, relatively few patients have long survivals. Acute myelocytic leukemia, in particular, is a devasting disease with a poor prognosis. This may be due in part to poor tumor immunity in a disease involving the lymphoid organs, but there is also evidence from human marrow transplantation that reinduction of disease may be a factor. The use of immunotherapy directed against viral as well as tumor antigens in an attempt to increase the length of survival provides great promise because it enables the individual to use his own host mechanisms to control the disease without the toxic effects from chemotherapy that are required to eliminate the last leukemic cell. The findings from this contract,

which uses drug protocols currently in effect in humans, will be directly applicable to the control of human leukemia. The ability of this investigator to achieve better results with spontaneous AKR leukemia, a disease of known viral etiology which is associated with a poor immune response to the disease in the host, indicates that this method for management of human leukemia may be successful.

Proposed Course: Continued attention will be given to determining the specificity of the immunotherapy with particular emphasis on the Gross-AKR system. Antiviral antibodies will be sought by other techniques, including elution of complexes from the kidney in collaboration with other SVCP contractors. A correlation between the effectiveness of cell-mediated and humoral factors in control of the disease will be pursued. The treatment protocols necessary to obtain the maximum response from immunotherapy will be evaluated.

Date Contract Initiated: September 15, 1971

Current Funding Level: $137,985

GEORGE WASHINGTON UNIVERSITY (NIH 72-3251)

Title: In Vivo and In Vitro Studies of the Immune Response to EBV-Associated Antigens in Lymphoma Patients and Controls

Contractor's Project Director: Dr. T. Crandall Alford

Project Officer (NCI): Dr. Ronald Herberman

Objectives: The purpose of this project is to study the immunological reactivity of tumor patients to antigens on their neoplastic cells. Several in vitro methods are used to assess specific anti-tumor and anti-viral reactivity in collaboration with other NCI and SVCP investigators. The results of these tests will be correlated with the clincial course of the disease. In addition, skin tests with extracts from cell lines containing antigens related to the tumor as well as suspected oncogenic viruses will also be used.

Major Findings: Assays have been established, and the macrophage migration inhibition test yielded the following findings: (1) Increase in concentration of some membrane extracts increase inhibition. This may be a non-specific effect or may reflect the presence of a common environmental antigen. (2) Some membrane extracts do not inhibit even at very high concentrations. (3) A patient-specific (CA of the larynx) inhibition has been demonstrated. (4) Some preparation of tumor membrane sonicates or column fractions may stimulate migration. In the chromium release assay and other cytotoxic assays such as modifications of those of the Hellstroms and Takasugi and Klein, results indicate that many normal subjects have

peripheral leukocytes capable of killing established cell lines from breast colon, lung, and bladder cancer patients, as well as lymphoid lines derived from normal individuals.

Significance to Biomedical Research and the Program of the Institute: The studies are expected to contribute information regarding the possible viral etiology of human tumors. Studies relating the clinical course of the patient with the response to antigens of viruses suspected as etiologic agents provide important information as to the nature of the virus' role in the disease process. The potential findings of common antigenicity of tumors on this contract providing strong indirect evidence for viral etiology. This contract should allow the study of several immunological parameters in patients with neoplastic diseases. By developing specific in vivo and in vitro tests for immunity to tumor associated and virus associated antigens, it should be possible to obtain direct information on the etiology as well as the control of tumors suspected of being virus-induced. Such information will not only provide a basis upon which to prevent possible reinduction of tumor by an etiologic agent, but also would provide a basis for early diagnosis and therapy.

Proposed Course: The contractor will test soluble extracts from cell lines containing antigens associated with the Epstein-Barr virus, and selected patients will be inoculated with these extracts to determine if the antigens are tumor specific and immunogenic. Materials from these patients will be studied in collaboration with biochemists and virologists within SVCP.

Date Contract Initiated: April 13, 1972

Current Annual Level: $102,228

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER (NIH 70-2976)

Title: Seroepidemiologic and Laboratory Studies of Nasopharyngeal Carcinoma and Burkitt's Lymphoma

Contractor's Project Director: Dr. G. Blaudin de The

Project Officer (NCI): Dr. Paul Levine

Objectives: To determine whether there is an etiological relationship between a herpes-type virus, the Epstein-Barr Virus (EBV), and Burkitt's lymphoma (BL); to study groups with high and low risk of nasopharyngeal carcinoma (NPC) with the aim of evaluating the possible interrelationship of genetics, environment, and viruses (especially EBV) in the etiology of NPC.

Major Findings: This international project has involved seroepidemiologic and environmental studies on BL in Uganda; seroepidemiologic, environmental genetic, and/or clinical studies on NPC in Hong Kong, Singapore, and Tunisia; and seroepidemiologic studies on EBV in France, with supporting laboratory work in Lyon.

West Nile District. The Burkitt's lymphoma (BL) study in the West Nile District of Uganda has continued according to plan. Approximately 18,000 sera had been collected by the end of March 1973. The rebleeding in the substudy was initiated and the first four clusters were covered. A case control study of the 25 most recent BL survivors (and their families) and 25 control families was initiated. The follow-up team was further strengthened for the continued detection and follow-up of BL cases in the entire West Nile District. No new BL case has yet been detected in the children already bled in the main study.

Hong Kong. During the last half-year the team continued the serum collection in the housing project of Sau Mau Ping, where 300 families had been randomly selected for the survery out of a total of 10,000 families. All 300 families (1840 individuals) were visited and interviewed, and 570 sera were collected during the first survey round which ended in November 1972. The team has made a concerted effort to overcome the reluctance to bleeding in the population of Sau Mau Ping. This is done by repeated visits by the doctor to the houses of the survey population and collecting blood on the spot. The rebleeding of children, whose cord blood was obtained at birth has continued. The search for aetiological factors other than the virus in NPC developed into a more explicit case control study both in Hong Kong and in Singapore during the last half-year. In both places a similar questionnaire is used for interviewing NPC patients and "controls" including matched hospital controls.

Singapore. The survey continued in the Toa Payoh housing scheme under Plan B. The expanded questionnaire (relevant to NPC and EBV epidemiology) was applied to the first 150 families who were bled in Toa Payoh in the previous half-year. The bleeding was resumed in February when Dr. Malcolm Simons

returned from home leave. Approximately 1200 persons (200 households) were bled before the end of April.

France. Professor Sohier at INSERM, Lyon, continued the serological survey in the community group in Nancy, in cooperation with the Centre de Medecine Preventive there. At the end of the present half-year a total of 2110 sera had been received from the family study in Nancy; of these 1850 have been Henle tested and complement fixation for EBV antibodies is also being carried out in Professor Sohier's laboratory. In addition to EBV studies a sample of the Nancy sera is also being tested (neutralization test and hemagglutination test) for herpes simplex one and two. This is done to compare the distribution of EBV and herpes simplex infection in a representative population. The serum collection in Nancy will continue throughout 1973.

Tunisia. A total of 149 questionnaires have been completed for NPC suspects interviewed at the Institut Salah Azaiz in Tunis. Of these 97 questionnaires with respect to confirmed cases, have been forwarded to BIOCA.

Significance to Biomediaal Research and the Program of the Institute: High EBV-antibody titers have been associated with nasopharyngeal carcinoma and Burkitt's lymphoma, but an etiological relationship has not been established. This study is an attempt at a direct determination of the etiologic association of EBV to Burkitt's lymphoma by determining the relationship of anti-EBV titers before and after development of the disease. The results of this study will provide data essential for the planning of any future trial of an EBV-related vaccine. This contract also comprises most of the work within the SVCP on nasopharyngeal carcinoma. It is hoped that an intensive study of the environmental and virologic factors associated with these diseases will help to determine the significance of such factors, and ultimately lead to preventive measures which may well be applicable to other malignancies. The information ascertained may also be valuable for determining which persons are most susceptible or are at risk for developing Burkitt's lymphoma and nasopharyngeal carcinoma, so that any known preventive or remedial measures can be applied directly to them.

Proposed Course: The projects described in Uganda, Hong Kong, Singapore, Tunisia, and France will be continued. Longitudinal studies on the immunity of NPC patients and controls will be initiated in Hong Kong.

Date Contract Initiated: January 1, 1971.

Current Funding Level: $532,000

UNIVERSITY OF MIAMI (NIH 73-3218)

Title: Immunity to Virus and Tumor-Associated Antigens in Breast Cancer Using Mouse Mammary Tumor as a Model

Contractor's Project Director: Dr. Paul Meyers

Project Officer (NCI): Dr. Paul Levine

Objectives: (a) To study cell-mediated immune reactions and humoral factors in MTV-infected mice and MTV-free mice, and (b) to initiate studies on the separation, isolation, and identification of antigens in MTV-containing and MTV-free tumors.

Major Findings: In the first six-months of this newly initiated contract, stocks of Balb/c CRGL MTV-free mice were developed from a small breeding colony and a breeding nucleus of Balb/cf C3H mice carrying MTV was obtained. Utilizing the blastogenic transformation assay of mouse spleen cells as a measure of cellular immunity evidence of tumor specific immunity to supernatants of a crude Dl-DMBA-3 tumor homogenate was obtained. Tumor-bearing animals appear to be relatively anergic, and surgical removal of tumor appears to relieve this condition, especially as regards response to PHA.

Significance to Biomedical Research and the Program of the Institute: Since the detection of virus-like particles from breast tumor patients, studies on mammary tumors in humans and relevant animal models have been given a high priority in the Special Virus Cancer Program. This project deals with cell-mediated immune reactions in the mouse mammary tumor system with the hope of utilizing the results of these studies for immunological investigations in humans. It attempts to compare the *in vitro* and *in vivo* parameters of immunity by comparing the various functions of lymphocyte and humoral factors. If the contractor can isolate and identify antigens present in purified virions, virus-free tumors and virus infected tumors, it should provide a set of reagents for future work on antigens of MTV-induced tumors. This will also have application to similar projects on the human response to tumors, and increase the chance of detecting human viruses.

Proposed Course: It is intended to perfect and standardize optimal assay conditions for blastogenesis as regards spleen cell reactivity to tumor antigens. This will include conce trations of antigen and serum. A study will be made of the effect of tumor and surgical excision on a longitudinal basis, including both time and tumor size. KCl extracts of tumor will be used as antigen in the assays. Cell separation will be done to determine what T cell populations are involved in response to tumor antigen, and mitogens. One of Dr. Blair's non-antigenic tumors (eg., Dl-DMBA-1,2 or 4) will be used as an additional control.

Date Contract Initiated: October 13, 1972

Current Funding Level: $70,000

ATOMIC ENERGY COMMISSION (CP-73-210)

Title: Studies on the Relationship of Fetal Antigens to the Etiology and Control of Cancer

Contractor's Project Director: Dr. Joseph Coggin

Project Officer (NCI): Dr. Ronald Herberman

Objectives: To determine the relationship of embryonic and fetal antigens to tumor and virus-associated antigens and to evaluate their role in the etiology, pathobiology, and control of cancer.

Major Findings: Work from this project successfully demonstrated that oncodnavirus tumors of syngeneic hamsters contained fetal embryonic or phase-specific antigen in the plasma membrane. Immunization with irradiated fetus from syngeneic or xenogeneic sources elicited humoral and cell-mediated responses against oncodnavirus tumors in male recipients. Many cancers in animals have since been shown to possess fetal antigens including mouse, rat and guinea pig. In the present contract period, work has focused on isolating fetal antigens from fetus and tumor cells and characterizing their biologic behavior in vivo. Soluble antigen which mimics the membrane bound antigen has been isolated and characterized. Its capability to induce antibody by several tests has been confirmed. Cell-mediated immunity can be produced in vivo in splenectomized female recipients with soluble antigen and with irradiated whole tumor cells. Soluble fetal antigens inhibit the cytotoxic action of specifically sensitized effector cells. Masking antibody associated with multiple pregnancy was described which augments the antigenicity of fetal antigen. Conditions for achieving fetal immunization against tumors in hamsters, rats and guinea pigs were described.

Basic studies in tumor immunology proceeded in parallel with the fetal antigen work. A study of why effector cells are not cytotoxic to autochthonous SV40 tumor cells demonstrated that effector cells from tumor bearers are coated with soluble inhibitors (presumably antigen) which can be removed by washing and incubation in vitro.

Significance to Biomedical Research and the Program of the Institute: The hypothesis of derepressed host genes being responsible for oncogenesis is being actively studied by several groups in the Viral Oncology Program. The relationship between fetal antigens and virus-induced antigens is an important issue here. To better understand the mechanism of neoplastic transformation, it will be important to develop the tools to distinguish between host gene re-expression and new viral gene expression. In addition to these fundamental questions, the study of fetal antigens in tumor cells is important because of the potential use of fetal antigens in cancer prevention, diagnosis and therapy.

Proposed Course: New work in 1973-74 will focus on (a) determining specificity of the fetal antigen using humoral assays; (b) further

characterization of the differences between male and female responsiveness to fetal antigens; (c) determination of the role of the fetal antigen in inhibiting cellular immunity in vivo and in vitro; (d) attempts at interruption of spontaneous autochthonous mouse cancer by fetal immunization and (e) further characterization of fetal antigen expression in the tumor cell and in the fetal cell.

Date Contract Initiated: January 1, 1965

Current Funding Level: $225,000

THE RESEARCH FOUNDATION STATE UNIVERSITY OF NEW YORK (NIH 71-2137)

Title: Application of Effective Immunotherapy to Studies on the Etiology and Control of Human Cancer

Contractor's Project Director: Dr. Edmund Klein

Project Officer (NCI): Dr. Charles Boone

Objectives: (1) To extend the application of skin sensitizer therapy proven curative for skin tumors to other types of neoplasms, and (2) to evaluate the mechanisms of successful immunotherapy for application to vaccine development.

Major Findings: In the last year results were obtained in four areas: (a) Regressions of primary metastatic and multifocal malignant tumors in the skin were induced by the stimulation of immunological memory developed prior to the onset of malignant disease through the use of bacterial and fungal sensitizing antigens such as PPD and BCG. (b) Regressions of untreated tumors in patients with multiple malignant lesions were induced following induction of a delayed hypersensitivity response with a recall antigen at a single tumor site. (c) Combinations of immunotherapy with chemotherapy were effectively applied. (d) In xeroderma pigmentosum an occurrence of new tumors was prevented by immunotherapy.

Significance to Biomedical Research and the Program of the Institute: The principal investigator is one of the few persons obtaining clinical cures of cancer with a type of therapy which appears to have an immunological basis. Using a technique that has proven to be successful in the field, it is possible to evaluate the mechanisms involved and apply them to other human tumors. In addition to providing data useful for the early diagnosis and treatment of cancer, the successful stimulation of immunity and breaking of tolerance can be applied to studies of etiology by increasing the chance of detecting immunity to possible oncogenic viruses. This contract also provides materials from patients that are well characterized so that the unusual genetic and immunological findings can be applied to studies being carried on by other areas in the Special Virus Cancer Program.

Proposed Course: In the coming year, the contractor will continue to search for new types of cutaneous neoplasms which can be affected by skin sensitizer therapy. Efforts will be accelerated to study mechanisms of the immune response to cutaneous neoplasms in relation to the development of materials that will suppress the growth of cancer. Collaborative studies with other SVCP investigators related to the specificity of the immune response, to the investigation of immune responses in patients with xeroderma pigmentosum, and to a study of the role of genetics in cancer, will be further developed.

Date Contract Initiated: May 25, 1971

Current Funding Level: $122,000

SUMMARY REPORT

SOLID TUMOR VIRUS SEGMENT CONTRACTS

July 1, 1972 through June 30, 1973

Introduction

Emphasis in VCB-STV programs has continuously been on those problems in cancer which appeared to be most amenable to attack. In trying to summarize the 1973 achievements of these programs, it became obvious that, with some exceptions, the work of most of the STV contract and inhouse research projects were so frequently intermeshed with those of others in efforts to achieve major objectives (cited below) that it was frequently impossible to discuss individual project accomplishments of one contract without at the same time discussing the contributions of two or more other groups. This is reflected in the foregoing VCB summary. It is also apparent in the publications made by VCB-STV scientists during the 1973 fiscal year.

The major targets of the STV programs can be listed as follows:

A. Determination of the specific roles of RNA and DNA viruses in the etiology of cancer, including those transmitted endogenously by genetic inheritance and those transmitted exogenously, either vertically or horizontally.

B. Identification at the cellular level of specific host gene control factors such as regulating mechanisms which either predispose to increased or decreased RNA virus and tumor expressions.

C. Studies of the specific host immune response factors which either predispose and/or increase virus and cancer expressions including immune responses that may eventually be used to prevent cancer or control its growth.

D. Studies of exogenous carcinogenic factors present in the human ecology of agents which precipitate virus expressions as well as cancerous responses. In this area special emphasis is placed on interactions between viral and chemical carcinogens.

Drs. Paul Arnstein and John Riggs (California State Department of Public Health) reported the growth of some 15 human tumor cell lines in brains of antithymocyte serum-treated NIH Swiss mice. When re-established in culture, a number of these tumor cell lines were found to contain type C RNA tumor virus despite the fact that neither the NIH Swiss mice nor the human tumors have ever yielded virus. Dr. George Todaro (VLLB), cooperating with Dr. Arnstein, showed that the new virus (AT 124) had murine gs and reverse transcriptase antigens but did not grow in mouse cells, preferring to grow in almost any human cell line. Dr. Janet Hartley (NIAID) identified an envelope antigen on the new virus as distinct from the Gross and FMR mouse viruses. This and several other similar mouse-human viruses also developed

in the ATS mouse systems provide opportunities for exploring interspecies tumor virus rescue and also for studies of immune responses in selected human cancer patients.

Dr. Kawakami and his associates at the University of California, Davis, reported that the primate woolly and gibbon type C RNA tumor viruses were related immunologically and also that these viruses readily infect human and rat cells. Additional lymphosarcomas from gibbons were shown to have gs antigen identical with those of the original gibbon virus. Approximately 5% of sera from two colonies of gibbons (SEATO Laboratories in Thailand and Smith, Kline and French in the U.S.) revealed neutralizing antibodies to the gibbon virus. Since monkeys and man are phylogenetically closely related the search for related viral antigens in human tumors becomes a new high priority endeavor. Similarly formalin-inactivated vaccines made of banded gibbon and woolly viruses grown in human tumor cells represent prime reagents for studies of immune responses in man as well as in the isologous primates.

Drs. Bishop and Varmus and their associates (University of California at San Francisco) have made significant contributions on the RNA's of a number of type C viruses, showing that the entire viral genome (70S RNA) can be transcribed by viral polymerase into single stranded DNA in the presence of actinomycin D. Application of this new finding to tests of infected or transformed mammalian cells (rat, mouse and hamster) reveals that the latter cells acquire one or more copies of the RSV specific DNA.

In similar studies with Dr. Walter Eckhart (Salk Institute), Dr. Bishop has been able to demonstrate RNA tumor virus specific RNA in polyoma-transformed cells. This finding provides considerable support for the viral oncogene theory.

In collaboration with Dr. Wallace Rowe (NIH) and Dr. Boyse (Sloan-Kettering Institute), Dr. Frank Lilly at Einstein Medical College obtained strong evidence that the Fv-1 gene (which is linked to brown in linkage group XII) exerts an influence on gs antigen expression in AK x B/c crosses. DBA mice were shown to have a dominant gene which protected them from leukemogenesis by their own endogenous RNA tumor virus; this important gene was identified in a DBA x AKR cross. Lilly's immunogenetic studies have also uncovered a gene independent of H-2 which influences H-2 antigen expressions, thus providing an opportunity for elucidating the basic mechanisms of the major histocompatibility gene affecting leukemia in laboratory mice.

Studies of RNA tumor viruses at Flow Laboratories, Inc. This contract serves as the major source in SVCP of reagents used for typing species specific type C viruses of some 10 different animal categories. In addition, Dr. Ray Gilden and his associates were the first to characterize many of the subunits of the mouse, rat, hamster, cat and primate type C viruses. This contract also carries major responsibility for producing and safety testing formalin-killed vaccines from concentrated banded mouse viruses (AKR, wild mouse, NZB, RLV) and hamster, rat and gibbon viruses. Good

progress was made in inducing virus specific immune responses with the vaccines, the resulting sera providing opportunities for studying virus specific antigens. For this purpose a newly developed radioimmune assay system was developed by Dr. Gilden and his associates. This versatile contract group collaborates regularly with at least 8 other STV contract programs who are, like Gilden's group itself, mostly concerned with studies of immune responses to specific RNA tumor virus expressions that may prove to be useful in the control of cancer.

Studies of DNA tumor viruses at Flow Laboratories, Inc. Dr. Hampar and his associates were amongst the first to report EB virus activation by BrdU in virus-free human lymphoblastoid cells. They have since studied the sequence of this activation during various stages of (1) early antigen (EA) expression produced by short term treatment of nonproducer cells; and (2) expressions in producer cells of both EA and viral capsid antigen (VCA).

Dr. David Allen (Harvard University) in cooperation with Dr.Padman Sarma (VCB) has succeeded in elucidating the amino acid sequences of one of the two avian virus type C gs antigens (gs-b). He is very close to achieving the same goal for gs-a. Knowledge of the complete chemical structure of the major proteins (30%) in an important RNA tumor virus must be regarded as a milestone in viral oncology.

Two major reports were made by Drs. Hans Meier and Ben Taylor (The Jackson Laboratory) in FY 1973. A two-year study of spontaneous tumors in F1 backcross and F2 progeny of the AKR x L cross-breeding study reported last year revealed that 166/180 or 92% of the mice which developed cancer (many different types) during their lifetime (up to 30 months) had gs antigen expressed in their spleens at 20-30 days of age. The 14 gs negative mice which developed tumors late in life revealed gs antigen in their tumors. Besides showing a linkage between gs antigen expression and the oncogene, the presence of gs antigen in early life was highly predictive of cancer later in life.

In the experiment above, gs antigen expression was determined by a dominant gene. In a rather experimental cross breeding experiment, it was found that the C57BL/10Sn carried a dominant gene for switching off gs antigen and virus, while an allelic recessive gene specified gs+. Preliminary studies suggest that the use of C57BL/10Sn for several generations on mice having originally 90-100% virus and leukemia incidences can be shriven of both these manifestations; thus one solution to cancer has been found, in a sense, since a known dominant gene for eliminating gs antigen and RNA tumor viruses should lead to eventual elimination of natural cancer. This hypothesis is now on test at The Jackson Laboratory.

Drs. Peters, Spahn (Microbiological Associates, Inc., Walkersville) and Kelloff (Project Officer, NCI) reported that RNA viruses isolated from spontaneous tumors (all types) of BALB/c mice were oncogenic when injected into newborn B/c mice, provided the viruses were tropic for B type cells;

N tropic viruses did not produce tumors. This was important because it revealed that endogenous mouse RNA tumor viruses, like nononcogenic RNA viruses, vary in pathogenicity and depend upon host cell responses. Dr. Peters, with Dr. Hartley (NIAID), found that N tropic viruses can be converted to B type on in vivo and in vitro passage in B/c mice and cells.

Drs. Peters, Nims and Kelloff are currently engaged in the production and testing of various RNA tumor virus vaccines in several strains of mice and rats with the purpose of determining immune responsiveness to virus specified antigens. The ultimate goal is to determine the effects of such immunity on spontaneous and chemically induced tumors.

The MAI contract in Bethesda embraces seven different research programs, several of them service projects. Major findings were as follows:

Drs. Freeman, Price and Zimmerman completed a blind test of 30 unknown carcinogenic and non-cancer-producing analogues submitted by Dr. Weisburger in their in vitro RNA virus infected rat cell cultures. When the specimens were decoded, it was found that the results in the in vitro test correlated nearly perfectly with that expected from in vivo carcinogen tests in mice and rats, thus establishing this rapid relatively simple procedure as the simplest and possibly the best for testing environmental carcinogens. This group, together with Dr. Rhim, showed that RNA viruses also provided determinants for accelerated transformation of rat cells by several DNA tumor viruses and that DNA tumor viruses (polyoma and SV40) switched on HaLV gs antigen in a significant proportion of hamster tumors. Tests of smog condensates in RLV-infected rat cells revealed nitrogenous as well as hydrocarbon fractions to be carcinogenic.

On another project, Drs. Whitmire and Salerno reported that purified RadLV vaccines inactivated by formalin, and interferon (given 3 x weekly for 4 months) reduced the incidence of 3MC-induced tumors in C57BL/Cum mice. Each of these observations, if confirmed in future studies, could represent significant breakthroughs.

In a third project, Dr. Lee Vernon completed a two-year study of type C particle expressions in embryos of 10 strains of mice. Typical budding (but not infectious) type C particles were observed on the various cells of the hematopoietic system (these same tissues were found by Huebner et al. [1970] to have gs antigen expressions). No particles were seen in muscle embryo cells which were also uniformly gs negative in Huebner's earlier studies.

Dr. Johng Rhim, on a fourth project, demonstrated that polyoma-transformed hamster and mouse cells, when transplanted into isologous hosts, produced tumors containing the appropriate HaLV and MuLV gs antigens.

A fifth project supervised by Dr. Padman Sarma (VCB) as an onsite project officer was summarized in the VCB summary.

Dr. Maurice Green and his associates at Saint Louis University reported a number of interesting findings: (1) Type C RNA tumor viruses from 5 different species were shown to have RNase H activity while 3 different non-oncogenic RNA viruses did not; thus this enzyme may prove to be another useful marker for RNA tumor viruses. (2) Dr. Green devised a cell-free protein synthesizing system based on polyribosomes both free and membrane bound; they synthesize two major viral polypeptides in vitro. (3) RD114 3H-DNA were found to form complexes with Hodgkins lymphoma RNA in hybridization experiments; however DNA from cat cells contained many more copies than did the Hodgkins tissue cells. (4) A system was developed for isolating virus specific RNA using nitrocellulose, thus providing a new means for extensive purification of intracellular viral RNA; these studies suggest that intracellular viral RNA also contains poly(a) traits. (5) Dr. Fred Rapp's Herpes type 2 transformed hamster cells were shown to have virus specific RNA sequences that hybridized with herpesvirus types 1 and 2.

This contract is equipped to rapidly explore human tumor and normal cells for putative human RNA tumor virus sequences when suitable human viral candidates are finally established and available for study; thus this contract, as in the past, should prove to be an extremely valuable resource. Recently Dr. Green began collaborative studies with Dr. Sol Spiegelman and hopes to confirm the latter's recent finding of virus-like RNA-DNA in human leukemic cells.

Dr. Walter Eckhart at the Salk Institute, in studies of polyoma mutants with Dr. Renato Dulbecco, has clearly shown a number of viral gene determinants controlling expressions of both virus and tumor. More recently, Eckhart, with Dr. Michael Bishop (University of California at San Francisco), found that polyoma-transformed hamster cells contain the specific RNA of hamster type C virus (HaLV). This exciting finding complements other studies (Freeman, Kelloff, Huebner) which indicate that polyoma-induced hamster tumors and transformed cells frequently become switched on for HaLV gs antigen, thus suggesting that polyoma virus may induce tumors through the mediation of endogenous RNA tumor virus genomes.

Also at Salk, Drs. Lennox, Shier, Nicolson and Holley are engaged in four separate projects. Dr. Lennox devoted this first year to the development of immunogenic forms of tumor cells that would specifically prevent subsequent transplantation of such cells; the cells were used both with and without formalin. Paradoxical effects were observed.

Dr. Shier tested synthetic antigens having specificities similar to wheat germ agglutinin for effects on syngeneic tumors in rats. Two glycoprotein-like antigens having short sugar chains gave "significant" modification of tumor growth rates.

Dr. Nicolson used sophisticated EM procedures employing ferritin-conjugated Concanavalin A (Con A) to study the topography of antigens on cell surface membranes. This interesting new technique revealed that SV40-transformed

cells gave distributions of the ferritin-Con A that were more clustered than did normal cells. Neuraminidase failed to unmask additional sites for Con A agglutination while it did so for two other (Ricinis) agglutinins.

This contract was scheduled for phase-out during FY 1973.

Drs. Dixon and Lerner at the Scripps Institute have mounted comprehensive studies of the RNA tumor virus and viral antigens and antibodies uniquely found in the lupus-like disease of NZB mice. In this excellent model of human lupus erythematosis they hope to unravel the roles of type C RNA virus in both autoimmune disease and the high incidences of cancer associated with such disease as follows:

In their initial studies they showed, with Dr. Oldstone, that the NZB autoimmune kidney disease is due not only to anti-DNA antibodies (Dixon) but to a complex of a type C RNA viral specific antigen and antibodies to this protein. With Hartley, they showed the virus to be N-B tropic and to have Moloney virus viral envelope antigens. The autoimmune mice were also shown to have antibodies to viral RNA, viral DNA, viral reverse transcriptase, soluble viral antigens found in plasma (Mellors and Aoki), and possibly even gs antigens. Recently they reported purification of the viral specified membrane antigen believed to be responsible for the autoimmune disease.

With Dr. Jensen they have established a number of lymphoblastomatous, sarcomatous and epithelian cell cultures from NZB tissue; all have large amounts of NZB specific virus which produce leukemia within 3 months in NZB x BALB/c F1 progeny. With Dr. Gilden (Flow Labs., Inc.) they are now studying the effects of formalin inactivated NZB banded virus vaccines, given early in life to NZB mice, on virus replication, immune responses, the autoimmune disease, and late cancers.

Dixon and his group also contributed importantly to the development of sensitive and specific radioimmune assay tests devised by Drs. Parks and Scolnick (VCB) and by Drs. Charman (USC) and Gilden (Flow Labs.).

At the University of Southern California, Drs. Murray Gardner, Brian Henderson, Earle Officer, Robert McAllister and Vaclav Klement described a number of possible new etiological factors in cancer of humans, wild mice, cats and rats.

Natural mouse and cat systems: (1) Studies of mice in large natural populations showed that one population (Lake Casitas) revealed, by one year of age, extensive expressions of type C viruses and also had extremely high incidences of spontaneous lymphomas and other cancers. Mice in a similar, equally large, virus negative population had less than 1/20th as many tumors by 30 months of age. (2) A large population of wild mice having >80% natural infection with polyoma had the same low incidence of cancer as a control polyoma virus-free population. (3) The highly infected and high lymphoma wild mice also manifested a high incidence (1% per month) of motor neuron paralysis similar to human amyotropic lateral sclerosis (ALS).

Each of these findings must be regarded as both unique and highly significant.

(4) Studies of cats by Drs. Gardner (USC) and Sarma (VCB) revealed the presence of RD114 virus (endogenous cat) in cat fetuses and possibly also in tumors. (5) In the meantime, Drs. McAllister, Gilden, Sarma, and Todaro and his associates, Drs. Livingston and Fischinger, found that the RD114 virus was indeed a completely novel endogenous virus of the cat. This virus, like those representative of other inherited type C RNA virus genomes, grows very poorly or not at all in cells of its natural host (cat).

Epidemiological studies: (1) Drs. Henderson, Gardner and Gordon report that information on over 20,000 Los Angeles cancer patients is now being incorporated in the USC Cancer Surveillance Unit, and have exploited this unique possibility to explore industrial and other envirnomental factors in the prevelance of various types of cancer in various parts of Los Angeles counties. Already in the first year it seems likely that increased incidences of certain cancers (Hodgkins) will be traced to inter-human contracts, and other types of cancer (lung and others) may be attributable to high levels of environmental carcinogens in certain highly industrialized areas. Unexpectedly high incidences of sarcoma and other solid tumors in broiler chickens in Los Angeles abattoirs were linked to the use of Marek's viral vaccines given at birth. The implications of this observation are now being explored in cooperative studies with Drs. Groupé and Fraenkel (Life Sciences, Tampa, Florida).

Recently Dr. McAllister derived a new sarcoma tumor cell line (Hickey) that produced type C particles (confirmed by Dr. A. Dalton, NCI) after IdU treatment. Although the particles as expected failed to grow continuously, the induction of clear-cut type C particles in human cells inspires considerable confidence in the proposition that such viruses will eventually be established from human tumor cells.

Dr. Peter Vogt and his associates at the University of Southern California are engaged in a 5-year study during which time they plan to acquire and examine 300 temperature sensitive mutants with the purpose of obtaining a complete mapping of the Rous Sarcoma Virus genome. He has already employed several ts mutants in proving that viral genetic activity is necessary for initiation and maintenance of the transformed (neoplastic) state in avian cells. This study should provide much needed information on the mechanisms by which RNA viral genomes cause cancer.

Drs. Hayflick and Stanbridge at Stanford University have devised a method using cytochalasin B and high g forces for removing nuclei from 95% of mass cell cultures. Such cells are critical to studies designed to determine cytoplasmic contributions to RNA viral replication and in the rescue of defective RNA tumor viruses.

Despite extensive studies with 5 different carcinogens, Hayflick and his associates have been unable to transform human cells in vitro (these include

cells from patients with the highest risks of spontaneous cancer--Down's, Trisomy D, Franconi's, Xeroderma). However, this effort will not be abandoned; new studies are planned using lower temperatures recently reported to favor cell transformation.

This contract also continued to serve as a major resource for mycoplasma testing; over 2700 specimens were examined during the year.

Dr. Henry Kaplan's program (also Stanford University), funded at a modest level late in FY 1973 by a supplement to the Hayflick contract, has not been in operation long enough to make a semi-annual report. Dr. Kaplan's main purpose is to mount in depth studies of etiological factors in Hodgkin's and other lymphomatous disease; he plans studies at both cellular and whole organism levels. He is particularly interested in elucidating the basic cause of the immunological aberrations which characterize Hodgkin's disease. He is impressed with the similarity of Hodgkin's to certain graft versus host reactions (GVHR) which he believes may be due to the interactions of the host immune mechanisms to putative herpes and RNA type tumor viruses.

Drs. Karl and Ingegard Hellstrom at the University of Washington have recently made the very important finding that cell mediated immunity, blocking and unblocking serum activity observed in vitro correlates very well with similar immunological activity observed in clinical studies of human cancer patients. Their findings were most clearly exemplified in 10 patients with malignant melanoma. Patients with little or no residual tumor had high lymphocyte mediated tumor immunity: patients with large tumors had low cell mediated immune responses. Blocking antibodies which abrogated the lymphocyte mediated immunity were found in patients with clinical tumors, but this blocking activity disappeared with clinical improvement. Sera from patients with no current clinical melanoma potentiated the cytotoxic activity.

Similar findings were made in virus induced rat tumor systems. Cell mediated cytotoxicity, blocking and unblocking antisera and modifications of tumor growth were also influenced by the clinical state of the tumored rats.

The most recent reported observation by this group (Pollock and Hellstrom) was the arming of normal lymphocytes by exposure in vivo to sera from tumor-bearing mice. Specific cytotoxicity activity was conferred on the normal lymphocytes taken from normal lymph nodes. Thus antisera to MSV tumor cells armed normal cells so that they were cytotoxic for the MSV cells in vitro.

Dr. Leo Sachs of the Weizmann Institute reported three new findings in FY 1973. (1) Concanavalin A selectively agglutinates tumor cells and can kill such cells more rapidly than normal cells, thus suggesting a new approach to chemotherapy. (2) Sachs also postulated that tumors were controlled by expressor and repressor genes in cells. Reversion of certain tumor cells to the normal phenotype has provided opportunities for studying gene repression factors. (3) Sachs had identified a molecule with a molecular weight of 68,000 (MGI) which is secreted by normal cells but which can turn blast cells

into macrophages and granulocytes. This factor, however, also requires a low molecular weight cofactor having adenine as a substrate. Modifications of this factor "may cause acute leukemic cells to stop developing and differentiate normally".

Dr. Anthony Girardi at the Wistar Institute succeeded in establishing the SV40 virus isolated from a human leukoencephalopathy case directly into human fetal brain cell cultures. The virus isolated was shown to be a typical strain of SV40, producing SV40 tumors in 100% of newborn hamsters; T and transplantation antigens were also identical with SV40 viruses isolated from monkey kidneys. This "human" SV40 virus transforms human cells readily. Girardi plans to study in more depth the role of this virus in immunosuppressed humans also having leukoencephalopathy.

Dr. Girardi is continuing his studies of related fetal-tumor antigens, but his interests now are to study mouse embryonic antigens (gs and transplantation) focusing particularly on the 2 to 64 cell stages. However he plans to continue to identify virus-induced tumor-specific transplantation antigens in animal model systems.

SOLID TUMOR VIRUS PROGRAM SEGMENT

Dr. Robert J. Huebner, VCB, Division of Cancer Cause and Prevention, Chairman
Dr. James T. Duff, VCB, Division of Cancer Cause and Prevention, Vice-Chairman

CALIFORNIA STATE DEPARTMENT OF PUBLIC HEALTH (PH43-NIH-NCI-E-68-997)

Title: Studies on the Possible Role of Oncogenic Viruses in the Causation of Cancer in Man

Contractor's Project Director: Dr. Edwin H. Lennette

Project Officer (NCI): Dr. James T. Duff

Objectives: To apply the newer knowledge of the nature of RNA viruses to the study of neoplasms of animals and man.

Major Findings: Direct tumor transplants: A total of 19 human tumors were inoculated into ATS-treated mice. In four instances tumors were produced in the mice although only one of these could be serially transplanted. In vitro cell cultures could not be established from these four tumors.

Monolayer cell cultures derived from human tumors: Eleven high passage (>60) human tumor cell cultures were inoculated into ATS-treated mice. All 11 produced tumors in the mice of the type from which the cells were established and were serially transplantable. Cell cultures were established from the tumors induced in the mice and were determined to be of human origin. Ten low passage (<50) human tumor cell cultures were inoculated into ATS-treated mice and 8 of the 10 produced tumors which were serially transplantable. Cell cultures were re-established from the 8 tumors.

Suspension cell cultures from human tumors: Nine suspension cultures derived from human tumors were inoculated into ATS-treated mice, with four of the cultures producing tumors in the mice.

Isolation of a Type-C virus: One Type-C virus which was isolated from one of the human cell cultures re-established from the tumor produced in the mice has the gs-1 antigen and polymerase of murine viruses but the host range of a hypothetical human agent.

Vaccine studies: In a pilot study in a small number of ATS-treated mice, a vaccine from RD-114 virus was used to determine the feasibility of utilizing this system for vaccine studies. The vaccinated animals produced antibody to the virus as determined by CF and IFA tests. Tumors were induced in 3/13 (23%) of the vaccinated mice and 9/14 (64%) of the placebo injected mice.

Passive hemabsorption studies: Use was made of the passive hemabsorption procedure to determine the feasibility of utilizing this system for the detection of viral antigens in monolayer cell cultures and antibodies to the viral antigens in sera. Preliminary results indicate the reaction is highly sensitive although absorption of the sera is needed to produce viral antigen specificity.

Electron microscopy: Two antisera were used to show the antigenic differences between RD-114 and feline leukemia viruses. Guinea pig and rabbit anti-RD-114 virus sera tagged RD-114 virus when tested by the indirect ferritin-labeled antibody technique. Feline leukemia virus was not tagged when treated with the same antisera. Also, tumor cells selected by the studies of ATS-treated mouse system are being monitored for the presence or absence of Type-C virus.

Significance to Biomedical Research and the Program of the Institute: The immunosuppression of mice with ATS and their subsequent tolerant state presents unique opportunities as an experimental system, not afforded in other methods of studying human cancers. One obvious advantage is the availability of a solid tumor, in which the human neoplastic tumor cells comprise the entire "malignant" component. These neoplasms are produced in the mice in 10-30 days and resemble architecturally well-known clinical tumor types. Thus, experiments can be designed to test chemical, physical or biologic influences on human solid tumors without the involvement of human subjects. The solid tumors might respond in a manner more closely resembling spontaneous human neoplasms than tumor cells in the more restrictive in vitro culture environment.

Proposed Course: (1) Growth of human tumor cells in ATS-treated mice for the purpose of (a) verifying that they are tumor cell lines instead of the usual fibroblasts and (b) trying to "switch-on" human Type-C virus by in vivo passage similar to the switch-on which occurred in RD cells on passage through a cat; (2) utilization of the hemagglutination inhibition technique to detect antibodies to available Type-C viruses in animals and man; (3) utilization of the fluorescent antibody (FA) procedure to look for gs-1 antigens in human tumors and tumor cell cultures and also utilization of the indirect FA procedure to look for antibodies in human cancer patients or laboratory personnel working with candidate human Type-C viruses; and (4) utilization of the ATS-treated mouse system for the study of viral vaccines.

Date Contract Initiated: June 24, 1968

Current Contract Level: $205,979

CALIFORNIA STATE DEPARTMENT OF PUBLIC HEALTH (NIH-NCI-E-69-87)

Title: Human-feline Cancer Household Study

Contractor's Project Director: Dr. Robert Schneider

Project Officer (NCI): Dr. James T. Duff

Objectives: To determine if a significant etiological association exists between human and feline cancer using a retrospective study of households in which a malignant cancer has been diagnosed in a cat, and concurrently to look for and test the significance of apparent horizontal transmission of lymphoma virus from cat to cat.

Major Findings: A study on the serological survey of veterinarians for titers to FeLV has been completed. The presence of antibody was tested for by Dr. John Riggs using an indirect immunofluorescent test against cell surface viral antigens. There was one reactor among the 626 veterinarians and the 67 non-veterinarians tested. The one reactor was negative on a subsequent serum sample taken 8 months later.

Incidence data indicate that between 5 and 10 of the 626 veterinarians will develop one of the leukemias or lymphomas during the reaminder of their lives. Thus, assuming that the positive reactor was not a false positive one, a single reactor where 5 to 10 would be expected to get various forms of the disease, does not support the probability that FeLV is associated with the occurrence of human leukemias or lymphomas in this particular group.

Significance to Biomedical Research and the Program of the Institute: The Animal Neoplasm Registry (ANR) of the California State Department of Public Health has been functioning since July, 1963. All cases are histopathologically confirmed and information is kept about the animal and the owner. This epidemiological study is important for determining the possible effects of exposure to cat leukemia and sarcoma viruses on human cancer and the importance of horizontal spread of virus from cat to cat and from cat to dog in maintaining the natural history of the feline viruses.

Proposed Course: The contract was terminated April 30, 1973.

Date Contract Initiated: June 19, 1969

Current Contract Level: $35,000 (funds carried forward from FY '72)

CALIFORNIA, UNIVERSITY OF (NO1-CP-33242; formerly NIH-NCI-E-70-2048)

Title: Comparative Leukemia and Sarcoma Viral Studies

Contractor's Project Director: Dr. Leo K. Bustad

Project Officer (NCI): Dr. Wade P. Parks

Objectives: To continue studies of the *in vitro* characterization of the woolly, gibbon and other simian Type-C viruses, especially by antigenic and biochemical characterization; to continue studies of the pathogenicity of the simian viruses; to evaluate the natural history of the gibbon

Type-C virus in specimens from Thailand and San Francisco Zoo populations; to employ serologic studies to detect shared antigenic components between the simian viruses and putative human Type-C viruses; to isolate or identify viruses or viral antigens in spontaneous human tumors as well as in nonhuman primate tumors using a wide variety of in vitro techniques.

Major Findings: In vitro studies have shown a similar host range for the woolly and gibbon viruses. These viruses infected a wide variety of cell cultures of rodent, bovine, simian and human origin as measured both by reverse transcriptase activity or morphologic alteration of human and rat cells. Using complement-fixation, immunodiffusion or measurements of antipolymerase activity, woolly and gibbon viruses were shown to be highly related immunologically. It was surprising, but of great interest, that the direct fluorescence-antibody test for viral envelope antigens showed that the woolly and gibbon viruses showed common determinants. As expected, the protein composition and RNA analysis of the two viruses was like that of other well-characterized mammalian Type-C viruses. Attempts to isolate other simiam viruses have included comprehensive studies of 11 spontaneous simian and human tumor samples. Of these, three simian samples and a human tumor had particles suggestive of Type-C morphology by electronmicroscopy. Further studies in progress are necessary before any conclusions are possible.

Analysis of 83 serum samples from gibbons, the Thailand SEATO Laboratory, revealed that approximately 5 percent had evidence of antibody against the gibbon virus. Studies using the sera from the San Francisco Zoo and from a small gibbon colony at Smith, Kline and French have also shown evidence of antibodies directed against the gibbon Type-C virus. These data suggest natural antibody may be prevalent among gibbons. Other studies are in progress to determine if human sera react with a comparable antigenic determinant.

Significance to Biomedical Research and the Program of the Institute: The finding at this laboratory of two Type-C viruses associated with tumors of different primate species is evidence that the higher animals, including man, are likely to be among the growing number of species harboring Type-C viruses. Since monkeys and man are closely related phylogenetically, the proposed studies, which are oriented toward characterization of the primate viruses and seeking possible relation with human tumors, are of direct relevance in establishing the etiology of human cancer.

Proposed Course: (1) To define, in molecular terms, the viral antigen which reacts with sera from gibbons and to determine if similar activities are present in humans. (2) Continued examination by electronmicroscopy and in tissue culture primate (including human) tumors and further characterization of existing primate lines. (3) Natural history studies of gibbon Type-C virus in gibbons and studies of its oncogenic potential in other species.

Date Contract Initiated: November 1, 1969

Current Contract Level: $420,000

CALIFORNIA SCHOOL OF MEDICINE, UNIVERSITY OF (NO1-CP-33293; formerly NIH-NCI-E-71-2147)

Title: Studies on the Role of Virion-Associated DNA Polymerases in Malignant Transformation by Tumor Viruses

Contractor's Project Directors: Dr. J. Michael Bishop
Dr. Warren Levinson
Dr. Leon Levintow

Project Officer (NCI): Dr. Robert J. Huebner

Objectives: Conduct investigation on the molecular biology of the avian and other RNA tumor viruses, particularly (a) the virion-associated DNA polymerase and (b) the virus-specific DNA and RNA in normal and transformed cells.

Major Findings: (1) The DNA polymerase of several RNA tumor viruses transcribe the entire viral genome (70S RNA) into single-stranded DNA in the presence of actinomycin D. This provides a valuable reagent for studies with molecular hybridization. In the absence of actinomycin D, transcription into double-stranded DNA occurs primarily from a limited portion of the template irrespective of whether the template is 70S RNA or other naturally occurring RNA's.

(2) At least 90 percent of the initial product of transcription from the 70S RNA of RSV (Schmidt-Ruppin) begins with the olignucleotide pdAdAdTdGdAdAdGdC. The occurrence of a nucleotide sequence of this length cannot be explained as a random event, and the homogeneity of the sequence implies the existence of a common and highly reiterated initiation site on template RNA.

(3) The "primer" RNA on which DNA synthesis initiates with 70S RNA of RSV (Schmidt-Ruppin) as template has been identified and purified. It is a unique fraction of the 70S-associated 4S RNA with a homogenous nucleotide sequence containing the distinctive oligonucleotide TψCG and terminating (3') with rA.

(4) Virions of RSV (Schmidt-Ruppin and Prague strains) and RAV-2 contain a tRNA nucleotidyl transferase which adds part or all of the sequence pCpCpA to the 3' termini of susceptible tRNA's, including the 4S RNA's found in virions of RSV. The enzyme has been purified and found to have properties typical of similar enzymes isolated from eukaryotic cells in the past.

(5) Infection and transformation of mammalian cells (rat, mouse, hamster) by RSV results in the acquisition of one or more copies per cell of RSV-specific DNA. The viral DNA is covalently "integrated" into host chromosomal DNA, as shown by the use of a newly developed assay based on reassociation properties of denatured cellular DNA. Hamster cells reverted from transformed to normal phenotype retain RSV DNA.

(6) The tissues of all mouse strains examined to date contain equal amounts of DNA homologous to the genome of MMTV virus, irrespective of the incidence of virus-associated carcinoma of the breast in the various mouse strains. Other rodent (rat, hamster, European field vole) and human (HeLa, normal breast, carcinoma of breast) DNA's contain no detectable MMTV-specific nucleotide sequences.

(7) All mouse tissues examined to date contain detectable MMTV-specific RNA irrespective of whether the tissues are producing virus, and irrespective of the incidence of virus production and virus-induced tumors in the mouse strains. Thermal denaturation of hybrids formed between tissue RNA's and MMTV-specific DNA has provided evidence for a qualitative difference between the MMTV-specific RNA's in tissues of low and high tumor incidence mice. Spontaneous production of virus and the presence of relatively large amounts of MMTV RNA segregate together in the progeny of hybrid genetic backcrosses.

(8) RNA from 5 normal and 25 carcinomatous human breasts have been tested and MMTV-specific nucleotide sequences were found in only one instance (an infiltrating ductal carcinoma).

Significance to Biomedical Research and the Program of the Institute: These studies are providing an important insight into the mechanism by which RNA tumor viruses bring about malignant transformation, and perhaps will lead to significant advances in the understanding of the causation and control of human neoplastic disease.

Proposed Course: (1) The 70S RNA of newly cloned RSV will be examined for major redundancies in its nucleotide sequence. This constitutes a test of the hypothesis that the genomes of RNA tumor viruses may be polyploid.

(2) Studies of transcription from various 70S RNA's will be continued in an effort to delineate the factors which constrain the extent of that transcription.

(3) The 4S "primer" RNA on which transcription from 70S RNA initiates will be further characterized in order to determine what elements distinguish this RNA from the other 4S RNA's associated with 70S RNA.

(4) Attempts will be made to implicate the tRNA nucleotidyl transferase of RSV in the biological activities of the virus by demonstrating that the 3' terminal nucleotide sequence pCpCpA is essential for both the binding of certain 4S RNA's to 70S RNA and the ability of these 4S RNA's to serve as primers for initiation of DNA synthesis.

(5) Continue to define the factors which regulate the incidence of carcinoma of the breast and spontaneous production of MMTV in mice. The next steps in this effort are as follows: (a) The relatedness of the genomes of several MMTV strains will be characterized by molecular hybridization. (b) The initial conclusion that the DNA of all mouse strains

contains the same amount and the same set of MMTV-specific nucleotide sequences will be subjected to more rigorous tests. (c) Surveys of MMTV-specific RNA in mouse tissues will be continued, with emphasis on geneticallyhybrid mice in which tumor incidence and virus production vary predictably. Attempts will be made to correlate the particular set of MMTV nucleotide sequences expressed (by transcription) with the genotype of the mouse, and to define the functional status of these sequences.

(6) Additional samples of DNA and RNA from normal and carcinomatous human breasts will be tested for nucleotide sequences homologous to the MMTV genome.

Date Contract Initiated: June 2, 1971

Current Contract Level: $84,000

EINSTEIN MEDICAL COLLEGE (NO1-CP-33249; formerly PH43-NIH-NCI-E-65-612)

Title: Genetic and Immunological Factors in Viral Leukemogenesis

Contractor's Project Director: Dr. Frank Lilly

Project Officer (NCI): Dr. Robert J. Huebner

Objectives: To gain a better understanding of the genetic events and underlying mechanisms that play a role in the oncogenic process.

Major Findings: Studies to date have confirmed that the F-T virus, a variant of the Friend virus, is B-tropic. These studies also revealed an irregularity in viral host range, suggesting the presence of another virus-susceptibility gene (Fv-3?).

In studies of the Fv-1 gene, in collaboration with Dr. Boyse (Sloan-Kettering) and Dr. Rowe (National Institutes of Health), strong evidence was obtained (1) that this gene was linked to brown in L.G. VIII, and (2) that the Fv-1 gene exerts an influence on gs antigen expression in crosses of AKR X BALB/c mice.

Tissue culture experiments showed that when Friend virus was grown in mouse embryo cells, its SFFV component grew about 200-fold less efficiently than its XC-active component. Extracts of chemically-induced DBA/2 leukemias were found to contain low levels of XC-active virus, but treatment of susceptible mice with physical and chemical leukemogens did not induce detectable virus in the early post-treatment period. Hairless and normal-haired mice of the HRS strain differed, at most, very slightly in the levels of XC-active virus in their tissues prior to the appearance of leukemia.

DBA mice were found to have a gene which protected them from leukemogenesis by their endogenous MuLV. This gene appeared to be dominant in the cross

DBA X AKR, since these mice were highly resistant to spontaneous leukemia but susceptible to MCA-painting, as are DBA/2. H-2 type was found to exert a moderate influence on skin tumor induction by MCA and DMBA painting.

In collaborative studies with Dr. Nathenson, the FMR antigens were found to be a major internal component of the FV virion which could be released by freeze-thawing and by ether or detergent treatment. The antigen appeared to be a feature of a carbohydrate-free protein.

Immunogenetic studies provided strong evidence for the existence of a gene, independent of H-2, which influences the expression of some H-2 antigens on erythrocytes. Experiments involving "capping" of membrane antigens with fluorescent-labeled antibodies demonstrated that the major H-2K and H-2D antigen molecules were not strongly associated with each other on the cell surface.

$H\text{-}2^d$ mice were found to make anti-FMR antibodies at a slower rate than $H\text{-}2^b$ mice. These studies may reveal the basic mechanism of the H-2 influence on leukemogenesis.

Significance to Biomedical Research and the Program of the Institute: One of the basic facts about tumor biology is that genetic mechanisms of the host exert major control over the expression of oncogenicity. By defining the loci and markers associated with leukemogenesis, it should be possible to undertake systematic studies of the precise immunochemical mechanisms governed by individual loci, with the objective eventually of encouraging or altering their immunogenetic effects to provide maximum resistance against cancer.

Proposed Course: (1) Analysis of the components of leukemia viruses: SFFV, leukemogenicity, XC-active virus, helper activity; are any of these activities inseparable from each other? (2) Studies of tissue culture-grown FV when reintroduced *in vivo*. (3) Genetic control of chemical leukemogenesis and X-irradiation: virus induction and genes governing the process and immune response. (4) Further studies of the F-T virus and the new (?) genes revealed by its host range. (5) Further studies of the FMR antigen.

Date Contract Initiated: May 13, 1965

Current Contract Level: $180,000

FLOW LABORATORIES, INC. (NO1-CP-33247; formerly NIH-NCI-E-71-2097)

Title: Studies of Tumor Viruses in Relation to Oncogenic Potential

Contractor's Project Director: Dr. Raymond V. Gilden

Project Officer (NCI): Dr. Robert J. Huebner

Objectives: To conduct immunological and serological studies of oncogenic Type-C RNA viruses and herpesviruses associated with neoplasia. To develop immunologic, biologic, and biochemical reagents and techniques for identification of Type-C viruses and their gene products. To delineate the mechanism of herpesvirus latency and to determine the relevance of herpesviruses associated with human tumors.

Major Findings: (1) Type-C Viruses: (a) Feline origin of RD-114 virus. A possible human origin for RD-114 virus was suggested from its history and by its distinctive major internal virion antigen (gs-1) and reverse transcriptase when compared to homologous proteins isolated from Type-C viruses of various mammalian species, including cat. Despite the distinctive gene products of RD-114 virus and conventional cat Type-C viruses, molecular hybridization studies using well-characterized DNA probes demonstrated RD-114 viral RNA sequences in several cell lines, normal tissues, and tumors from cats, but not from other species. Since similar findings have been reported by other laboratories, it would appear that RD-114 virus is of cat origin. This significant finding indicates that the previously held view that all Type-C virus isolates from any one mammalian species contain identical gs-1 antigens is invalid. Further, the finding that cat cells may contain full RD-114 RNA equivalents in the absence of detectable virus-specific proteins suggests a unique instance of translational control, the significance of which has yet to be explored. (b) Demonstration of Type-C virus species-specificity at the molecular level. Molecular hybridization studies using sensitive and stringent techniques; e.g., S-1 nuclease digestion, indicate a high degree of Type-C virus species-specificity. Cat and mouse viruses, for example, show less than 5 percent cross-hybridization. While virus isolates from any one species may lack homology at the nucleic acid level, e.g., RD-114 and FeLV, each isolate demonstrates species-specificity. This could serve as a baseline against which to evaluate inter-species cellular hybridizations using viral probes, and when used in conjunction with amino acid analysis of specific virion proteins, should give some insight into the evolution of Type-C viruses. (c) Development of bioassays for RD-114 virus. A sensitive plaque test, using Rous sarcoma virus transformed human glial cells (the KC cell assay) and a focus-forming assay using a MSV pseudotype of RD-114 produced by a fusion technique were developed. (d) Fusion of human lymphoblastoid cells by RD-114 virus and FeLV with activation EB virus. Infection of EB virus producer or nonproducer cells with RD-114 virus or FeLV resulted in rapid but transient cell fusion followed by the establishment of carrier cultures. A second and final cycle of syncytia occurred after 3-4 weeks. During the periods of cell fusion, EB virus was activated in the syncytial cells; with early antigen (EA) being synthesized in nonproducer cells and both EA and viral antigen (VCA) being synthesized in producer cells. Aside from this indirect effect of Type-C viruses attributable to cell fusion, continued replication of RD-114 virus or FeLV had no appreciable effect on the synthesis of EB virus. (e) Developmental procedures and biologic testing of killed Type-C virus vaccines. Experimental testing of Type-C virus vaccines in animals and the potential usefulness of comparable vaccines in humans for immunoprevention and immunotherapy of tumors requires standardization of procedures for the preparation of killed virus vaccines and adequate measures for determining biologic

activity and safety. Initial studies with various purified murine virus isolates indicated the effectiveness of formaldehyde (1:4,000 at 5°C for 7 days) for obtaining immunogenic killed virus without affecting viral integrity. Additional studies suggest that subviral fractions obtained from agarose chromatography using guanidine-HCl may prove useful as vaccines. One significant finding was that B-propiolactone (BPL) was not effective for preparing killed virus vaccines. Infection of human lymphoblastoid cells, but not RD cells, with BPL inactivated RD-114 virus induced syncytia and resulted after several weeks in the appearance of infectious virus. Whether this was due to residual infectious virus in the BPL treated material or was due to multiplicity reactivation in syncytia has yet to be determined. (f) Production of virus and reagents. This contract has the capability for purifying virus stocks in large scale, and has developed procedures for purification of subviral components and preparation of monospecific antisera for use by the Program. (g) Collaborative programs. These include: (1) identification of indigenous rat Type-C viruses with Dr. V. Klement; (2) thorough characterization of RD-114 virus with Dr. R. McAllister; (3) establishment of radioimmunoassay procedures at the Scripps Clinic and Research Foundation (contract 72-3264), Dr. F. Dixon, and (4) identification of rat gs antigen in cells transformed by BrdU and chemicals with Dr. A. Freeman. The contract, in addition, has carried out a number of collaborative programs with the NCI in-house staff.

(2) Herpesviruses: (a) Sequence of spontaneous EB virus activation. This was studied in a producer cell line (P3HR-1) made resistant to BrdU. The P3HR-1(BU) cells lack thymidine kinase (dTK), but the enzyme appears in cells after spontaneous virus activation. Initiation of the virus activation sequence requires cell DNA synthesis; occurs in dTK negative P3HR-1(BU) cells; and, is inhibited by both ara-C and hydroxyurea. Once activation is initiated, the cells synthesize EA in the absence of additional DNA synthesis. Synthesis of EA is followed by the appearance of dTK and a cycle of DNA synthesis where both cell and viral DNA are made. Synthesis of this DNA is inhibited by ara-C, but not by concentrations of hydroxyurea which inhibit DNA synthesis in non-activated cells. Synthesis of viral DNA is followed by synthesis of VCA and cell death. (b) Activation of EB virus by thymidine analogues. The activation of EB virus in nonproducer cells treated with thymidine analogues (BrdU or IdU) was described. Short-term treatment with analogues results in synthesis of only EA in nonproducer cells, while similar treatment of producer cells results in synthesis of both EA and VCA. In contrast, long-term treatment of nonproducer cells with increasing concentrations of BrdU results in the continued synthesis of EA, VCA, and virus particles. This conversion of a nonproducer cell to a producer cell by analogues probably involves a heritable mutation, while the transient appearance of EA in nonproducer cells treated for short periods with analogues probably involves a mechanism other than mutation. (c) Identification of a critical period of cell DNA synthesis for EB virus activation by IdU. Using cells synchronized by the double thymidine blocking technique, activation by IdU was localized to a period 60 minutes into the cells' S phase. Incorporation of analogue into cell DNA during other periods of the S phase resulted in little, or no, activation above control cell levels. The critical period in the S phase for virus activation occurred prior to the periods of maximum drug

incorporation. The results suggest that cellular DNA, synthesized at approximately 60 minutes into the S phase, contains unique sequences which control repression and activation of the EB viral genome.

Significance to Biomedical Research and the Program of the Institute: The contract is the major source of reagents for typing Type-C tumor viruses. The expertise developed by the contract for purifying viruses and subviral components and for preparing antisera of known specificity has allowed rapid and reliable identification of viruses isolated from various species. These reagents are especially important in instances where virus isolates have been obtained from human tissues. Since the human Type-C virus has yet to be isolated and characterized, it is important to have reagents available for testing isolates to exclude contamination by nonhuman viruses. The contractor is also involved in biological and biomedical studies relating to the mechanism of Type-C virus persistence in cells and the differentiation of events leading to cell transformation. The existing expertise of the contractor and the programs presently under development will prove useful in attempts to develop vaccines for immunoprevention and immunotherapy of human tumors. The herpesvirus studies are directed towards delineating the mechanism of latency in human cells and determining the relevance, if any, of herpesviruses associated with human tumors.

Proposed Course: (1) Continue studies on the mechanism of EB virus latency in human cells with emphasis on determining the state of the repressed viral genome; (2) initiate studies to determine the site of latent herpesviruses in humans and to determine critically whether the presence of herpesviruses is of significance in the etiology of tumors; (3) studies on the ability of Type-C viral vaccines to induce protection against carcinogenic stimuli including transplanted cells and chemical carcinogens; (4) search for Type-C viral gene products by immunological and molecular hybridization methodology in human tissues, concentrating on reagents prepared with primate viruses, woolly monkey, and gibbon ape.

Date Contract Initiated: February 1, 1971

Current Contract Level: $3,210,000

HARVARD UNIVERSITY (NIH-NCI-E-73-3265)

Title: Primary Structure and Synthesis of Avian Leukosis Virus Proteins

Contractor's Project Director: Dr. David W. Allen

Project Officer (NCI): Dr. Padman Sarma

Objectives: (1) To complete the determination of the primary structure of the gs-a and gs-b antigens of avian myeloblastosis virus (AMV), (2) to isolate and sequence additional group-specific (gs) antigens from AMV, (3) to compare the structure of these antigens with those of other avian leukosis viruses, (4) to study the distribution of avian leukosis gs antigens in "leukosis-free" chick embryos.

The information obtained from these studies on the detailed chemical structure of the antigens will then be used directly in two further areas: (5) the chemical synthesis of gs antigens and of selected peptides representing specific regions of their primary sequences, and (6) the use of these synthetic peptides in a detailed study of the immunological properties of the antigens, and in the development of sensitive and specific radioimmunoassays for the detection of antigen in embryonic tissues and in the course of oncogenesis.

Major Findings: Recent advances in the techniques of protein sequencing have allowed substantial sequence determination on small amounts of group-specific antigens from avian and mammalian oncogenic RNA viruses. These techniques have been applied to the structural analysis of the MW 11,000 gs-antigen (gs-b or gs-3) and the MW 20,000 gs-antigen (gs-a or gs-1) from avian myeloblastosis virus.

The use of limited cleavage methods such as tryptic digestion following either lysyl-modification by acylation or arginyl-modification by 1,2-cyclohexanedione, mild oxidative cleavage at tryptophan residues with BNPS-skatole, and CNBr methionyl cleavage, has allowed the isolation of a complete set of overlapping peptides in nanmolar quantities has been accomplished through a combination of a modified manual Edman procedure and high sensitivity automated degradations. These methods have permitted the elucidation of the complete sequence of gs-b. Using these same approaches the major portion of the sequence of gs-a has been completed.

Significance to Biomedical Research and the Program of the Institute: The determination of the primary structure of the gs-a and gs-b antigens of AMV are a prerequisite to the chemical synthesis of one (gs-b) or both of these antigens. The avian myeloblastosis virus is being used as a model system prior to initiating similar studies with the interspecies antigen (gs-3) of mammalian viruses. The availability of a highly specific and purified antibody against the interspecies antigen would considerably aid in finding antigenic fingerprints of a potentially oncogenic virus in human neoplastic tissue.

Proposed Course: (1) Complete the amino acid sequencing of the gs-a antigen of AMV, (2) synthesize gs-b antigen of AMV, and (3) initiate studies on the sequencing and synthesis of the gs-3 (interspecies) antigen of mammalian viruses (in collaboration with Dr. Gilden, Flow Laboratories, Inc. [NO1-CP-33247; formerly 71-2097] and Dr. Sarma, NCI).

Date Contract Initiated: September 18, 1972

Current Contract Level: $67,077

THE JACKSON LABORATORY (NO1-CP-33255; formerly PH43-NIH-NCI-E-67-744)

Title: Natural Occurrence of RNA Tumor Viruses (Genomes) and Host Gene Control of Their Expressions

Contractor's Project Director: Dr. Hans Meier

Project Officer (NCI): Dr. Robert J. Huebner

Objectives: The primary objective of this contract is to achieve an understanding of the mechanisms underlying the genetic determination of susceptibility and resistance to cancer and the RNA tumor viruses. The Jackson Laboratory is a unique source of highly inbred mouse strains. These are used to define specific gene influences on Type-C RNA virus/genome/tumor expressions under natural conditions, and the influence of environmental and other factors (carcinogens, aging) on host gene controls of oncogene and virus expressions.

Major Findings: (1) Tumorigenesis in a strain of dwarf mice (DW/J) and the induction of endogenous Type-C RNA tumor virus group-specific antigen by prolactin. A dwarf mouse mutation has proved very valuable in elucidating the normal and oncogenic functions of the endogenous Type-C RNA viral genome. Homozygotes dwarf mice have reduced body weights and a shortened life span, with primary anterior pituitary endocrine defects and an absence of Type-C genome expression. The normal-sized heterozygote has a high incidence of Type-C group-specific antigen (85%, which increases with age) and a concomitant high tumor incidence. The administration of prolactin reverses growth retardation in the homozygote and organ atrophy. At the same time, it stimulates RNA and protein synthesis and induces the Type-C group-specific antigen. The incidence of tumors in the homozygotes whose lives are prolonged by treatment matches that of the heterozygotes. The dwarf mutation is a classic case of a single gene mutation causing primary endocrine defects providing an excellent assay system for endogenous tumor virus induction by hormones. The development of tumors is closely associated with Type-C virus antigen expression, lending support to an etiologic relationship. The antigen-negative untreated dwarf homozygote does not develop tumors, but this may be related to their short life span. Tumors have not been observed in untreated dwarf mice; possibly they do not survive long enough.

The administration of ovine prolactin has been shown to repair a number of specific biochemical defects in the dwarf mouse. It also stimulates the mammary gland, which is the primary target organ of prolactin. Similar effects were obtained with other pituitary hormones as well, including a remarkable synergism between growth hormone plus prolactin, and thyrotropic and adrenocortico-tropic hormone in terms of weight increase.

Prolactin has been found to stimulate group-specific antigen expression in young, normal dwarf strain mice. In light of these findings, the Jackson group will be using prolactin in other strains of mice which have low or absent group-specific antigen viral expression to see what effect this will have on stimulating not only antigen expression, but Type-C virus and tumors as well. Unlike many compounds used in chemical carcinogenesis, prolactin and other hormones are "natural" biological compounds occurring in all mammalian organisms, and the implications for human cancer could be very important.

(2) Negative inheritance of group-specific antigen of murine leukemia virus. The congenic strain B10.D2(58N) differs from its partner strain C57Bl/10Sn at the histocompatibility-one (H-1) locus and the closely linked Hbb locus. Spleens from weanling mice of the 58N strain are consistently positive for the Type-C viral antigen, while those from the C57Bl/10Sn are invariably negative. Complete replicating virus is not detectably present in either strain. In contrast to results obtained from crosses of AKR/J and C57L/J mice, where expression of virus and antigen was inherited in a dominant fashion, the absence of antigen was dominant in crosses of the C57Bl/10Sn and 58N. Results from backcross and F_2 generations of the latter revealed that presence of antigen was determined by a single recessive gene. The apparent suppression of the viral gene that codes for antigen in heterozygotes suggests that the allele contributed by the C57Bl/10Sn mice produces an inhibitor of viral gene expression. Studies are now in progress in the Viral Carcinogenesis Branch on viral inhibitors and the Jackson strains under study will be invaluable in efforts to isolate and define the inhibitory mechanism.

(3) Relationship between genes controlling murine leukemia virus. In experiments utilizing crosses of high (AKR/J) and low (C57L/J) leukemia virus strains, the Jackson group observed independent segregation of two dominant genes essential to the expression of endogenous leukemia virus, and demonstrated that either of the genes alone could control the presence or absence of complete infectious virus. Further studies are now underway in a number of RNA virus and tumor-defined strains to genes, loci and linkage relationships associated with tumor virus expression, natural and carcinogen-induced cancer.

(4) Host-genetic rescue of murine leukemia virus. Complete infectious murine leukemia virus has been recovered by host-genetic rescue in F_1 hybrids of two virus-free recombinant inbred lines of mice. It was determined that two autosomal dominant genes are required for the presence of complete virus. The two genes apparently regulate the expression of two complementation regions containing the structural genes for latent viral constitutents.

(5) Biochemical characteristics of primary liver cell cultures from mouse fetal livers in chemically defined medium. The diverse metabolic functions of liver have been extensively investigated in whole animals, and in vitro for short periods of time. However, it appeared that some of the systems that regulate the synthesis of certain enzymes and the production and secretion of serum proteins and lipids would be more amenable to study in liver cells maintained in culture for the relatively long periods of time needed for expression of the regulatory systems. A number of biochemical determinations were made. Although only limited aspects of metabolism have been investigated so far, this test system promises to be useful in studies of liver functions and for comparison with hepatomas and nonhepatic cells in culture. The fact that growth occurs in the absence of serum will be helpful in defining the factors that affect liver metabolism and the mechanisms involved.

(6) Additional findings of significance. In general, the risk of developing tumors is greatest in mice which carry the gene for group-specific antigen and virus expression. In addition to determining susceptibility, it appears the tumor types which develop are gene-determined, thus accounting for the great preponderance of specified tumor types in individual mouse strains.

In addition to genetic marker studies, work is now underway to develop biochemical markers to identify stages of transformation. Preliminary findings indicate that unlike normal lymphoid cells, leukemic cells could not be stimulated by phytohemagglutinin to incorporate exogenous thymidine. More refined methods of lymphocyte culture and whole blood culture are now underway which make it unnecessary to sacrifice the animals under study, a fact of importance in analyzing genetic traits.

Studies are now underway in a number of strains and recombinant inbred lines to determine the influence of the gene which determines inflammatory response and papilloma induction by topical carcinogens, and inducibility of an enzyme, aryl hydrocarbon hydroxylase (Ahh) to chemical induction of tumors. It appears that the genes involved must be identical because of their virtually identical strain distribution. Follow-up experiments will be done to determine the specificity of the Ahh-dependent enzyme to learn whether it relates to all polycyclic hydrocarbons and whether other carcinogens work through the same genetic control mechanism.

One of the more significant findings revealed an inverse relationship between teratogenic and oncogenic effects of transplacentally administered 1-ethyl-1-nitrosourea. The fact that certain strains of mice develop both mesenchymal (leukemias) and epithelial (pulmonary adenomas) tumors within a few months provides an excellent assay system for testing newly developed anti-Type-C viral vaccines. In addition, this approach is also being utilized to test a number of suspect compounds, including hormones.

Studies on immune responsiveness and tumor susceptibility were done in 15 inbred strains, 13 mutants and 2 random-bred mouse strains. Based on their antibody responses to tetanus toxin, the strains could be grouped into two categories: (1) early and high responders and (2) late and low responders. The high tumor strains showed little, if any, antibody at 14 days and responded poorly to adsorbed toxoid. This work is being pursued in the original and additional strains selected to provide insight into the nature of the genetic control of immune responsiveness and tumor susceptibility, progression or regression.

Significance to Biomedical Research and the Program of the Institute: This program has contributed much of the basic data concerning the genetic determinants of oncogenesis and the natural expressions of the endogenous Type-C virus. It has pointed up the overwhelming influence of genetic predisposition in the development of natural cancer and susceptibility to environmental carcinogens. The contractor has developed sophisticated systems for defining and locating the genes and loci involved in murine oncogenesis, and virus and antigen expression, and has rescued complete virus through gene complementation by hybridization of two virus-free mouse strains.

Information derived from this research bears direct relevance to the human cancer problem; definition of the heritable nature of cancer is essential to eventual control or prevention of the disease. These well-defined murine systems and tissue cultures derived from them provide valuable vehicles for assay of environmental carcinogens and experimental therapeutic measures.

Proposed Course: (1) Further development, characterization, and uses of recombinant inbred lines. (2) Study of genetic control of endogenous murine leukemia virus. (3) Study of association of the viral group-specific antigen with tumor development. (4) Genetic analysis of a dominant gene inhibiting expression of the group-specific antigen. (5) Mapping of viral structural genes by use of defective sarcoma virus mutants: complementation classes. (6) Additional marker studies for mapping structural genes of viral components. (7) Induction of complete or partial virus synthesis by drugs. (8) Chemical co-carcinogenesis studies. (9) Marker studies (immunogenetic and biochemical). (10) Study of genetic control of embryonic and post-natal, normal and abnormal cell proliferation. (11) Study of the effect(s) of hormonal stimulation of RNA metabolism in dwarf mice, and (12) host genetic rescue of murine leukemia virus.

Date Contract Initiated: May 2, 1967

Current Contract Level: $400,000

MICROBIOLOGICAL ASSOCIATES, INC. (NO1-CP-33248; formerly PH43-NIH-NCI-E-67-697)

Title: Immunoprevention of Spontaneously Occurring Neoplasms

Contractor's Project Director: Dr. Robert M. Nims

Project Officer (NCI): Dr. Gary J. Kelloff

Objectives: This contract continues to place primary emphasis on a systematic study of the parameters involved in spontaneous neoplasia including the incidence, progression and histological types of neoplasms in several strains of laboratory mice, and has concomitantly measured the various Type-C virus expressions, as well as establishing their relationship to the occurrence of the neoplastic state. This experience has enabled the contractor to develop the expertise necessary in these areas to do the large scale studies required in any study of spontaneous neoplasia. In the past contract year, the contractor has established the additional methodologies needed to effectively continue the pursuit of these objectives. This has included the development of continuous flow ultracentrifugation, sucrose density gradient banding of virus, radioisotope techniques including tritiated uridine labelling and reverse transcriptase assays for Type-C virus, protein determinations, efficient means of virus production, techniques for lymphocyte culture and the development of systems for automated data accumulation, analysis and retrieval. These techniques, with the established background of experience has allowed the contractor to initiate studies to determine the extent to which the

incidence and progression of spontaneous neoplasia can be influenced by treatment with viral and cellular vaccines and the relationship of the host immune responses to the observed results.

In addition to the research described above, the contract constitutes an important service to the SVCP. For specimens submitted for testing by NCI supported scientists, the contractor provides services which include storage and inventory of biological specimens, bioassays of leukemia and related virus, tissue culture and serological assays; as well as an extensive histopathology laboratory and pathological diagnostic service.

Major Findings: In studies of tumor bearing BALB/c CR mice the natural Type-C virus expression in the spleen and tumor tissues has been determined serologically by measurement of its species-specific gs antigen and for infectivity by the COMAL test. Extracts of tumor tissue in mice with non-leukemic solid tumor malignancies revealed a significant incidence of gs antigen exceeding that found for normal muscle and connective tissue from the same tumor bearing mice. The gs antigen incidence in spleen extracts from these tumor bearing animals did not exceed that found in similar preparations from age matched controls; however, spleen extracts from mice with neoplasms of the reticuloendothelial system (all involving the spleen) had a highly significant increase of gs antigen incidence when compared to the same controls. These findings as well as others have established an association of Type-C virus with spontaneous neoplasia and have suggested an etiologic role for these viruses. Such data and the extensive background information obtained on the BALB/c CR mouse strain led the contractor to study the effect of exogenously introduced Type-C virus obtained directly from the tumor bearing host on the incidence of the wide variety of spontaneous neoplasms observed in this strain. A total of 2,250 mice of the natural host, BALB/c CR, were inoculated at birth with viral preparations and observed up to 24 months of age. The spontaneous tumors from which the viral inocula were obtained included various histologic types (including lymphoreticular neoplasms, sarcomas, mammary tumors, carcinomas, benign tumors). Of these tumors a total of 51 percent yielded isolatable virus. Significant induction of lymphoreticular neoplasms were observed in those animals receiving virus positive inocula as compared to those receiving virus negative inocula or uninoculated controls. Similar induction of solid non-lymphoreticular neoplasms that are known to occur naturally in the BALB/c CR mouse was not observed. The histologic type of donor tumor that the viral inocula was derived from and the route of inoculation used did not affect these observations.

From these studies it was also found that the BALB/c CR mouse harbors both N-tropic and B-tropic MuLV naturally. Of primary interest were the findings that the N-tropic variant (which grows poorly in BALB/c cells *in vitro*) was found predominantly in early life (>90% for mice under 12 months of age), much less so in later life and very infrequently in the mouse's naturally occurring tumors; whereas the B-tropic variant was rarely found in early life, increased with age, and was the predominant isolate from spontaneous neoplasia. Furthermore, it was subsequently found in the induction studies that the B-tropic variant was oncogenic in its

natural host whereas the N-tropic virus, even in high doses, was not.

A naturally occurring rat Type-C virus has been isolated and characterized with all the parameters of measuring Type-C virus activity. Since this is the first isolate from a spontaneously occurring rat tumor, a small field survey is being conducted to determine its natural incidence in untreated laboratory rats.

A naturally occurring sarcoma virus has been isolated from a spontaneous hemangiosarcoma of the BALB/c CR mouse attesting to the presence, however rare, of naturally occurring sarcoma genomes.

Immunoprevention studies to date have included pilot studies to determine the optimal means of virus production and characterization and the optimal fixation and storage of viruses. These preparations have been evaluated by the XC test, protein determinations, CF titer, and polymerase assay. These studies have been completed and have led to pilot studies to determine the optimal route, dose and schedule of immunization of the BALB/c CR mouse with banded Type-C virus. These studies have been accompanied by studies aimed at establishing techniques to measure the host's cellular immunity; these to date have included evaluation of a footpad assay, in vivo lymphocyte transfer tests, and an in vitro blast transformation assay. All three techniques to date have shown promise and are under development and will be used with techniques already established for measuring circulatory humoral antibody. Additionally, the optimal means of fixation of spontaneously occurring tumors is being studied to be used in cellular vaccine studies.

Significance to Biomedical Research and the Program of the Institute: This contract has contributed significantly in experimental findings from which the oncogene hypothesis was formulated. It has also contributed importantly in ruling out the liklihood of virtual spread of Type-C viruses and has provided much of the large body of information implicating the Type-C viral genome in normal embryogenesis. It has contributed greatly to our understanding of the endogenous Type-C viruses' relationship with its host and its genetic and epigenetic relationship to the host's naturally occurring neoplasm. The contractor's current studies on immunoprevention of spontaneously occurring tumors are designed to answer the question of whether neoplastic disease can be prevented or treated by classical virological vaccine or cellular vaccine techniques. The service aspects of this contract are in direct support of the SVCP.

Proposed Course: (1) Continuation and completion of the characterization and oncogenicity studies of the two naturally occurring variants of MuLV in the BALB/c mouse. (2) Completion of the characterization of the naturally occurring rat Type-C virus and naturally occurring sarcoma virus of the BALB/c mouse described above. (3) Continuation of the studies designed to develop usable techniques for monitoring the host's cellular immunity including the footpad assay, in vivo lymphocyte transfer test and the lymphoblast transformation assay; and completion of the pilot studies to define the optimal preparation, handling, fixation and storage of viral and cellular vaccines and the optimal route, dose and schedule of

host immunization. (4) Initiate immunoprevention studies with viral vaccines. (5) Initiate immunoprevention studies with cellular vaccines.

Date Contract Initiated: November 15, 1961

Current Contract Level: $620,000

MICROBIOLOGICAL ASSOCIATES, INC. and CHILDREN'S HOSPITAL OF AKRON (NIH-NCI-E-70-2068)

Title: Studies of Viruses and Chemicals in the Etiology of Cancer

Contractor's Project Directors:
Dr. Riley Housewright (Tech. Manager)
Dr. Johng S. Rhim
Dr. Ilan Shif
Dr. Paul Price
Dr. Carrie Whitmire
Dr. M. Lee Vernon
Dr. John C. Parker
Dr. Padman S. Sarma (on site NCI assistant project officer)
Dr. Aaron E. Freeman (Children's Hospital of Akron)

Objectives: Project A - Dr. Rhim: Project A is concerned with the development, evaluation, standardization and application of in vitro systems for studying the effects of known and suspected carcinogens in the environment. The systems developed under this Project and Project B will be used to test new murine Type-C RNA viral vaccines vs. transformation effects in vitro. New emphases include the development of sensitive in vitro assay systems for Type-C RNA viruses, including candidate human viruses; characterization of clonal lines of Ki-MSV transformed guinea pig embryo cells in vitro and in vivo; and efforts to detect, isolate and characterize the endogenous guinea pig, rabbit, bovine, dog, feline and rat Type-C RNA viruses and viral genomes.

Project B - Drs. Shif, Price and Freeman: This project complements Project A in developing rapid, sensitive in vitro assay systems for identifying carcinogens in the environment. Particular emphasis is being given to developing in vitro transformation systems for studying the effects of chemical carcinogens on human cells, and "normal" epithelial cell lines for studying the role of Type-C RNA viruses in carcinoma production. In collaboration with other contract and NCI in-house investigators, studies are underway to correlate chemical transformation effects in vitro with tumorigenesis in vivo to establish the validity of individual in vitro cell systems for carcinogenic screening. This project also characterizes all of its own cell lines, as well as those of interest to the program, and serves as an overall repository.

Studies on the biochemistry of transformation are underway in a biochemistry unit established within Project B. In addition, the unit services the

entire contract program (i.e., testing for reverse transcriptase), working in a wide variety of cell lines and animal species.

Project C - Dr. Sarma: This project is concerned with the development of sensitive in vitro assay systems for studying the prevalence and behavior of naturally occurring Type-C RNA viruses in a number of avian and mammalian systems. Present emphasis is on developing immunological and biochemical techniques for the quantitation of virus and antibody for suspected human candidate viruses, and two newly-described simian Type-C viruses (Kawakami, NO1-CP-33242).

Project D - Dr. Whitmire: This program is currently concentrating on the development and testing of Type-C RNA viral vaccines in vivo vs. naturally occurring and chemically induced tumors in a number of mouse strains defined with their respective natural tumor experience and susceptibility to chemical carcinogens. The large body of data generated under this program is providing the baselines essential to evaluate a number of trial vaccines in a variety of mouse strains in terms of prevention and therapy. These studies will serve as direct models for human trials with vaccines designed for human use.

Project E - Dr. Vernon: This project provides electron microscopy support to the entire contract program, contract NO1-CP-33248, and selected specimens referred by Project Officer. This service has been invaluable in natural history studies of the Types-A, -B, and -C RNA viruses in all species under study, and in evaluating the viral switch-on effects of carcinogens, hormones, etc.

Project F - Dr. Parker: Dr. Parker's group provides serological support to this and related programs.

Major Findings: Dr. Rhim: Three crude extracts of city smog, provided under contract PH43-NIH-NCI-E-68-1030, were tested in an NIH Swiss embryo cell line chronically infected with AKR virus, a highly sensitive in vitro transformation system developed in Dr. Rhim's laboratory. All three extracts produced transformation more efficiently than was found in prior parallel experiments in RNA virus-infected hamster and rat cell systems (Freeman), and in in vivo experiments (Kotin). The results in all three laboratories correlated well, revealing transformation and carcinogenic potential in environmental smog. The transformation potential of the crude smog extracts was found to be 100 to 1,000 times higher than could be accounted for by Benzo-a-pyrene (BP), a carcinogen known to be present in smog, pointing up the necessity for better fractionation and analysis of smog to define additional carcinogenic components. The latter, in turn, could be tested in Dr. Rhim's and Dr. Freeman's in vitro systems.

Dr. Rhim and his group have produced morphological transformations in a guinea pig embryo cell line using the Kirsten murine sarcoma virus (Ki-MSV). The transformed cells contained both infectious Ki-MSV and gs antigen, and produced tumors when transplanted into newborn homologous hosts. The virus derived from the transformed cells, however, produced cell transformation in half the time of the original Ki-MSV, indicating the virus may have acquired new properties.

Transformation of guinea pig lines was also effected using DMBA and BrdU. The latter compound also switched on a virus presently being characterized. The virus particle resembles, but is not identical to, the guinea pig leukemia virus.

Studies to determine the possible role of the Type-C virus in DNA tumor virus oncogenesis yielded several interesting observations. Dr. Rhim's group found that transformation by the DNA virus, in this case polyoma, was facilitated by the use of established rat cell lines as compared with primary or secondary cultures; transformation was further accelerated in rat cells chronically infected with RLV. In another system, polyoma-transformed hamster embryo cells regularly led to the presence of hamster leukemia virus gs antigen after transplantation into newborn hamsters; similar observations were made in an NIH Swiss mouse system. The foregoing evidence supports the premise that the Type-C RNA virus genome is the oncogenic determinant of DNA virus cell transformation as well as of chemical transformation.

Drs. Freeman, Price, Zimmerman and Shif: In collaborative study with Drs. Elizabeth and John Weisburger (NCI Carcinogenesis Area), this group tested some 30 compounds of known carcinogenic activity, as well as six unknown compounds whose oncogenic activity in vivo was unknown, in an RLV-infected Fischer rat embryo cell system developed under this project. The correlations with the Weisburgers' in vivo results were nearly perfect, providing strong support for the validity of the in vitro systems developed on this contract for large scale screening of environmental compounds for carcinogenic potential.

Fractions of smog condensate and the four major cannabinoids found in marijuana were also tested in the rat system. Fractions of smog condensate (done in parallel with similar tests in Dr. Rhim's laboratory) were found to be very carcinogenic; and one fraction of marijuana, the delta ^{9}THC, was found to be weakly carcinogenic. As noted above, the smog fractions under study represented crude extracts. Efforts are currently underway under contract PH43-NIH-NCI-E-68-1030 to develop more sensitive fractionation procedures; these materials will be provided to contract 2068 for further study in order to define the specific carcinogenes in smog, and their effects, singly and synergistically, in vitro.

This group previously reported the transformation of hamster embryo cultures by 3-methylcholanthrene (3-MC) and cigarette smoke condensates. Although the original as well as the transformed cultures were free of hamster Type-C virus (HaLV), extracts of hamster tumors induced by the transformed cells were usually CF positive. These observations have now been extended to chemical, viral and spontaneous activation of antigen expression in vivo and in vitro in a number of hamster strains. These studies support the conclusion that the hamster leukemia virus is ubiquitous in hamster cells but is virtually never expressed under natural, normal conditions. HaLV has been isolated by this group from tumors induced by chemically and polyoma transformed hamster cells, although the cells themselves were demonstrably free of virus both before and after transformation. Interestingly, tumors produced by spontaneously transformed cells were serologically

negative for HaLV antigens; however, cell lines derived from these tumors contained the virus.

Studies were undertaken in a wild mouse (feral) system with similar results, demonstrating the universal presence of the Type-C viral genome in a non-inbred animal population.

In a series of timed sequence experiments in which low and high passage Fischer rat embryo cultures were treated with 3-MC before or after inoculation with RLV, it was found that transformation in low passage cells was dependent on the presence of virus at the time of chemical treatment, but that at high passage levels 3-MC could transform the cells in the absence of virus, although not as efficiently.

Uninfected low passage rat cultures treated with BrdU were transformed with 3-MC. The transformed cultures were positive for reverse transcriptase but virus particles have not as yet been demonstrated.

Dr. Zimmerman, using labeled 3-MC, found no difference in the uptake kinetics of virus infected and uninfected cells at low passage levels, but at higher passage levels virus infected cells accumulated more chemical. However, the passage level and viral infection were demonstrated to have no effect on the persistence of the chemical in cells.

Cellular contamination is a major problem in transformation studies. In collaboration with Dr. Samuel Baron (NIAID), Dr. Freeman's group developed an interferon assay for determining the genus of origin of cell cultures. This short, simple test involves the interferon-mediated protection of cells growing in vitro from CPE produced by vesicular stomatitis virus (VSV) or Sindbis virus. Along with karyotype analysis, the test can determine routinely the genus type of most cell cultures.

As noted, Dr. Freeman assumed the position of Research Director at the Children's Hospital in Akron. In collaboration with Dr. Howard J. Igel, his primary effort will be devoted to developing a human cell transformation system for studying carcinogenic and potentially carcinogenic chemicals utilizing the principles and methodology. It appears Drs. Igel and Freeman have successfully transformed a human cell line with chemicals, but results are preliminary. Dr. Freeman is particularly well qualified to undertake this task. He has developed or contributed to many of the viral-chemical in vitro model systems, and has recently written a definitive paper setting down the criteria and determinants of cell transformation (JNCI). As a member of a hospital staff, Dr. Freeman will have access to human cancer and control tissues representing a number of different cancers under diverse conditions.

Dr. Ilan Shif, a microbiologist with a wide background in chemical carcinogenesis, recently joined Project B from the laboratory of Dr. Frederick Bang at Johns Hopkins University. Within the past several weeks, Dr. Shif has devised a number of experiments designed to define the role of the Type-C RNA virus in chemical carcinogenesis using biochemical, genetic and virologic approaches. One approach of particular interest is being done

in collaboration with Dr. Aaronson (NCI). The effects of chemical carcinogens will be evaluated in combination with a number of temperature-sensitive RNA viral mutants to determine whether cell transformation can be effected with non-transforming mutants. Dr. Shif is highly regarded by his co-workers, and he promises to add an important dimension to the overall contract program.

In addition to the research projects underway, this group performs important service functions by establishing, maintaining and storing cell lines of particular interest, particularly those which prove labile in less experienced laboratories.

Dr. Zimmerman runs reverse transcriptase assays for all of the professionals on this contract and a sister contract with MAI (NO1-CP-33248).

Dr. Sarma: This group has addressed itself to delineation of the natural histories and characterization of Type-C RNA viruses of man, rat and the mouse. Working in close collaboration with a number of contract and NCI in-house groups, Dr. Sarma and his associates have made some very significant observations during the past contract year.

Dr. Sarma played a key role in characterization of a candidate human Type-C virus, RD-114, in collaboration with a number of other contractors (Drs. Robert McAllister, Murray Gardner, Raymond Gilden, Masa Hatanaka and Steven Oroszlan). This virus, derived by passaging a human rhabdomyosarcoma line through fetal cat brain demonstrated unique properties not shared by the feline Type-C viruses or isolates from other mammalian species. Subsequently, Dr. Sarma, in collaboration with Drs. McAllister and Gilden, and members of the NCI, Drs. George Todaro, David Livingston and Peter Fischinger, were able to rescue a similar agent (CCC virus) from a number of feline cultures. The evidence would now indicate that the RD-114 or CCC is the endogenous cat Type-C virus. The RD-114 may represent a hybrid of a cat virus with some human information on the viral envelope which would still be of intrinsic interest in terms of future human cancer vaccines.

Studies of envelope antigen relationships in feline Type-C viruses yielded information on the occurrence of divergent serotypes. The studies were performed using a viral interference test. The results suggested that feline Type-C viruses contain distinct viral envelope antigens, similar to those described by Vogt and others for the avian Type-C viruses. Many field strains of feline leukemia as well as sarcoma viruses were found to occur in nature as antigenic mixtures. These studies permitted subgroup classification of the feline viruses and is providing valuable clues as to the mechanism of infection of feline cells. It would also appear from these developments that the highly prevalent feline leukemia virus (FeLV) is spread epigenetically in the cat.

In previous studies, Dr. Sarma and his associates found that overt expression of the endogenous rat Type-C virus was rare. Present studies were more successful. Established rat cell cultures, including two Rous sarcoma virus transformed cultures, were found to spontaneously release low quantities of

non-infectious rat Type-C virus. Virus production was consistently enhanced by treatment of these cultures with IdU and BrdU. Similar viral induction efforts with human lines derived from cancer tissue have been unsuccessful, although there was some evidence of reverse transcriptase enzyme activity. In view of the normally repressed endogenous rat Type-C virus, the isolation of a Type-C virus recovered from an untreated established tissue culture line of Wistar-Furth rat embryo fibroblasts by Bergs and his associates was of particular significance. Dr. Sarma's group characterized the biological, immunological and biochemical properties of the virus which was demonstrated to have the rat gs-1 antigen, 70S RNA and reverse transcriptase, and was found to be infectious for a variety of rat embryo cultures. The ready availability of high titers of this virus now makes it possible to produce the gs-1 antigen and corresponding antisera, which, in turn, will be of great value in studies of the natural history of the Type-C rat viruses and for routine assays in vitro by a CF antigen induction test.

Dr. Sarma's group has also been collaborating with Dr. Adi Gazdar (NCI) on a new strain of murine sarcoma virus (Gazdar MSV) isolated from an NZB/NZW mouse which was found to differ substantially in its biologic and antigenic interactions with the heterologous hamster host than other known MSV's. The latter fail to induce the mouse leukemia viral gs antigens or corresponding antibodies in the hamster, despite the incorporation into the transformed hamster cells of rescuable MSV genome. The Gazdar MSV was found to induce mouse gs-1 in the hamster tumors produced. Thus, hamsters bearing primary or transplanted tumors consistently developed the mouse leukemia gs CF antibodies. These hamster antisera are proving as useful as MSV rat sera for detection and assay of murine leukemia gs antigens and the noncytopathogenic mouse leukemia viruses for use in the COMUL test.

In one of its more important efforts, Dr. Sarma's group has demonstrated the presence of the avian Type-C viral gs antigen (gs-a) in RIF-free chick embryos. In collaboration with Dr. D. Allen (Massachusetts General Hospital), Dr. Sarma found the gs-a largely confined to the microsome fraction of the cell. Their studies also suggested that although part of the antigen detected may be present in non-infectious C particles present in the microsome fraction, much of the antigen may be present in other cell structures. The exact cellular locus and function of the gs-a viral gene product is currently under study. These observations, taken in context with similar findings reported in murine embryos (Huebner, et. al.) supports the evidence that Type-C viral genes play a role in normal ontogeny as well as oncogenesis.

Dr. Whitmire: Perhaps some of the most important developments in the SVCP in terms of cancer control were reported by this group during the past contract year. Major emphasis during this period was focused on the effects of anti-viral treatments on chemical carcinogenesis in mice. These approaches were based on evidence that Type-C RNA virus and group-specific antigens were present in virtually all chemically induced tumors in 20 strains of mice, implicating the RNA viral genome in chemically induced oncogenesis. Studies included viral immunization, interferon and thymosin.

Viral Immunization: A single injection of two "natural" Type-C RNA viruses, one from a radiation-induced leukemia virus (RaDLV) and one from a wild MuLV derived from a 3-MC-induced tumor, reduced the incidence of sarcomas from 86% to 33% and 37%, respectively, in the C57BL/6 mouse. A laboratory strain of the Type-C virus, RLV, on the other hand, decreased the effect of 3-MC induction of tumors from 78% to 50% in the BALB/c CR mouse. These results, while all very significant, demonstrate a markedly enchanced response with the wild type Type-C viruses. It would thus appear a number of specific antigens are not shared by the wild and laboratory strains of the Type-C viruses, an important consideration in evaluating model systems and designing vaccines or other anti-viral cancer therapy. These experiments were of an exploratory nature and consequently were limited in scope. Experiments are now being intensively pursued in this and other laboratories in a number of mouse strains with a variety of vaccine preparations, dosages and procedures, to test the efficacy of these vaccines in (1) preventing tumors, and (2) viral immunotherapy against induced and spontaneous tumors. In addition to the Type-C RNA murine vaccines being tested, vaccines of the hamster, rat and candidate human Type-C viruses are being prepared for trials, as well as a murine mammary tumor virus (MTV) vaccine.

Interferon studies: In preliminary experiments, CF-1 mice were treated with mouse serum interferon beginning at 5 days of age, followed by inoculation of 3-MC at 8 days of age. Treatment with increasing doses of interferon was continued for 4 months. The interferon treatment not only inhibited sarcoma induction at the site of 3-MC treatment but also prevented the development of expected lung tumors. However, interferon did not reduce the incidence of levels of gs antigen detected by the CF test, indicating the inhibitory action of interferon may be specific for the viral oncogene. This would be consistent with reports from other laboratories that interferon inhibits viral induced neoplasia with or without reduction in viral replication. The CF-1 mouse, a non-inbred strain, was chosen for this experiment because it had been shown to harbor high levels of Type-C RNA gs antigen and infectious virus. Further experiments are now in progress to determine the effects of interferon on 3-MC sarcoma induction in several genotypically different strains of mice having different endogenous Type-C virus expressions.

Dr. Vernon: Over 800 specimens were submitted for electron microscopy from project directors on this contract and contract NO1-CP-33248, as well as occasional specimens referred by the Project Officer. This service provides important support to a number of key projects and has proved enormously efficient and valuable. In addition, Dr. Vernon recently completed a study in which she demonstrated budding Type-C particles in 85 percent of the visceral tissues of embryo or newborn mice. The data offered supportive evidence for the endogenous nature of the viral genome and a role in normal development.

Dr. Parker: During the past year, approximately 8,000 specimens were received, on which various serodiagnostic tests (MSV, polyoma and LCM) were performed in support of contract operations NIH-NCI-E-70-2068, NO1-CP-33248 and PH43-NIH-NCI-E-68-1030 and the Viral Carcinogenesis Branch laboratories. This group has competence in complement fixation, hemagglutination

inhibition and XC testing. In addition to its service function, the laboratory did a comparison study to compare the hemagglutination-inhibition (HI) test with the complement fixation (CF) test to determine the better assay for detecting evidence of polyoma infection. The HI test was found to have much greater validity. The CF test is most efficiently used for diagnosis of recently acquired infection, and the HI test for past infections since HI antibodies persist indefinitely in recovered animals.

Significance to Biomedical Research and the Program of the Institute: This program has contributed profoundly to the development of new approaches and concepts, which in turn have provided new insights into the nature of the oncogene, and mechanisms of viral and chemical carcinogenesis. Specifically, the *in vitro* tissue culture systems for assaying environmental carcinogens will make possible large scale, rapid screening of environmental compounds, drugs, food additives, etc., at a fraction of the cost of equivalent tests *in vivo*. In view of the screening burden posed by literally thousands of new compounds each year, the newly developed tissue culture systems represent the only feasible hope of recognizing possibly dangerous carcinogens before the public has been harmed.

The development of viral vaccines capable of immunizing against chemical carcinogenesis, and optimistic results with mouse interferon against chemically induced tumors in animals, have important implications for cancer prevention and control in the immediate future. The initial tests have established that the vaccines which confer greatest protection are those derived from "natural" Type-C viruses, leading to the conclusion that immunity is antigen-specific. These results are particularly encouraging with the availability of new candidate human Type-C viruses. Plans are now underway to produce inactivated vaccines for possible trials in humans within the next several months.

In summary, this contract is a core part of a coordinated, comprehensive, targeted research program to define the role of the Type-C RNA genome as a determinant of oncogenesis, to develop diagnostic systems for screening carcinogens, and most importantly to continue studies with vaccines and interferon for cancer prevention and control.

Proposed Course: Work will be continued on (1) the development, evaluation and standardization of *in vivo* and *in vitro* systems for studying the effects of known and suspected environmental carcinogens; (2) development and application of sensitive *in vitro* assay systems for studying the natural history of Type-C tumor virus infection and its relationship to carcinogenesis in a variety of avian and mammalian systems; (3) development and testing of Type-C virus vaccines to evaluate their efficacy in prevention of spontaneous and chemically-induced tumors in genetically defined mouse strains; and (4) electron microscopic and serological studies in support of the above and related projects within the SVCP.

Date Contract Initiated: February 1, 1970

Current Contract Level: $2,080,000

PRINCETON UNIVERSITY (NIH-NCI-E-71-2372)

Title: Studies on Surface Alterations in RNA Tumor Virus Transformed Cells

Contractor's Project Director: Dr. Max M. Burger

Project Officer (NCI): Dr. Gary J. Kelloff

Objectives: To study the surface alterations in transformed cells, as manifested by agglutinability with plant lectins.

Major Findings: Several mouse lines (TG-8 and another 3T6 line) and three different human lines (HESM, WI-38 and RD_O) were studied after infection with MSV and RD-114 and MSV(RD-114) viruses. TG-8, 3T6, HESM and WI-38 agglutinated less than RD_O and that in turn less than the RLV and those less than the MSV and RD-114 infected cells. So far, only increased agglutination with wheat germ agglutinin and Concanavalin A was demonstrated. Other lectins have not been studied so far.

It was shown that escape from contact inhibition of growth, usually seen in transformed fibroblasts and mimicked by a brief treatment with low doses of protease, can be inhibited with low doses of dibutyryl-c-AMP. This suggests that the signal to escape from growth control may be a transient decrease in total intracellular cyclic-AMP. Such a drop was earlier found not only permanently in transformed cells, but also transiently after protease treatment of the cell surface which leads to growth stimulation.

A change in the cell surface membrane of normal cells was found during mitosis. The change noted was similar to that found permanently in transformed cells. Additional studies showed a complete disappearance of unbound intracellular cyclic-AMP during mitosis.

Other agglutinins will be tested on the RNA virus transformed cells to see whether it is possible to find agglutinins which differentiate between RLV and MSV infected cells. A lectin binding assay will be applied to RNA virus transformed cells. The assay is not yet fully completed and some parameters will still have to be studied before it can be applied to RNA virus transformed cells *in toto*.

Significance to Biomedical Research and the Program of the Institute: These studies aid in understanding the viral-induced changes that occur in the molecular architecture of the cell surface upon transformation by RNA viruses. The surface changes of transformed cells are undoubtedly involved in the phenomena of unrestrained growth and metastasis of tumors.

Proposed Course: Dr. Burger and his laboratory have moved to the University of Basel, Switzerland. The contract with Princeton will be moved to the University of Basel. No additional funding is contemplated.

Date Contract Initiated: June 28, 1971

Current Contract Level: $48,435 (funds carried forward from FY '72)

SAINT LOUIS UNIVERSITY (PH43-NIH-NCI-E-67-692)

Title: Search for Viral-Specific Genetic Material in Human Cancers and Studies on the Mechanism of Oncogenesis by RNA and DNA Tumor Viruses

Contractor's Project Director: Dr. Maurice Green

Project Officer (NCI): Dr. Robert J. Huebner

Objectives: This research program is aimed at understanding in detail the mechanism of cell transformation by RNA and DNA tumor viruses, applying new information on viral carcinogenesis and on the molecular biology of human cells directly to the problems of human cancer, and searching for inhibitors of polymerase that may control the expression of cancer specific genetic information.

Major Findings: (1) Two structurally distinct forms of RNA-directed DNA polymerase (RDDP) were isolated from avian myeloblastosis virus (AMV). In addition to RDDP activity, both enzymes had RNase H activity which degraded the RNA moiety of RNA-DNA hybrids. One form had two subunits, alpha and beta, with a molecular weight of 65,000 and 105,000, respectively. The other had a single subunit, alpha with a molecular weight of 65,000. Both enzymes have similar antigenic determinants and were not distinguishable by differential responses to several different RNA and DNA templates. These data suggest that alpha which contains both RDDP and RNase H activity is derived by dissociation of alpha-beta; the function of the beta subunit is unknown.

Ten RNA tumor viruses grown in five different host cell species were shown to have RNase H activity while three non-oncogenic viruses from three different virus groups possesses no RNase H activity. RNase H, therefore, may be a ubiquitous activity in the virions of RNA tumor viruses and serve as a sensitive diagnostic tool for detection of RNA tumor virus particles.

(2) The 3'-terminal polynucleotide of MSV(MLV) 70S viral RNA appears to be $(A)_nA$-OH, as shown by periodate oxidation-tritiated borohydride reduction, RNase digestion, and oligo(dT)-cellulose chromatography. All MSV(MLV) and MLV-producing cells contain distinct 35S and 20S viral RNA species while non virus-producing transformed hamster and mouse cells contain either 35S or 26S RNA. The viral 70S RNA genome contains 35S RNA, no 20S RNA, and a small amount of 5S and 7S RNA. Hybridization competition suggests that 35S RNA from virus-producing cells contain nearly all sequences present in the 70S RNA genome.

Both intracellular MSV(MLV) 35S and 20S viral RNA species are retained on nitrocellulose filters, providing a means for extensive purification of intracellular virus-specific RNA, and suggesting that intracellular viral RNA species contain poly(A) tracts.

(3) The in vivo and in vitro translation of RNA tumor virus mRNA into virus-specific proteins was studied. Antibodies to disrupted MSV(MLV) were used to study the in vivo synthesis of viral polypeptides in the MSV

transformed virus-producing rat cell line, 78A1. Intracellular viral proteins were labeled with radioactive amino acids isolated by immunoprecipitation and analyzed by electrophoresis in SDS polyacrylamide gels. In addition to the major virion polypeptides, proteins of cellular origin were associated with virions. These cell derived polypeptides are found in the membrane fraction of the cell and probably have a higher turnover rate.

Virus-specific RNA was detected in free- and membrane-bound polyribosomes by hybridization with the ^{3}H-DNA product of RDDP. Membrane-bound polyribosomes contain four times as much virus-specific RNA per unit of RNA as did free polyribosomes. A peak of virus-specific RNA and nascent viral polypeptides was detected in polyribosomes with the sedimentation rate of about 350S.

A cell-free protein synthesizing system composed of free- and membrane-bound polyribosomes from virus-producing cells was established. These polyribosomes synthesize two major viral polypeptides in vitro.

(4) A system for rapid transformation of 3T6 cells by MSV(MLV) was used to analyze the molecular events of cell transformation. Most cells are infected and gave rise to transformed foci after exposure to 1-2 ffu for 60 minutes. Virus-specific DNA was synthesized and found on chromosomes by 5 hours after infection, providing the first direct evidence for the functioning of RDDP and the association of viral DNA with cellular chromosomes. The transcription of virus-specific RNA was detected at 6-7 hours, virus replication by 14-15 hours, and surface alterations by 22 hours after infection.

(5) Chinese hamster ovary cells were induced to produce virus-like particles by cyclic AMP. 3T3 cells cryptically transformed by the Kirsten strain of MSV were induced by treatment with BrdU and the system was optimized for molecular analysis. Human embryo cells in culture were induced to form RNase sensitive particles with DNA polymerase activity by altered culture conditions.

(6) The search for inhibitors of polymerase molecules to control gene expression of viruses and cells concentrated on two series of derivatives: (1) Twenty-nine derivatives of rifamycin SV with substituted cyclic amine side chains in position 3 of the ansa ring are strong inhibitors of RNA-directed and DNA-directed DNA polymerase activity of RNA tumor viruses of murine, feline and avian origin, especially those with cyclohexyl and cyclohexyl alkyl substituents. Cell transformation and cellular DNA polymerases were inhibited. Studies on mechanism show that the drugs interact with the enzyme and not with the template or substrates. Inhibition is non-competitive with regard to template and the drug does not affect the affinity of the viral polymerase for substrate molecules. A cooperative effect exists among drug molecules and there is evidence suggesting that a step in the initiation of DNA synthesis may be blocked. (2) Some fluoranthrene di-substituted cationic derivatives and analogs are strong inhibitors of RDDP and of cell transformation. Here the mechanism of action probably involves specific binding to the template.

(7) End fragments of molecular weight 1-2 x 10^6 daltons prepared from the DNA molecules of human adenovirus 2 and simian adenovirus SA7 contain only 15-30 percent of the sequences present in the entire genome, thus eliminating the possibility that adenovirus DNA molecules are circularly permuted. Reassociation kinetics were utilized to show that the multiple viral gene copies are present in cells transformed by adenovirus 2, 7 and 12. Hybridization-competition experiments show that distribution of cell-specific mRNA in the nucleus and cytoplasm alters after infection with adenovirus 2. The presence of poly(A) tracts in adenovirus mRNA was used to develop a method for the purification of milligram amounts of adenovirus mRNA. A transcription complex was isolated from adenovirus infected cells and shown to transcribe adenovirus RNA in vitro and to possess poly(A) polymerase activity. Cytological hybridization was used to demonstrate the association of adenovirus DNA with chromosomes of transformed cells. Nuclear membranes from adenovirus transformed cells were isolated and found to possess nascent adenovirus DNA.

(8) The transcription of the herpesvirus genetic information was demonstrated in virus-free summer tumors of the frog Lucké adenocarcinoma, thus providing strong evidence for the viral etiology of this tumor. The hamster cell line transformed by ultraviolet inactivated herpesvirus Type-2 by Duff and Rapp was shown to transcribe virus-specific RNA sequences that hybridized both with human herpesvirus Type 2 and Type 1.

(9) Human cancers were analyzed for RNA sequences specific for RNA tumor viruses. Approximately 700 cesium sulfate density gradient centrifugations and numerous hydroxyapatite analyses were performed with the MSV(MLV) and RD-114 ^{3}H-DNA products. Many of RNA specimens from human tumors of several types formed distinct complexes with the ^{3}H-DNA products of MSV(MLV). However, RNA from embryonic human cells and normal tissues also formed complexes, although in some cases to a relatively lower extent. RD-114 ^{3}H-DNA formed complexes with Hodgkin's lymphoma RNA. With certain tumors and with certain ^{3}H-DNA preparations, DNA-RNA complexes were found with the properties of true DNA-RNA hybrids; but the nature of many other complexes which were detected on Cs_2SO_4 is still unknown. The DNA from cat cells contains 220 copies and RD-114 cells contain 30 copies of some RD-114 ^{3}H-DNA sequences.

Significance to Biomedical Research and the Program of the Institute: Within the Special Virus Cancer Program sequential scientific activities, which must be conducted prior to the development of a means for the prevention of virus-induced neoplasia in man, include (a) detection of the virus or virus product in human materials, (b) identification of the virus as a known or new agent, (c) selected biochemical characterization of the agent, and (d) verification of oncogenicity for man. The basic research on the molecular biology of normal and virus infected cells may provide the basis for understanding the mechanism of animal virus infection and carcinogenesis, and for developing a rational chemotherapy for viral diseases and cancer.

Proposed Course: The following investigations are planned: (1) The analysis of human cancers for (a) viral sequences of primate, murine and

feline origin, (b) DNA polymerase-template complexes of the type found in RNA tumor viruses (c) DNA polymerase activities which respond to templates specific for RDDP, such as methylated polynucleotides. (2) The study of the specificity antipolymerase molecules currently under study, usefulness for chemotherapy, mechanism of action, and the search for new anti-polymerase agents. (3) The mechanism of RNA tumor virus replication and cell transformation utilizing as model cells which are rapidly transformed by infection with the Harvey strain of MSV(MLV) and cells that are induced synchronously to produce virus particles. (4) The characterization of the purified polypeptide subunits of AMV RDDP with regard to chemical, physical and enzymological properties and comparison with purified RDDP of other RNA tumor viruses. (5) The analysis of transcription and regulation of RNA and DNA tumor virus genetic information in vivo and in vitro by transcription complexes, the characterization of viral RNA species and their partial base sequences. (6) The biosynthesis and regulation of RNA and DNA tumor virus-specific proteins using appropriate in vitro translation systems. (7) The large scale propagation and isolation of appropriate tumor antigens and transforming proteins from cells transformed by RNA and DNA tumor viruses and study of their mechanism of action. (8) The application of basic information derived from studies on tumor virus replication and cell transformation to the analysis of the neoplastic lesion in human cancers.

Date Contract Initiated: March 20, 1967

Current Contract Level: $1,200,000

SALK INSTITUTE (PH43-NIH-NCI-E-67-1147)

Title: Interactions Between RNA Tumor Viruses and Other Viral Agents

Contractor's Project Director: Dr. Walter Eckhart

Project Officer (NCI): Dr. Stuart A. Aaronson

Objectives: To determine whether infection with a DNA virus induces the appearance of RNA tumor virus RNA in infected cells.

Major Findings: These studies are being approached in the following two ways: first, to determine whether infection by a murine leukemia virus overcomes any temperature-sensitive mutation in polyoma; and, second, to determine whether polyoma infection induces the appearance of RNA tumor virus RNA in cells carrying inducible viral genomes.

The work conducted thus far on the synthesis of endogenous RNA tumor virus RNA in BALB/3T3 cells infected with polyoma, or transformed by SV40 viruses suggest that new RNA virus-specific sequences are transcribed after polyoma infection or SV40 transformation. Further studies are in progress to determine whether the new RNA sequences correspond to "oncogenic" information. DNA probes containing sequences corresponding to the sarcoma information of murine sarcoma or leukemia viruses are being used.

Significance to Biomedical Research and the Program of the Institute: The expression of genes (viral or cellular) causes an alteration of cell growth. If it is possible to understand the factors that govern expression of RNA virus genes in the mouse system (where the molecules involved can be identified), much of that information should be applicable to human cells. Thus, these studies serve as a model for the human situation.

Proposed Course: (1) To characterize the RNA sequence of the endogenous RNA virus in BALB/3T3 cells, and of hamster sarcoma viruses, in order to identify the sequences expressed in polyoma infected and transformed cells; and (2) to isolate cell mutants or variants that have different abilities to allow expression of an endogenous RNA virus, and to look for the biochemical basis of the differences.

Date Contract Initiated: June 5, 1967

Current Contract Level: $35,000 (funds carried forward from FY '72)

SALK INSTITUTE (NIH-NCI-E-72-3207)

Title: Growth Regulation of Normal and Transformed Cells and Immunological Approaches to Tumor Rejection and Prevention

Contractor's Project Director: Dr. Edwin S. Lennox

Project Officer (NCI): Dr. Robert J. Huebner

Objectives: (1A) Use of antigenically modified tumor cells as immunogens (Drs. Lennox and Dulbecco), (1B) development of a chemical anti-tumor vaccine (Dr. Shier), (2) role of the cell surface in escape from immunological surveillance (Dr. Nicolson), and (3) serum factor requirements of temperature-sensitive viral-transformed mammalian cells.

Major Findings: (1) A. Use of Antigenically Modified Tumor Cells as Immunogen. Two ways have been sought to develop immunogenic forms of tumor cells. In one, a rat sarcoma, polyoma induced, that grows in tissue culture and produces tumors in Wistar-Furth rats (PW-13 from Sjögren) was used. From this line several lines were isolated by cloning. Mass cultures prepared from the isolates were tested for ability to produce tumors in adult rats. Several isolates were like the parental line and gave progressively growing tumors when 10^5 or more cells were injected subcutaneously. One isolate, however, yielded no tumors at 10^5 or 10^6 cells and at 10^7 gave tumors that later regressed. The capacity of this isolate to immunize against subsequent implantation of the tumorigenic lines is under study. In addition, in order to seek ways of selecting such variants, their agglutinability by a panel of lectins is being compared with the parental line and attempts are being made to correlate with immunogenicity.

The other approach has been to explore the utility as immunogens of formaldehyde treated tumor cells. The rat tumor line, PW-13, and a mouse mastocytoma P815 have been treated with formaldehyde and tested for their

ability to block growth of subsequently implanted tumor cells. In both cases, the response is complex. In some animals tumor growth was long delayed but eventually resumed. In others growth was immediately stimulated but later slowed down.

Preliminary data from an antigenic analysis of the cell mediated immune response to rat lymphomas induced by the murine leukemia virus suggest that while purified whole MSV and certain fractions block complement dependent lysis of cells by antiserum, they seem to stimulate cell mediated immunity in vitro.

(1) B. Development of a Chemical Anti-Tumor Vaccine. A synthetic antigen designed to elicit an immune response with the same specificity as wheat germ agglutinin has been tested extensively with a solid syngeneic tumor in rats. It gives a statistically significant modification of tumor growth rate. Since this antigen has a structure that is typical of one of the major classes of glycoprotein except that it has an unusually short sugar chain, another antigen was prepared with a short sugar chain typical of another major class of glycoproteins. Testing to date indicates that it is at least as effective as the first antigen. Careful, extensive work indicates that these antigens do not cause significant suppression of the immune response to a second antigen.

(2) Role of the Cell Surface in Escape from Immunological Surveillance. Recently developed electron microscopic techniques were used to study the topographic distribution of ferritin-conjugated Concanavalin A (Fer-Con A) on normal, virus-transformed and protease-treated 3T3 fibroblasts. All lines of cells bound equivalent amounts of ^{125}I-Con A, but SV40-transformed 3T3 (SV3T3) and trypsinized normal 3T3 cells had topographic distributions of Fer-Con A that were more clustered than found on normal cells. Subsequently, the clustered agglutinin sites were related to the agglutinability of the cell lines by experiments designed to identify the distribution of Fer-Con A sites directly involved in cell agglutination. This indicated that proteolysis converts normal cell surfaces to a topographic state similar to that maintained by oncogenic virus transformation and that membrane topography is related to cell agglutinability. We have also been developing methods to determine the topographic distributions of anionic residues on normal and tumor cell membrane surfaces. Initially, erythrocyte and MOPC-70A membranes were studied for pH-sensitive changes in membrane gross morphology and topography. Using erythrocyte ghost membranes as a model system, we found that shifting the pH to 4.5-5.5 modified ghost morphology and membrane topography in a completely reversible manner.

Several tumor specific plant agglutinins were purified by affinity techniques and characterized. One of these, Ricinus communis agglutinin was found to be, in fact, two agglutinins (RCA_{60}, mol. wt. 60,000 and RCA_{120}, mol. wt. 120,000) with slightly different saccharide specificities. Further characterization revealed that the two R. communis agglutinins shared some immunological determinants, peptides and subunits, but also had some distinct ones.

Using RCA_{120}, the contractor investigated the effects of cell density, contact and enzyme treatment on the quantities of RCA_{120} sites on 3T3, SV3T3 and 3T12 cell lines. Rapidly growing 3T3 cells (sparse, not touching) bound one-half to one-third the number of RCA_{120} molecules compared to density-inhibited (confluent) 3T3 cells. The increase in RCA_{120} sites occurs just at the point when the cells make contact, and then no further quantitative changes occur. SV3T3 and 3T12 cell lines did not show quantitative changes during cell growth and contact and the number of RCA_{120} sites remained constant. Neuraminidase "unmasked" RCA_{120} sites (increasing the number of sites 2-3 fold) on these cells and also on murine S-49 lymphoma cells, but proteolytic enzyme treatment decreased slightly the number of RCA_{120} sites. Neuraminidase and protease treatment failed to "unmask" Con A sites on these cells.

(3) Serum Factor Requirements of Temperature-Sensitive Viral-Transformed Mammalian Cells. Control of the length of the S phase of the cell cycle by the serum concentration in the medium has been used as a criterion for determining whether the temperature-sensitive transformed cell lines are "transformed" at the non-permissive temperature. The results indicate that by this criterion the cells are "transformed" at both permissive and non-permissive temperatures.

Significance to Biomedical Research and the Program of the Institute: The contract focuses on the antigenic properties of cancer cells which are involved in the phenomena of unrestrained growth and metastasis of tumors.

Proposed Course: (1) Mutagenize the PW-13 tumor cell line to give a large collection of variants for comparison of growth in rats as well as agglutinability with lectins, and develop further the antigenic analysis of the MuLV system, by examining blocking or stimulation of complement dependent or cell mediated immunity against G and M rat tumors.

(2) Continue to investigate the topography of several normal and virus-transformed cell lines with ferritin-conjugated antibodies and plant agglutinins to see if topographical modifications in membrane structure are general properties of tumor cells. Develop techniques to look at surface charge distribution of normal and tumor cells in culture.

(3) Continue studies on the nutritional and serum factor requirements of the various temperature-sensitive transformed cells and the mechanisms by which cell cycles are controlled at permissive and non-permissive temperatures.

(The contract terminates during the next fiscal year.)

Date Contract Initiated: January 4, 1972

Current Contract Level: $150,000 (6 months)

SCRIPPS CLINIC AND RESEARCH FOUNDATION (NIH-NCI-E-72-3264)

Title: Immunologic Study of RNA Tumor (Type-C) Viruses

Contractor's Project Director: Dr. Frank J. Dixon

Project Officer (NCI): Dr. Robert J. Huebner

Objectives: (1) To develop quantitative, specific and sensitive radioimmune assays for detection of envelope antigens of mammalian Type-C viruses and for antibodies to these antigens. To apply these assays to the study of experimental materials developed in the course of work on this and other contracts in the SVCP.

(2) To observe the effect of immunization of mice with various antigens derived from the Type-C virus produced in lymphocyte lines isolated from New Zealand mice in the Scripps laboratory--Scripps leukemia virus (SLV). Immunization of NZ mice early in life will be attempted in order to suppress viral growth and the associated oncogenic and autoimmune diseases. Immunization with viral antigens will also be attempted in order to prevent or delay induction of tumors by graft vs. host reactions.

(3) To characterize serologically the SLV and to develop a sensitive infectivity assay for this agent.

(4) To isolate and quantitate the various nucleic acid intermediates involved in the replication of RNA tumor viruses.

(5) To develop electrophoretic, chromatographic and immunochemical procedures to isolate soluble envelope antigens from SLV in purified form and in sufficient amounts to produce highly specific antibodies against these antigenic substances.

(6) To determine whether the isolated soluble envelope antigens are products of the host cell or the virogene with subsequent development of methods to isolate antigens which are virus-specific and lack host cell components.

(7) To define the chemical composition of highly purified virus-specific envelope antigens, including their carbohydrate and amino acid composition.

Major Findings: In its first year of operation, the contractor has enlisted the participation of a knowledgeable professional staff of 10 with expertise in immunology, virology, cell biology, tissue culture, protein chemistry, nucleic acid chemistry and pathology. In addition, Dr. Dixon's group is working closely with Dr. Raymond Gilden (NO1-CP-33247), Dr. Berge Hampar (VCB), and Dr. Robert McAllister (PH43-NIH-NCI-E-68-1030).

Immunoassays: Modifications and improvement of the radioimmune assay (RIA) for screening human and animal tumor materials for the presence of the group-specific RNA tumor virus antigen were among the important priorities for the first contract year. Several methods of iodination of the gs

antigen were investigated in order to determine the mildest conditions consistent with efficient iodination. Gs preparations passed through a G100 column after electrofocusing and then lyophylized were freed of materials which interfered with iodination and were readily iodinated under mild conditions. The lactoperoxidase method of iodination modified by attaching the lactoperoxidase to sepharose 4-B beads was also found to be effective in iodinating gs and may provide the mildest possible conditions. In addition, this method should permit labelling of only a few µg of protein which is not easily done with the chloramine T method. This will be important in work with transcriptase and perhaps other antigens available in only very small amounts. Dr. Dixon's group also found that it was important that care be taken to rid gs preparations of contaminants which interfere with iodination. Gs taken directly from the electrofocusing procedure may have large amounts of such contaminants which necessitates a 100-fold increase in chloramine T concentration in order to achieve even a very low level of iodination (<1%). Iodination under such conditions carries with it the danger of denaturing the protein and results in levels of labelling so low that the RIA cannot be carried out properly.

The RIA procedure described by Dr. Oroszlan, et. al., was modified to employ ammonium sulfate as precipitating agent rather than sodium sulfate because of the better precipitation characteristics of the former.

For determination of gs in serum and tissue samples, it was found necessary to expose all gs for measurement. Tween treatment was found to be very efficient in exposing a maximum of gs, whereas ether treatment offered no advantage. Tween treatment of tissues will be used on all unknown murine samples in future gs assays. In addition to tumor materials, gs assays were also run on uterine strips in a study of viral activation by sex hormones (collaboration with Dr. A. Hellman, NCI). The test promises to have important applicability in determining the role of the RNA tumor virus and viral antigens in normal growth and development as well as in oncogenesis.

Study of lymphoid lines from New Zealand mice: The New Zealand mice, which suffer an autoimmune disease, and leukemia when treated with immunosuppressants, develop natural antibody to a variety of antigens of RNA tumor viruses. In view of their interest and importance for tumor immunology, continuous suspension lymphoid lines were established from NZB, NZW and NZB x NZW hybrid mice. After establishment, all of these lines produced a Type-C virus and in addition had karyotypic abnormalities including a marker chromosome and an X-deletion. These cells have been made available to investigators within the SVCP and appear to be of wide interest and use.

Properties of the virus produced by the New Zealand cell lines (Scripps leukemia virus--SLV): Virus isolated from the New Zealand lines has been characterized biochemically and biologically. Isolates from the NZB cells were found to be infectious for NRK, NZB, the hybrid NZ line and BALB/c 3T3 cells, but not for NIH Swiss cells. By contrast, virus isolated from NZW cells was infectious for all of the above cells. Mice inoculated with SLV as newborns developed strong anti-nuclear antibodies by 2 months of age. Half of the mice developed leukemias and/or lymphomas within 3

months, and 3/20 developed glomerular lesions resembling immune complex glomerulonephritis by 3 months.

Isolation of tumor-specific cell surface associated polypeptides from the New Zealand cells: Methods have been developed in the Scripps laboratory for specific radioiodination of the plasma membrane of lymphocytes and isolation of specific polypeptides in relatively pure form. The availability of large amounts of lymphocytes and virus made it possible to study some of the tumor-specific polypeptides of the cells. A component of remarkably homogenous molecular size was isolated from NZB cells; further study revealed the molecule to be a glycopeptide. Studies employing different antisera and/or cells are in progress to attempt to relate this polypeptide to either GCSA or MCSA or any TSTA. Results may be of considerable significance since cell surface glycopeptides may be primary targets of the host immune defenses.

Infection of human lymphoblasts with RD-114 virus: In collaboration with Drs. Berge Hampar (VCB) and Robert McAllister (PH43-NIH-NCI-E-68-1030), a series of experiments were undertaken to determine the effect of RD-114 virus, a virus derived from a human cell passed through a fetal cat, on human lymphocyte lines. The virus proved to be infectious and the infected cells produced RD-114. More interesting, however, was the observed alteration in the cell growth pattern and morphology after infection. It produced a unique CPE, which appeared to induce either syncytial formation and/or endoreduplication of the nucleus; further studies will be needed to decide between these alternatives.

Significance to Biomedical Research and the Program of the Institute:
Dr. Dixon: The immunological mechanisms associated with tumors provide a major key to the detection and eventual control of cancer. The degree of sensitivity needed requires a great deal of improvement and refinement of existing tests. With the isolation of simian and candidate human Type-C and -B viruses, Program will require large scale screening of specified human and animal materials. Dr. Dixon's group has the experience and wherewithal to meet these needs. The only other laboratories now engaged in somewhat similar programs are those in VCB and at Flow Laboratories. However, neither laboratory is equipped for large scale screening, and each is also importantly involved in other areas of research. Input by Dr. Dixon's group adds an impressive dimension to a very difficult but essential area of research. The contractor provides an important service, and serves as an outstanding laboratory for confirmatory tests of new candidate human isolates.

Dr. Reisfeld: The major objective of Dr. Reisfeld's research is to obtain soluble coat antigens of mammalian Type-C viruses in highly purified form and in sufficient amounts to produce highly specific antibodies against these antigenic substances. Such specific reagents directed against individual virion coat proteins greatly facilitate the immunologic identification of different intraspecies Type-C viruses. In addition, the clear delineation of envelope antigens provides better insight into host contributions to the viral envelope and antigenic modulations following passage of mammalian Type-C viruses through heterologous species. A

mapping of viral envelope antigens and their chemical characterization will greatly aid in the search for human cancer viruses and their gene products.

Dr. Lerner: The studies outlined are of importance in understanding the mechanism of replication of RNA tumor viruses.

Proposed Course: This contract has been in operation for less than a year. All of the projects and approaches outlined above will be continued in the renewal year.

Date Contract Initiated: June 29, 1972

Current Contract Level: $250,000

SOUTHERN CALIFORNIA SCHOOL OF MEDICINE, UNIVERSITY OF and CHILDREN'S HOSPITAL OF LOS ANGELES (PH43-NIH-NCI-E-68-1030)

Title: A Comprehensive Field and Laboratory Research Program on the Etiology and Epidemiology of Human Cancer

Contractor's Project Directors: Dr. Murray B. Gardner (USC)
Dr. Robert M. McAllister (Children's Hosp.)

Project Officer (NCI): Dr. Robert J. Huebner

Objectives: To mount a multifaceted, highly interrelated program designed to determine the roles of viruses, physical and chemical carcinogens, and other factors in the etiology of human and animal cancer in a natural urban ecology. These studies are carried out at USC School of Medicine and at Children's Hospital of Los Angeles.

Viral Studies: Human, pet and feral animal cancer and fetal materials are under intensive study for RNA tumor virus expression, utilizing all the modern in vitro as well as in vivo test systems. Extensive field studies and procurement efforts provide large numbers of tissues derived from cancer patients, genetically defective individuals, and spontaneous and therapeutic abortions. These materials are utilized for in vitro and in vivo biological studies are subjected to serological, immunological, biochemical and electron micrographic analyses designed to detect, isolate and characterize the RNA viruses and virus-specific antigens associated with naturally occurring animal and human neoplasms.

Epidemiological Studies: This program is providing, through hospital record surveys and community questionnaire surveys, up-to-date information of the natural occurrence of human cancer as it may be influenced by genetic, viral, environmental or other factors, including exposure to variable smog components in differing ecological areas of Los Angeles County, industrial and household carcinogens, and pets with and without cancer. Other factors such as occupation, aging, genetic defects, smoking, hormone therapy, and immunosuppressants are being studied using classical epidemiological methods combined with virological and serological surveillance.

Environmental Studies: This program is concerned with monitoring focal environmental areas for levels of carcinogens and other air pollutants. Materials collected are characterized and supplied to laboratories at USC as well as to NCI and other SVCP contract programs, e.g., Contract NIH-NCI-E-70-2068, for studies to determine the carcinogenic activities of such pollutants in tissue culture and in animals.

Major Findings: Epidemiology and Environmental Studies: 146 hospitals representing 95% of the acute beds in Los Angeles County are now participating in the Cancer Surveillance Program. Our preliminary data suggests that the rate of lung cancer is increased in the southern part of Los Angeles County where chemical and petroleum refining industries are concentrated. Our air monitoring studies have shown increased levels of benzo(a)pyrene and benz(a)anthracene in this area.

Biochemistry Studies: 14% of human milk samples tested showed low levels of reverse transcriptase activity as measured by the simultaneous detection technique. There was no correlation between detection of these complexes and a family history of breast cancer. None of 29 milk samples screened by EM showed the presence of Type-B or-C particles. A good correlation between morphologic particles and reverse transcriptase activity was found in wild mouse milk.

Virology Studies: Accumulating evidence strongly suggests that RD114 is an endogenous cat Type-C virus different from any of the described strains of feline oncornavirus. FeLV and KiMSV were unaltered by growth in RD cells. Attempts to repeat the isolation of RD114-like virus by transplantation of RD and other human tumor cell cultures into the brains of fetal and newborn kittens were unsuccessful. RD114 exhibited a wide in vitro host range for humans but not cat cells and induced no morphologic alterations in the infected cells. CF antibodies to RD114 gs or envelope antigen were not detected in human or cat sera.

Type-C virus strains of wild mouse origin were shown to be the cause of a neurogenic type of hind limb paralysis occurring under natural conditions in 2 lymphoma prone demes of wild mice. Paralysis and lymphoma, together or separately, could be transmitted experimentally to other wild mice or laboratory mice with these purified and well characterized virus isolates. The paralysis appears to result from a direct neurotropic effect of Type-C virus upon anterior horn neurons in the lower spinal cord.

Endogenous rat Type-C viruses have been isolated by chemical or spontaneous induction from rat cells of 3 different strains.

Significance to Biomedical Research and the Program of the Institute: This program searches for causes of human, pet and other animal cancers in a natural ecology, utilizing (1) experimental animal systems; (2) basic viral and chemical carcinogenesis studies; and (3) epidemiological profiles of cancer incidence in Los Angeles area humans and animals in relation to exposure, and to environmental carcinogens.

In addition, the program continues as a prime resource for supplying human and animal materials to the Viral Oncology in-house and SVCP contract programs, particularly to Microbiological Associates (NIH-NCI-E-70-2068), Flow Laboratories, Inc. (NO1-CP-33247), St. Louis University (PH43-NIH-NCI-E-67-692), University of California (Naval Biomedical Research Laboratories) (NO1-CP-33237), and the California State Department of Public Health (PH43-NIH-NCI-E-68-997).

Proposed Course: Continuation of (1) efforts to rescue a human candidate Type-C virus and eventual development of vaccines for trials in cancer patients; (2) studies of the Type-C virus isolated from a feral house mouse which is responsible for naturally-occurring early lymphoma incidence and amyotrophic lateral sclerosis-like paralysis in the colony under study and in other mice as well under experimental conditions; (3) epidemiological studies of factors influencing cancer incidence and type; (4) characterization of air pollutants; and (5) procurement, growth and distribution of human and animal materials within the SVCP.

Date Contract Initiated: June 26, 1968

Current Contract Level: $2,499,040

SOUTHERN CALIFORNIA SCHOOL OF MEDICINE, UNIVERSITY OF (NIH-NCI-E-72-2032)

Title: Conditional Lethal Mutants of RNA Tumor Viruses

Contractor's Project Director: Dr. Peter K. Vogt

Project Officer (NCI): Dr. Gary J. Kelloff

Objectives: (1) To continue the isolation of temperature-sensitive mutants of avian sarcoma and leukosis viruses; the goal of this effort is to build a collection of about 300 well-characterized mutants of different avian tumor virus subgroups. Special emphasis is being placed on mutants which are affected in their transforming ability. (2) To characterize each newly isolated mutant with physiological and genetic techniques. (3) To determine the number of viral genes involved in transformation and identify the products of these viral genes.

Major Findings: The methodology of isolation of the ts mutants has been completely defined by the contractor so that the isolation of ts mutants is now straightforward and the contractor has succeeded in isolating about 50 ts mutants to date. All mutants have been derived from helper-independent avian sarcoma viruses--the virus strains used for mutant isolation have been B77, PR RSV-A and PR RSV-C. Three major classes of mutants have been characterized. Class I contains those mutants which are temperature-sensitive for focus formation as well as virus replication; Class II encompasses mutants which are temperature-sensitive for viral replication but are still capable of inducing foci at the nonpermissive temperature. Not only do these mutants induce transformation at 41°, but they have also been shown to maintain the transformed state in the presence of drastically reduced virus production.

Class III consists of mutants which are unable to transform cells at 41° but produce a normal yield of virus under nonpermissive conditions. One of the most significant findings to date in this field has been this contractor's demonstration that viral transformed cells stay transformed only by virtue of continued viral genetic activity indicating that viral genes are required not only for the initiation of the transformed state, but also for its maintenance.

A systematic physiological and genetic characterization of the ts mutants is being carried out. The physiological parameters for monitoring neoplastic transformation have included focus formation, colony formation in agar, and tumor formation in the animal; evidence has been obtained that these parameters may measure independent manifestations of neoplasia. Other physiological parameters measured include the mutant's ability to carry out viral specific DNA, RNA and protein synthesis in infected cells, as well as the mutant's ability to induce viral tumor specific transplantation antigens. Specific studies designed to search for viral mutants which are affected in RNA dependent DNA synthesis has yielded two isolates, ts 335 and ts 337, which appear to have a genetic lesion in their polymerase. The purified polymerase product of these mutants is currently under study. Temperature shift experiments are also being carried out for each mutant to determine the time after infection when individual viral genes are active. These physiological characterizations of the mutants are resulting in several groupings. These groups can then be compared to genetic groupings obtained in complementation and recombination tests. These physiological studies are also providing a time table of viral gene action. The genetic characterization of the mutants has thus far resulted in at least six complementation groups definable in neoplastic transformation, suggesting that several viral genes are involved in this transformation. The contractor has discovered that genetic reassortment does occur among avian tumor viruses and the property is being utilized as a tool to allow pairwise complementation experiments with viruses of different subgroups, thus avoiding viral interference. The contractor has also demonstrated complementation of input mutants with endogenous avian leukosis viruses and to investigate and control this (in other experiments), has defined and utilized (1) cells which show both gs antigen and helper factor activity, (2) cells which are free of detectable gs but still show helper factor and (3) cells which have significant levels of gs but are helper factor negative. The contractor has also demonstrated that avian leukosis viruses can complement sarcoma mutants of Class II (with ability to transform, but not replicate) to produce complete viral progeny. Similar experiments with Class I and Class III mutants are in progress.

Significance to Biomedical Research and the Program of the Institute: The work on avian sarcoma virus ts mutant has provided and will provide detailed information on the function of individual viral genes, on their timetable of action and their location on the viral genome. Of particular value will be the characterization of viral transforming genes, their number, position on the genome and sequence of activity. Eventually this work should lead to the identification and isolation of the primary gene products of transforming genes, the hypothetical transforming proteins. The characterization of such proteins will be a major step towards understanding viral carcinogenesis.

Proposed Course: Assuming 50 complementation groups in RNA tumor viruses, one would need about 200-300 mutants randomly distributed over the genetic map to achieve the degree of map saturation required for a rough genetic definition of individual viral genes. The work, therefore, will proceed with the isolation of 200-300 mutants which will require 2 to 3 years. Physiological and genetic studies will be conducted in parallel with mutant isolation and can be expected to require 5 years.

Date Contract Initiated: October 15, 1971

Current Contract Level: $200,000

STANFORD UNIVERSITY (NIH-NCI-E-69-2053)

Title: (A) Study of Human Tumor Cell Cultures; (B) Operation of a Central Mycoplasma Diagnostic Laboratory; (C) Studies of Hodgkin's Disease and other Human Malignant Lymphomas

Contractor's Project Directors: Dr. Leonard Hayflick
Dr. Henry Kaplan

Project Officer (NCI): Dr. James Duff

Objectives: Part A is for studies on the cultivation and characterization of human tumor tissue, growth of human tumors in mice treated with anti-lymphocytic serum, and transformation of normal human cells by chemical carcinogens. Part B serves as a central diagnostic facility for the detection and identification of mycoplasma contamination in virus preparations, sera, cell cultures, and clinical materials submitted by other SVCP contractors. Upon request, identification of isolates is made as to species; and mycoplasma antigens are distributed to those investigators requiring these materials. Part C is for studies of Hodgkin's disease and other human malignant lymphomas.

Major Findings: A new biochemical technique for the detection of mycoplasma contaminants of cell cultures was devised. This technique utilizes the differential uptake of radioactively labelled uridine and uracil by contaminated and uncontaminated cells. A comparison of this technique with the agar/broth method indicated that a larger number of presumptive mycoplasma contaminated cultures were detected by the biochemical test. Preliminary data regarding the sensitivity of this technique showed that the agar/broth method was more sensitive. It is recommended that a combination of biochemical and microbiological tests be performed to maximize the detection of mycoplasma contaminants in cell cultures. During the past year approximately 2,500 samples were received from SVCP and NCI intramural laboratories and were tested for mycoplasma contamination.

No transformation of human cells by treatment with chemical carcinogens at high drug concentrations was observed. Cytotoxic effects were prominent; the most prominent morphological changes and cytotoxicity were observed with 4'nitroquinoline 1-oxide. As yet, no transformation of normal human cells has been possible with the following oncornaviruses: feline sarcoma virus,

feline leukemia virus and RD-114 virus. In studies of surface changes in SV40-transformed human cells, a new antigen was detected by micro adsorption which cross-reacted with HL-A5. This finding is of interest because the HL-A5 haplotype has been linked with the frequency of Hodgkin's disease.

Significance to Biomedical Research and the Program of the Institute: The mycoplasma diagnostic facility is a testing and monitoring service available to all SVCP contractors and Viral Oncology intramural staff. Many of the most important viral specimens, cell cultures, sera, etc., used in the Viral Oncology Program are sent to this facility for mycoplasma testing. Transformation of a human diploid cell line by chemicals could lead to a more direct testing of the oncogene theory, provide an in vitro assay system for testing the carcinogenic effect of chemicals on human cells, and could lead to a "switch-on" of a human tumor virus. The system of C57/L mice treated with anti-lymphocyte serum is being used to tell whether cells from neoplastic tissues, grown in vitro, are "tumor cells or fibroblasts."

Proposed Course: (1) Continuation of mycoplasma testing of samples received from other SVCP laboratories. (2) Continuation of studies on (a) effects of various chemical carcinogens on normal human cells in vitro, alone and in combination with oncornaviruses; (b) human tumor growth in immuno-suppressed mice; (c) histocompatibility expression of human cells transformed by oncogenic viruses; and (d) enucleation of human normal and cancer cells with Cytochalasin B. The studies by Dr. Kaplan on Hodgkin's disease will become a separate contract.

Date Contract Initiated: June 19, 1969

Current Contract Level: $172,369

WASHINGTON, UNIVERSITY OF (NIH-NCI-E-71-2171)

Title: Studies on Tumor-Specific Transplantation Antigens

Contractor's Project Directors: Dr. Karl Erik Hellström
Dr. Ingegerd Hellström

Project Officer (NCI): Dr. Robert J. Huebner

Objectives: To detect and characterize tumor-specific antigens, plus serum-mediated and cell-mediated immune responses to these tumor-specific antigens, in human tumors.

Major Findings: (1) Ten human patients with malignant melanoma were followed with respect to two in vitro parameters of tumor immunity, the ability of the patients' blood lymphocytes to destroy cultivated melanoma cells, and the ability of the patients' sera to block such destruction, and with respect to changes in the clinical status, such as increases or decreases in detectable tumor mass. Allogeneic and (to a much lesser extent) autochthonous melanoma cells were used as targets.

The degree of lymphocyte mediated tumor immunity was higher in patients with little or no residual tumor than in patients with large tumor loads, although also the latter patients were found to be reactive. Sera from patients with clinically detectable melanoma could block the cytotoxic lymphocyte effect, as could sera from patients who developed melanoma shortly after the serum harvest. Disappearance of blocking serum activity was seen to accompany clinical improvement. Sera from some patients without clinically detectable tumor could potentiate the cytotoxic effect of lymphocytes from melanoma patients.

The findings support the view that cell-mediated tumor immunity and blocking serum activity, as studied in vitro, are important correlates of the patients' immunological defense against their tumors in vivo, and they suggest that monitoring of these parameters may be prognostically useful. They also suggest that procedures capable of improving cell-mediated tumor immunity without increasing blocking serum activity may be therapeutically beneficial.

(2) It was shown that normal syngeneic lymph node cells can have a cytotoxic effect on normal, cultivated brain cells and that this cytotoxic effect can be blocked by normal syngeneic serum, except when the serum has been absorbed with cultivated brain cells (when the cytotoxic effect is not blocked). Analogous, albeit less regular, findings were made in reciprocal experiments, using serum absorbed on normal, syngeneic kidney cells. The findings suggest that blocking serum factors may play a role in protecting against immunological destruction by lymphocytes sensitized to normal tissue antigens, thus helping to maintain immunological tolerance to "self" antigens.

(3) Lymph node cells of non-sensitized mice were "armed" (i.e., made specifically cytotoxic) by exposures in vivo to passively transferred sera from tumor bearing mice. Four hours after an intraperitoneal injection of antitumor serum, the lymph nodes were removed from recipient mice and the activity of the lymph node cells tested on tumor cells in an in vitro micro cytotoxicity test. Specific cytotoxicity was demonstrated to the respective target tumors (Moloney virus or 3-methylcholanthrene-induced sarcoma) carried by the tumor bearing serum donors.

(4) Administration of BCG at the time of rat polyoma tumor isografting or 2 weeks previously inhibited tumor growth and induced an increased level of lymphocyte cytotoxicity and an increase in the number of circulating lymphocytes; i.e., it resulted in an increased level of cell-mediated immunity. A similar inoculation of BCG at the time when the tumor isograft had already grown out to a palpable nodule did not inhibit tumor growth, but rather increased it. Furthermore, it did not induce any production of cytotoxic antibodies detectable with rat complement. BCG treatment of rats which had the same day undergone incomplete tumor excision increased the blocking activity in serum. On the other hand, BCG treatment of analogous rats which had received "unblocking treatment" in the form of splenectomy and inoculation of unblocking serum caused tumor regression in some animals and increased the cell-mediated immunity in all animals tested.

(5) Immunotherapy, in the form of inoculations of "unblocking" antitumor sera alone or combined with BCG and splenectomy, was tried in a metastasis model in animals. W/Fu rats were exposed to progressively growing polyoma tumor isografts which were subsequently excised and the rats rechallenged with an isograft of the same tumor as a "metastasis." The immunological manipulation was initiated at the time of rechallenge and although it was not capable of preventing tumor outgrowth, it caused a complete regression of the outgrowing tumor nodules within a few weeks in 7/7 and 6/16 rats in 2 different experiments. An attempt was also made to inhibit well-established spontaneous tumor metastasis to the lung by repeated inoculations of unblocking antitumor serum. A prolonged survival was obtained in 4 out of 9 rats and one of these animals was free of tumor when killed after 4 months. The spread of the tumor to various organs was significantly restricted by the therapy indicating a partially inhibited tumor growth.

An attempt was made to establish correlations between inhibition of tumor growth in vivo and certain alterations in the antitumor immunity as analyzed by in vitro techniques. Disappearance of serum blocking activity and appearance of rat complement-dependent cytotoxicity in serum was seen in all rats with regressing tumors but not in the other animals, although they had received the same treatment. The lymphocyte cytotoxicity was increased to about the same extent in regressor and progressor rats.

(6) In collaborative studies with Dr. M. Hayami, it was found that sera from Japanese quails with progressively growing Rous sarcomas can block destruction of cultivated Rous sarcoma cells by specifically sensitized lymphocytes, and that the blocking effect can be removed by absorption with Rous tumor (but not with control) cells, also when blocking is tested for by having the serum present with the lymphocytes and target cells during the whole incubation period, indicating that the major blocker in this system has affinity for the target cells and is an antigen-antibody complex or an antibody, rather than an antigen. The effect of bursectomy on the blocking serum activity (and on Rous sarcoma growth in vivo) is presently investigated.

Additional studies in progress involve (1) an attempt to prevent (or delay) the induction of urinary bladder tumors in mice and rats by "vaccination" with tumor-specific antigens common to such tumors and (2) studies to determine if Rous sarcoma cells (transformed with a temperature-sensitive mutant of the Rous virus) will express the antigens which act as targets in lymphocyte-mediated cytotoxic reactions, when the transformed cells are tested at either permissive, or non-permissive, temperatures.

Significance to Biomedical Research and the Program of the Institute: This important and productive study of human and animal tumor antigens, as well as cell-mediated and humoral immune responses to the antigens, has already given, and promises to continue to give, insights to the understanding of the body's immune response to tumors, and ultimately may lead to immunotherapy of established tumors in man.

Proposed Course: Continued investigation of interaction between lymphocytes and serum factors in animals and patients with tumor, on the nature of

immunity to Rous sarcoma in birds and on attempts to prevent and treat tumors by immunological means.

Date Contract Initiated: April 14, 1969

Current Contract Level: $110,000

WEIZMANN INSTITUTE OF SCIENCE (NIH-NCI-E-69-2014)

Title: Study of Virus-Induced Tumor-Specific Transplantation Antigens

Contractor's Project Director: Dr. Leo Sachs

Project Officer (NCI): Dr. Robert J. Huebner

Objectives: To investigate the differences between the structure of the surface of normal cells and of cells transformed by viral and non-viral carcinogens by studying the differential binding of plant lectins.

Major Findings: Experiments were carried out to determine the mobility of Concanavalin A (Con A) binding sites on the surface membrane in various cells. These studies incorporated normal and transformed fibroblasts as examples of normal and malignant cells that form a solid tissue, and normal lymphocytes and lymphoma cells as examples of normal and malignant cells that are in suspension in vivo. The fibroblasts were transformed after virus infection or treatment with a chemical carcinogen, and the lymphoma cells were obtained after infection with the Moloney leukemia virus. It was found that binding sites for Con A are floating in the surface membrane in a random distribution. Given the appropriate fluidity, the distribution of sites can be changed by binding of con A, followed by movement of the Con A membrane site-complexes on the surface membrane to form a new distribution. The final distribution after Con A binding was clusters of sites in transformed fibroblasts and lymphoma cells, a concentration of most of the sites on one pole of the membrane to form a cap in normal lymphocytes and what appeared to be no or almost no change from the original distribution in normal fibroblasts. The order of site mobility was therefore normal fibroblasts < transformed fibroblasts and lymphoma cells < normal lymphocytes. The mobility of Con A sites was increased by increasing cross-linking with anti-Con A antibodies or glycogen and by trypsin treatment of the cells before the binding of Con A.

The results suggest, that the mobility of Con A binding sites can be used as a probe for the fluid state of this specific carbohydrate structure on the surface membrane. Using this probe, the results suggest that in cells that form a solid tissue, the transformation of normal into malignant cells is associated with an increase in membrane fluidity of the carbohydrate structure where the Con A sites are located. However, in cells that are in suspension in vivo, the malignant transformation is associated with a decrease in fluidity of this carbohydrate structure on the cell surface. This increased fluidity of the membrane in transformed fibroblasts can explain their lack of contact inhibition, their ability to grow in soft agar and

their malignancy in a solid tissue. Differences in membrane fluidity can also explain the differences in the behavior of cells in mitosis.

In other studies with normal lymphocytes, it was found that activation of lymphocytes by lectins so that they undergo DNA synthesis is dependent on a temperature-sensitive activity on the surface membrane that may also be required for membrane fluidity.

Significance to Biomedical Research and the Program of the Institute: Compounds that interact differentially with the surface membrane of normal and tumor cells are of value in elucidating the chemical nature of the differences in the surface that are associated with cell malignancy. They are also of potential value for tumor chemotherapy.

Proposed Course: The studies will (1) continue to explore differences in fluidity of the surface membrane to explain the differences between normal and tumor cells, using lectins with different specificities, (2) develop new methods for studying this fluidity, (3) further study to what extent fluidity and subsequent mobility is essential for the activation of normal lymphocytes in the immune response, and (4) explore the potentialities of changing membrane fluidity so as to change the behavior of malignant cells.

Date Contract Initiated: April 22, 1969

Current Contract Level: $60,000

WISTAR INSTITUTE OF ANATOMY AND BIOLOGY (NO1-CP-33250; formerly NIH-NCI-E-71-2092)

Title: Extraction and Characterization of Virus-Induced Transplantation Antigen and Rescue of Virus from Sarcomas and Leukemias

Contractor's Project Director: Dr. Anthony J. Girardi

Project Officer (NCI): Dr. James T. Duff

Objectives: The area of major effort is tumor immunology and involves two parts; one, studies of fetal antigen expression and virus expression in very early embryos (days 0-10) and two, studies of shared transplantation antigens between MSV non-producer cells and regular shedding MSV tumors.

Other studies involve attempts to isolate viruses from human sarcomas and leukemias through co-cultivation, fusion, and activation.

Major Findings: (1) A new problem initiated during the past year concerns a study of the biological and immunological characterization of the human SV40 isolates from the disease progressive multifocal leukoencephalopathy (PML). The SV40 isolated directly from human brain tissue into human fetal brain cells (no monkey passage) is almost typical of regular SV40 being similar in producing tumors in 100% of hamsters inoculated when newborn via the subcutaneous route; such tumors contain T antigen, do not shed

infectious virus or do so at a very low level but the SV40 is rescuable after fusion with CV-1 (AGMK). The tumors contain SV40 transplantation antigen and protect hamsters against development of tumors induced by regular SV40 virus (the reverse test is being done) and tumor-bearing hamsters make T antibody. In vitro the virus seems to have the same host range and induces the same antigens after infection of cells; human cells are transformed in typical fashion by the SV40 (PML) virus. Various species of small animals were inoculated via the intracerebral route; however, neither newborn hamsters, guinea pigs nor Lewis rats became ill. Such hamsters on long term tests are now being "modified" as adults in an attempt to induce brain tumors or other CNS disease. It is known that PML occurs in people who are immunosuppressed either purposely (transplants, etc.), or naturally, through having a disease which immunosuppresses. Our "modifying" procedures include chemical immunosuppression and infection by a second virus. Future studies will try to determine the role of this agent in human disease other than PML (for instance in human leukemia).

(2) The fetal antigen study has been directed at determining the location and nature of the "active" cells--fetal liver, the site of most of the hematopoietic cells during early embryogenesis, is being tested separately from other embryonic elements. The role of mouse embryonic antigen in prevention of spontaneous mouse tumors will be an area of future study.

(3) Virus expression in 2-cell stage to 64-cell stage mouse embryos is being performed in collaboration with non-contract personnel in the electron microscopy and embryology areas at the Wistar Institute. It has been found that there is early viral particle expression (which seems to vary with different mouse strains) and this is being studied by gs antigen staining, by direct infectivity assays, and by electron microscopy.

(4) An immunologic study with MSV-BALB/c non-producer cells (BNP) indicated their potential to induce in vivo protection and in vitro lymphocyte cytotoxicity. The sera of BNP immunized mice augmented the cytotoxicity of sensitized lymphocytes and may augment the cytotoxicity of normal lymphocytes as well against BNP target cell, and sera raised in MSV regressor mice similarly augmented the cytotoxic response of MSV regressor lymphocytes against BNP target cells. However, MSV regressor mice did not display protection in vivo when challenged with BNP cells.

(5) Human sarcomas in cultures have been negative for mammalian gs-3 antigen and have not shed agents capable of transforming target cell either spontaneously, after fusion, and (in less frequent tests) after chemical induction.

Significance to Biomedical Research and the Program of the Institute: The treatment of cancer by immunologic methods has been an attractive hypothesis for decades, but it is only recently that new and fundamental discoveries in immunobiology have made cancer immunotherapy a real possibility. The work being conducted by the contractor is part of a larger effort of the SVCP to isolate and test virus-induced tumor-specific transplantation antigens in animal model systems. The virological studies involve attempts to rescue a human tumor virus.

Proposed Course: The majority of the immunological studies will be continued under an NCI grant at the Wistar Institute.

The contract supported effort will involve the rescue of human tumor viruses utilizing cell fusion and chemical induction techniques. Attempts are being made to obtain brain tissue at autopsy of cancer and leukemia cases since this tissue with its partial immunological privilege and variety of target cell types may be a clearing house for agents which can not survive freely in other organs. (The spontaneous transformation of human brain cells derived from some "slow" virus diseases as well as claims of transforming properties of "agents" obtained from such brain autopsies warrants this study.)

Date Contract Initiated: February 1, 1971

Current Contract Level: $49,500 (for 9 months)

TUMOR VIRUS DETECTION SEGMENT

CONTRACT SUMMARY REPORT

Human Studies

Cells from a case of childhood acute lymphoblastic leukemia contain an apparent DNA polymerase activity which was not found in any other cells except thymus cells. The enzyme has the properties of terminal transferase, an enzyme known to be found in thymocytes. (Contract #71-2149)

The Immunodeficiency Cancer Registry has been continued and expanded, and cell cultures continue to be established from these and other patients. Attempts to find or induce type C virus from these cells, or any other human cells, in a number of laboratories, has been unsuccessful to date. (Contract #71-2261)

Measuring the in vitro transformation frequency of human diploid fibroblasts by SV40 a number of highly susceptible groups were discovered. These included patients with Fanconi's Anemia, Down's Syndrome and radiation treated cells. Besides increased SV40 transformation these groups also have chromosome anomalies and a high incidence of spontaneous neoplasms. (Contract #72-2034)

Poly (G)-Sepharose columns were used to separate viral from cellular polymerases. All cells examined contained a minor peak of activity that eluted like the viral reverse transcriptase. Human acute leukemia cells do not appear to contain more of this enzyme than normal cells. (Contract #72-208)

A human breast tumor cell line, HBT-3, has been isolated and characterized biologically. Virus particles were not seen in HBT-3 cells even after treating with inducing agents such as IdU. Column chromotography using dT-cellulose was used to demonstrate a reverse transcriptase in HBT-3 cells having many of the properties of known viral transcriptases. (Contract #73-3230)

Primate Studies

The virulence of Herpesvirus saimiri for owl monkey kidney (OMK) cultures was attenuated by serial passage of the virus in dog fetal lung (DFL) cell line. Two findings suggest that oncogenicity can be activated by simultaneous inoculation with two different DNA viruses: (1) cinnamon ringtail monkeys inoculated with H. saimiri and a likely new adenovirus named Cebus isolate developed a disease similar to Hodgkin's lymphoma when they were inoculated with both viruses; when either of these viruses is inoculated alone similar disease is not observed. (2) H. saimiri positive squirrel monkeys developed a disease similar to malginant lymphoma when inoculated with Cebus isolate. (Contract #72-3246)

Using antisera to the viral reverse transcriptase, it was shown that the Mason-Pfizer monkey virus (MP-MV) was unrelated to either known type C viruses or the primate syncytium-forming ("foamy") viruses. Among the type C viruses, the woolly monkey and gibbon ape viruses were found to be very closely related immunologically, but the two were only distantly related to RD-114. (Contract #72-2006)

The major internal protein (gs antigen) from each of the following type C viruses -- mouse, cat, woolly monkey, gibbon ape, and RD-114 -- was purified to homogeneity, and used in species specific radioimmunoassays. The close relationship of gibbon ape and woolly monkey virus, and their difference from RD-114 was demonstrated. (Contract #72-2006)

A clonal isolate of a primate sarcoma virus analogous to the S+L- strain of murine sarcoma virus has been obtained. It contains woolly gs antigen and woolly polymerase. Primate and murine helper viruses have equal ability to rescue primate or murine sarcoma viruses. (Contract #72-2006)

Feline and Other Studies

An endogenous virus (CCC) of a single cell clone of feline fibroblasts has been isolated. It has a reverse transcriptase, gs antigen and host range distinct from FeLV and similar to RD-114. CCC and RD-114 both grow well in human and primate cells and poorly on feline cells. FeLV, in contrast, grows well on feline cells and poorly on monkey and human cells. (Contract #72-2006)

RNA from RD-114B virus hybridized ten to twenty times more with normal cat DNA than with normal human DNA and hybridized to the same extent with DNA from normal cat and DNA from leukemic feline cells producing Thielen leukemia virus. Also, the 70S RNA from RD-114B virus hybridized to the same extent with DNA from normal human cells and DNA from neoplastic human cells including the RD cells. As expected, however, DNA from RD-114B cells which are producing this virus hybridized two to four times more than the non-virus producing RD cells. It thus appears that RNA from RD-114B virus is not suitable as a probe for the search for putative human oncornavirus DNA in human tumors. It also appears that RD-114B virus contains RNA that is not homologous to that of Gardner, or Thielen, FeLV since it cannot differentiate between normal cat DNA and DNA from cat cells that are releasing these two feline leukemia viruses. RD-114 appears to be a feline oncornavirus, different than Gardner or Thielen FeLV. (Contract #33283)

The relatedness of type C viruses in hybridization experiments shows that within one family of viruses there is usually greater than 50% homology (for example, between woolly and gibbon viruses or between Rauscher and Kirsten viruses) with one exception: the endogenous cat virus (CCC) is not related to the Rickard or Thielen strains of feline leukemia virus. (Contract #72-2006)

A poly(U)-Sepharose chromatography method for fractionation and isolation of specific messenger RNAs was developed, based upon temperature program elution; it can fractionate polymers of (A) differing in chain length by only 4 or 5 nucleotides. Neither ribosomal nor transfer RNA will absorb to the column. Steroid-induced cells showed increases in synthesis of specific mRNA fractions. The technique has been applied to purified viral RNA subunits from various viruses. MuLV 36S RNA subunits are composed of two chromatographically distinct fractions consisting of poly A containing subunits representing approximately 60% of the viral RNA and non poly A containing subunits comprising 40% of the total RNA. (Contract #72-208)

A search to see if ppGpp was the "pleiotypic mediator" in mammalian cells, anagogous to its role in "stringent" microorganisms, led to the inability to detect it. Other experiments implicated cyclic AMP as the "pleiotypic mediator". (Contract #72-3236)

Attempts to obtain nonsense suppressor mutants of mammalian cells is progressing well. A number of HGPRTase$^-$ CRM$^-$ mutants were obtained which are excellent candidates for nonsense mutations. Reversion of the HGPRTase$^-$CRM$^-$ mutants to HGPRTase$^+$ CRM$^+$ was obtained. The individual revertant isolates from a given mutant differ in their characteristics. It is very likely that reversion is being achieved by a variety of mechanisms, among which will be suppression. (Contract #72-2058)

Murine Studies

Additional S+L- cell lines have been developed and characterized. The nature of the RNA and reverse transcriptase in S+L- particles has been described. A system for rapid quantitative transformation of 3T3 cells with MSV was developed. Glycolipid transferase activity is reduced following MSV infection in a manner similar to that observed for cells transformed by papovavirus, and it is apparent that these changes occur after cell transformation by MSV. (Contract #72-3230)

A virus has been isolated in immunosuppressed NIH Swiss mice bearing human rhabdomyosarcoma cells (RD). This virus, AT-124, has mouse virus polymerase and mouse virus gs-1 antigens, yet its host range is primarily human and primate. It grows in none of the known mouse cell systems. A pseudotype with this virus readily transforms human cells. (Contract #72-2006)

Some spontaneously transformed clones of Balb/3T3 were found to spontaneously and continuously release high titers of the endogenous type C virus. Other transformed subclones were found to be virus-free, but treatment with BrdU induced production of the endogenous virus in large amounts. Non-transformed Balb/3T3 clones never spontaneously released type C virus, and could be induced to release very small quantities only with BrdU; in contrast, cells transformed by mouse sarcoma virus, radiation, methylcholanthrene and also spontaneously were "superinducible", i.e., BrdU treatment caused virus production within

8 hours and resulted in secretion of very large quantities of virus with exponential kinetics. (Contract #72-2006)

All murine cell lines examined so far have been found to contain RNA which is homologous to 35S RNA of murine leukemia virus. After purification of cytoplasmic RNA by dT-cellulose chromatography to remove RNA not containing poly-A sequences, and hybridizing this RNA to the ^{3}H-DNA product, it was found that a "normal" murine cell line, A31, transcribes enough RNA to saturate 2-4% of the ^{3}H-DNA product. Murine cell lines transformed either spontaneously, by radiation, or by SV40 all transcribe an additional amount of RNA so as to saturate about 10% of the ^{3}H-DNA viral probe. Normal rat cell RNA, poly-A, or dT-cellulose purified RNA from an SV40 transformed human cell line do not hybridize to the ^{3}H-DNA murine probe. (Contract #72-2006)

Natural expression of the mouse gs antigen was found in all mouse tissues examined, strongly suggesting that a continuous synthesis of this polypeptide is an integral part of the murine cell macromolecular synthesis. (Contract #72-2006)

Poly(Am) inhibited tumor formation by MSV in vivo, in newborn Balb/c mice. With RLV in young adult Balb/c mice, at high virus dose there was little difference in the poly(Am)-treated animals and controls, but at low virus concentration the leukemic process was stimulated by poly(Am). (Contract #72-208)

Mouse cells which are permissive for mouse leukemia virus (3T3) were fused with nonpermissive human cells (Wl-38). Essentially all of the heterokaryons examined were nonpermissive for virus expression, indicating that the nonpermissive state is dominant and implying that the nonpermissive cell is able to repress virus synthesis specifically. Moloney virus is adsorbed with equal efficiency to Wl-38 and 3T3 cells, indicating that restriction is not at the level of virus adsorption. 12 human/mouse hybrids which have lost half or more of their human chromosome complement are permissive for virus synthesis. Restriction of host range of mouse leukemia viruses is not at the adsorption-penetration step, since fusion of nonpermissive (B) cells in the presence of the Gross (N) virus does not overcome the restriction. (Contract 72-208)

Murine leukemia virus (derived from AKR 3T3 cells) has been fractionated into its six major polypeptides. This was accomplished by two cycles of gel filtration in agarose in the presence of 6M guanidine hydrochloride and reducing agents. Each protein was dialyzed free of the guanidine hydrochloride and used for the immunization of a rabbit. Antisera have been prepared against each polypeptide and are presently being studied for serological specificity. (Contract #72-2022)

Six cell-surface antigens associated with infection by MuLV have been identified. Individual mice can be typed for these antigens by cytotoxic and immunofluorescence tests. In genetic crosses between mice of high and low leukemia strains these antigens segregate according to Mendelian ratios. (Contract #72-2022)

Autogenous immunity to endogenous MuLV related antigen(s) in RF mice was demonstrated. A marked decrease in detectable MuLV antigen in thymus and spleen at 1.5 months of age correlated with development of humoral immune components of the spleen, antigen localization, and serum immune elimination. This in turn correlated in time with the onset and increasing incidence and severity of glomerulonephritis. Some antibody eluted from kidney of aged mice was determined to be specific for MuLV antigens. (Contract 72-208)

Utilizing serum as well as kidney eluate in immunoelectronmicroscopy (IEM) it could be demonstrated that throughout life B6C3F1 mice possess significant levels of free antibody which is specific for MuLV envelope antigens; this antibody is capable of neutralizing AKR virus as well as Moloney virus. Immunoglobulin and MuLV antigens, presumably in the form of immune complexes, were localized in kidney glomeruli, and type C virus was demonstrated within the glomerular basement membrane matrix of glomerulonephritic kidneys of both B6C3F1 and AKR mice. (Contract #72-208)

A radioimmune precipitation (RIP) test using ^{3}H-leucine labeled AKR virus was developed. A marked difference between mouse strains of age associated levels of free antibody to GVEA was detected by the RIP test, and the differences were inversely related to the natural incidence of lymphoma. (Contract #72-208)

Immunologic crossreactivity of antigens common to tumor and fetal cells was demonstrated both with humoral and cell-mediated immunity. Suppression of syngeneic fetal cell growth can be obtained by immunization with either syngeneic, allogeneic, or xenogeneic tumor cell systems. (Contract #72-208)

Immunoelectron microscopy has demonstrated an MSV-associated cell surface antigen on the surface of nonproductively transformed cells infected by two different strains of MSV, Kirsten and Moloney. This antigen is distinct from previously described antigens on the surfaces of cells infected by murine leukemia virus, on the viral envelope, and on the surfaces of cell lines transformed spontaneously or by x-irradiation. (Contract #72-2006)

Murine mineral oil induced myeloma-associated virus (MuMAV) carries a specific viral envelope antigen, different from those on MuLV. Endogenous type C viruses released from spontaneously transformed Balb/3T3 cells can be classified into at least two different populations: (a) type C viruses which carry xVEA envelope antigens and (b) uncharacterized type C viruses which have neither xVEA nor MuLV envelope antigens. (Contract #72-2006)

Fibroblasts transformed by SV40 or Kirsten MSV showed no loss of HL-A antigens. By contrast, four of eight tumor cell lines showed no evidence of histocompatibility antigens. These findings suggested the possibility that antigenic loss could be related to immunoselection *in vivo* or antigenic loss associated with long term culture and that, contrary to earlier predictions, loss of histocompatibility and differentiation antigens was not necessarily a frequent event associated with

transformation. (Contract #71-2261)

Results suggest that the initial product of the viral reverse transcriptase reaction is covalently linked DNA-RNA molecules and that the primer for initiation of DNA synthesis is probably 4S RNA associated with 60-70S RNA. Identical results were obtained with mouse leukemia virus, avian myeloblastosis virus (AMV), and a reconstructed system consisting of purified 60-70S AMV RNA and purified AMV DNA polymerase. (Contract #71-2149)

Balb lines transformed by RNA tumor viruses showed agglutinability by wheat germ agglutinin or concanavalin A that correlated with transformation, as found previously in DNA virus transformed cells. (Contract #72-2028)

An extensive collection of temperature sensitive mutants of polyoma virus is being studied and characterized. (Contract #72-2031)

At least 3 peaks of RNA can be resolved when 4S subunits of 70S viral RNA are analyzed by reversed phase chromatography. They are located in the region where mammalian tRNAs are eluted. Narrow rather than diffused appearance of the peaks suggests that they are distinct molecular species (like individual isoaccepting tRNA species). Whether or not these are tRNAs remains to be determined. (Contract #72-208)

The RNA-directed DNA polymerase of murine leukemia viruses is competitively inhibited by single-stranded polyribonucleotides, and these polymers especially poly (A), and the relatively RNase resistant derivative 2'-O-methyl poly(A)[poly(Am)], inhibit the replication of leukemia viruses in cultured cells. Concentrations of poly(Am) which do not affect leukemia virus uptake are still effective in blocking replication. Activation of AKR virus by IdU is not affected by even high concentrations of poly(A) or poly(Am). (Contract #72-208)

Avian Studies

RNA of RSV harvested at 3 minute intervals from cells contains mostly 30-40S RNA. Upon incubation at 40°C the 30-40S RNA is converted to 60-70S RNA in the virus. This suggests that 30-40S is a precursor - and not a breakdown product - of 60-70S RNA. (Contract #71-2173)

For the avian tumor viruses, it was found that cloned sarcoma viruses contain only a subunits and nontransforming viruses only b subunits. (Contract #71-2173)

An affinity chromatographic procedure for the avian type C viral polymerase was developed. Using this procedure, viral enzyme can be selectively purified from extracts of virus producing avian cells. Extracts of Rous sarcoma virus transformed rat cells (XC cells) which synthesize no virus but do contain easily detected quantities of the avian gs antigen(s), failed to show any viral polymerase. (Contract #72-2006)

Noninfectious particles of a mutant of Rous sarcoma virus failed to exhibit DNA polymerase activity even with the use of the most sensitive synthetic template-primer complexes. A neutralization blocking test against antibody to DNA polymerase revealed that these mutants did not contain protein immunologically related to the DNA polymerase. (Contract #71-2149)

The glycopeptides of the viral envelope glycoproteins of different avian RNA tumor viruses were analyzed. It was found, that the glycopeptides of all viruses released from transformed cells are larger than those of all viruses released from phenotypically normal cells. (Contract #71-2173)

Dr. George J. Todaro, Chief, VLLB, DCCP, Chairman
Dr. Bernard Talbot, VLLB, DCCP, Vice Chairman

CALIFORNIA SCHOOL OF MEDICINE, UNIVERSITY OF (NCI-E-72-3236)

Title: Hormonal Control of Gene Expression in Tumor Viruses

Contractor's Project Director: Dr. Gordon M. Tomkins

Project Officer (NCI): Dr. Bernard Talbot

Objectives: (1) To establish relatively simple systems in which to study the effects of hormones on the expression of the genomes of various DNA- and RNA-containing oncogenic viruses. (2) To examine the range of cell types and viruses responsive to such effects. (3) To discover whether the observed effects result from alterations in host cell physiology or from direct effects on the expression of the viral genes themselves. (4) To determine whether hormones regulate the expression both of integrated "oncogenes" and of viral genes involved in productive infection. (5) To determine the molecular mechanisms of the observed hormonal effects.

Major Findings: A search to see if ppGpp was the "pleiotypic mediator" in mammalian cells, analogous to its role in "stringent" microorganisms, led to the inability to detect it. Other experiments implicated cyclic AMP as the "pleiotypic mediator".

Results suggested that the alterations brought about by integrated SV40 oncogenes lead to a decrease in the ability of cells to produce cyclic AMP under conditions where untransformed cells do so.

Experiments indicated that a "mediator" of cellular function is involved in growth control by serum, rather than that the function of serum is to promote the entry into cultured cells of required nutrients such as glucose and amino acids.

By continuous growth of a line of cultured Balb/c mouse lymphosarcoma cells in increasing concentrations of dibutyrl cyclic AMP, a cell population was selected which was resistant to concentrations as high as 10^{-3} M dibutyrl cyclic AMP in combination with theophylline. Experiments indicated that the resistance is associated with diminished concentrations of both catalytic and regulatory subunits responsive to the cyclic nucleotide.

Cyclic GMP antagonizes the inhibitory effects of cyclic AMP on membrane transport in 3T3 cells; thus cyclic GMP may play a positive role in control of cellular growth in contrast to the negative one played by cyclic AMP. Preliminary experiments suggest that serum addition to starved cells may increase the levels of cyclic GMP, as expected.

Significance to Biomedical Research and the Program of the Institute:

There is considerable evidence that the entire genome of both DNA and RNA oncogenic viruses may be integrated into the chromosomes of host cells, whether or not such cells show evidence of their presence. Malignant transformation appears to depend, to a large extent at least, on the degree to which viral "oncogenes" are expressed, which means that a major aim of viral oncology must be to understand the biological factors which regulate virus gene expression. A closely related problem concerns the mechanisms which control the cellular specificity both of viral transformation and productive infection. That is, what factors determine the types of cells in multicellular organisms which are susceptible to transformation or infection by particular oncogenic viruses? Hormones are tissue-specific effectors which selectively control gene expression in a variety of cell types. It is thus an important facet of the SVCP Program to investigate viral gene expression and cellular specificity in the light of the present (and rapidly evolving) information about the mechanisms of hormone action.

Proposed Course: In the coming year the following specific studies will be undertaken: (1) Examination of the effect of steroids on tumor virus functions. Preliminary data indicate that glucocorticoid treatment of 3T3 cells or mouse embryo fibroblasts increase the number and size of polyoma plaques. Studies will probe whether the effect is mediated by cytoplasmic glucocorticoid receptors. (2) The possible requirement for viral growth of the physiologically active glucocorticoid concentrations present in the serum used in tissue culture media, will be examined by adding glucocorticoid inhibitors. (3) Attempts will be made to derive viral mutants with altered response to hormonal stimulation, i.e., steroid-independent mutants, in an attempt to localize further the site of hormone virus interaction. (4) A number of other virus-cell systems will be examined for hormone responsiveness; these include SV40, adenovirus, herpesvirus, MuLV and RSV. (5) Experiments will be performed on the effects of the hormones on the activation of latent oncogenic viruses. Here, hormones will be used to attempt to induce specific viral functions (such as antigen production) in cell lines where other techniques (e.g.,BrdU or IdU induction, cell fusion, or nucleic acid hybridization) have demonstrated the presence of unactive viral genes. For example, attempts will be made to induce viral RNA synthesis, the appearance of gs antigens or reverse transcriptase in various mouse cell lines containing glucocorticoid receptors.

Date Contract Initiated: April 25, 1972

Current Contract Level: $72,432

BAYLOR COLLEGE OF MEDICINE (NCI-E-72-2058)

Title: Nonsense Suppressor Mutants in Mammalian Cells

Contractor's Project Director: Dr. Thomas Caskey

Project Officer (NCI): Dr. Edward Scolnick

Objectives: To develop suppressor mutants of mammalian cell lines and use these to investigate the genes involved in maintaining the transformed state in cell cultures.

Major Findings: Chinese hamster cells were treated with two chemical mutagens, (Ethyl-methanesulfate and N-methyl-N'-nitro-N-nitrosoguanidine), and mutants resistant to 8-azaguanine were selected and characterized. Hypoxanthineguanine phosphoribosyltransferase activity of a number of mutants were found to be extremely negative, making them suitable for reversion to HGPRTase+. Some of the extremely negative mutants revert at a frequency higher than 10^{-7} suggesting their point mutational character. Some mutants had no enzymic activity but reacted strongly with anti-HGPRTase (CRM^+), indicating the presence of a defective enzyme protein probably caused by a mutation in the HGPRTase structural gene. A number of mutants had little or no enzymic or immunologic activity. Among these $HGPRTase^-$ CRM^- mutants are excellent candidates for nonsense mutations. Reversion of the $HGPRTase^-$ CRM^- mutants to $HGPRTase^+$ CRM^+ was obtained. The individual revertant isolates from a given mutant differ in their characteristics. Thus it is very likely that reversion is being achieved by a variety of mechanisms, among which will be suppression.

Significance to Biomedical Research and the Program of the Institute:

Understanding of the mechanism of action of tumor viruses will require an understanding of the specific genes of the viruses that are responsible for transforming a normal cell into a tumor cell. For these studies, viral and cellular mutants will be essential. The system of suppressor mutants that Dr. Caskey proposes to develop has unique advantages over temperature sensitive mutants. They are simpler, less expensive, and produce "absolute, non-leaky" mutants.

Proposed Course: Now that mutants and their revertants are available in CHL cells a major effort will be devoted toward the genetic and biochemical analytical approaches for identifying the su^+ lines. The approaches being explored are (a) development of an *in vitro* analytical method for detecting low levels of $tRNA^{UAG}$ (su^+); (b) development of a group of Sindbis virus mutants of nonsense type which can be used for identification of 3T3, Hela and CHL su^+ clones; (c) analyzing the stability of the $HGPRTase^{+(rev)}$ mutants. Clones which have reverted via suppression will be less stable than those which reverted by intergenic changes.

Date Contract Initiated: January 15, 1972

Current Contract Level: $60,000

HARVARD UNIVERSITY (NCI-E-72-3246)

Title: Oncogenic Herpesviruses in Primates

Contractor's Project Director: Dr. Luis Melendez

Project Officer (NCI): Dr. Roy Kinard

Objectives: To further characterize two oncogenic herpesviruses, H. saimiri and H. ateles, known to be oncogenic in Aotus and Saguinus sp. and two new isolates, H. aotus and H. saguinus, and to determine if other new herpesviruses, isolated from primates indigenous to South and Central America, are oncogenic in the host or other species.

Major Findings: Four viral isolates have been obtained from Aotus species; the first two isolates are adenovirus; the second is a paramyxovirus and the last one is a new herpesvirus named H. aotus type 2.

Herpesvirus saguinus is a new virus from the cotton-top marmoset. Four other herpesvirus strains have been obtained from this species and are being tested with H. saguinus and H. ateles antisera.

Herpesviruses simplex type 1 (oral strain) and type 2 (genital) have been differentiated for the first time by applying the specificity differentiation (SPD) technique to immunofluorescent procedures. This allows the utilization of SPD to differentiate herpesviruses (oncogenic and non-oncogenic) for diagnostic purposes.

The virulence of Herpesvirus saimiri for owl monkey kidney (OMK) cultures has been attenuated by serial passage of the virus in dog fetal lung (DFL) cell line.

Two findings suggest that oncogenicity can be activated by simultaneous inoculation with two different DNA viruses: (1) Cebus albifrons (cinnamon ringtail) monkeys inoculated with H. saimiri and a likely new adenovirus named Cebus isolate have developed a disease similar to Hodgkin's lymphoma when they were inoculated with both viruses. Further studies have indicated that when any of these viruses is inoculated alone similar disease is not observed. (2) H. saimiri positive squirrel monkeys have developed a disease similar to malignant lymphoma when inoculated with Cebus isolate.

Significance to Biomedical Research and the Program of the Institute:

The knowledge gained in the study of these herpesviruses will provide insight into malignant processes of man in which herpesviruses are considered likely candidates for etiology: Epstein-Barr virus (EBV) in Burkitt's lymphoma, and Herpes simplex type 2 in carcinoma of the cervix, and will provide well characterized antigens and other reagents for testing for presence of related viruses in humans.

Proposed Course: Continuation to achieve the objectives described.

Date Contract Initiated: June 26, 1972

Current Contract Level: $72,650

UNIVERSITY OF MINNESOTA (NCI-E-71-2261)

Title: The Search for Tumor Virus Related Information in Human Immunodeficiency Patients with Cancer

Contractor's Project Director: Dr. John Kersey

Project Officer (NCI): Dr. George J. Todaro

Objectives: To search for viruses in cancer patients with immunodeficiency diseases and on immunosuppressive therapy.

Major Findings: The Immunodeficiency Cancer Registry has been continued and expanded.

The continuing search for complete tumor viruses in cancer patients with immunodeficiency diseases or on immunosuppressive therapy has, to date, been negative.

Cell cultures continue to be established from tumors reaching a total of 270 over the past two years.

In 32 cases of primary immunodeficiency disease, both B- and T-cell functions were evaluated and tablulated to aid in the characterization of various syndromes.

Nine patients with acute lymphocytic leukemia and 13 patients with lymphoma were studied by immunohistochemical methodologies to delineate their clonal origin. This may aid in more precise diagnosis and better classification of these entities and ultimately may have an impact on their treatment.

Immunosuppressed renal transplant patients were screened for virus infections. A clear cut association between virus infection and rejection of the renal allograft was shown.

Fibroblasts transformed by SV40 Kirsten MSV showed no loss of HL-A antigens. By contract, four of eight tumor cell lines showed no evidence of histocompatibility antigens. These findings suggested the possibility that antigenic loss could be related to immunoselection in vivo or antigenic loss associated with long term culture and that, contrary to earlier predictions, loss of histocompatibility and differentiation antigens was not necessarily a frequent event associated with transformation.

A difference in the distribution of intramembranous particles (as seen in electronmicrographs of freeze-etched membranes) of Balb 3T3 cells at

low density and after contact inhibition was demonstrated. At low density intramembranous particles showed a random distribution; after contact inhibition a marked aggregation of particles was noted.

Significance to Biomedical Research and the Program of the Institute:

The main premise behind initiation and continuation of this contract is the idea that there should be a much better chance of recovering a complete human tumor virus from a tumor in an immunologically incompetent patient than in an immunologically competent one. The contract provides information and materials from patients either with immunodeficiency diseases or iatrogenically immunosuppressed, which are investigated for the presence of tumor viruses. The recovery of a complete human tumor virus would greatly aid the study of the relationship of viruses to human cancer, leading towards an effective means of prevention and control.

Proposed Course: 1. Continuation of studies linking immunodeficiency, cancer, and oncogenic viruses; a) procurement of tumors arising in immunodeficient individuals for direct virologic analysis and establishment in tissue culture, b) continuation of clinical studies of tumors arising in immunodeficient individuals, c) studies of antitumor immune responses to tumors in immunodeficient individuals. 2. Continuation of human and model system studies of the clonal origin of cells and histocompatibility antigens.

Date Contract Initiated: May 13, 1971

Current Contract Level: $280,124

MASSACHUSETTS INSTITUTE OF TECHNOLOGY (NCI-E-71-2149)

Title: Studies of Leukemia Virus DNA Polymerases

Contractor's Project Director: Dr. David Baltimore

Project Officer (NCI): Dr. Edward Scolnick

Objectives: Characterization of DNA polymerases associated with oncorna viruses and normal and neoplastic cells; to investigate their function, mechanisms, and products.

Major Findings: Results suggest that the initial product of the polymerase reaction is a covalently linked DNA-RNA molecule and that the primer for initiation of DNA synthesis may be RNA. Identical results were obtained with mouse leukemia virus, avian myeloblastosis virus (AMV), and a reconstructed system consisting of purified 60-70S AMV RNA and purified AMV DNA polymerase.

One laboratory use of the RNA tumor virus DNA polymerase is the synthesis of DNA complementary to mRNA. The 10S RNA from rabbit reticulocytes, which stimulates the synthesis of globin chains in cell-free protein

synthetic systems, was transcribed into complementary DNA.

Recent studies have focused on how synthesis of the second strand of DNA in a double-stranded DNA is initiated. Purified, single-stranded DNA is an excellent template and is converted to double-stranded product with about 40% efficiency. The double-stranded product is made by "hair-pin" formation, that is, initiation of the second strand on the tail of the single-strand.

The contractor has confirmed the reported existence, associated with AMV DNA polymerase, of a ribonuclease H (which degrades the RNA portion of DNA-RNA hybrids). When the AMV DNA polymerase is purified, the ribonuclease H activity remains with polymerase at all steps. The nuclease therefore appears to be part of the same molecular complex as the polymerase.

Using infected and uninfected murine cells they have developed a simple screening procedure for quantitating the various DNA polymerases and for identifying the intracellular reverse transcriptase. This involves chromatography of extracts and assay with three templates: They call the various enzymes polymerase N (the major nuclear enzyme), polymerase C (the major cytoplasmic activity), polymerase A (the one responding to a poly(A) template) and polymerase V (the cell-associated form of the virion DNA polymerase).

Cells from a case of childhood acute lymphoblastic leukemia contain an apparent DNA polymerase activity which was not found in any other cells except thymus cells. The enzyme has the properties of terminal transferase, an enzyme known to be found in thymocytes. The cells also contain the three major DNA polymerases found in growing cells.

Noninfectious particles of a mutant of Rous sarcoma virus failed to exhibit DNA polymerase activity even with the use of the most sensitive synthetic template-primer complexes. A neutralization blocking test against antibody to DNA polymerase revealed that these mutants did not contain protein immunologically related to the DNA polymerase.

Significance to Biomedical Research and the Program of the Institute:

The characterization of the enzyme that produces DNA from the tumor viruses genetic material (RNA) has a very high priority in the SVCP. It may provide much more sensitive techniques for locating cancer virus genetic information in human tissues.

Proposed Course: Continuation to achieve the objectives described.

Date Contract Initiated: May 1, 1971

Current Contract Level: $65,000

CALIFORNIA, UNIVERSITY OF (NCI-E-71-2173)

Title: Comparative Studies on the Structure and Replication of Murine and Avian RNA Tumor Viruses

Contractor's Project Directors: Dr. Howard K. Schachman
Dr. Peter Duesberg

Project Officer (NCI): Dr. Bernard Talbot

Objectives: To study the RNA structure and the replication of avian and murine RNA tumor viruses.

Major Findings: It was suggested previously by Dr. Duesberg that the a subunit of avian sarcoma virus contains information for transformation of fibroblasts and that b subunits of nontransforming virus lacked this information. This hypothesis was submitted to two tests: First, the RNA of a temperature-sensitive mutant of Rous (T5) affecting transforming ability was investigated. It contained a subunit both if propagated at the permissive and at the nonpermissive temperature. It was concluded that a temperature-sensitive gene of the a subunit rather than a cellular gene determines transformation. Second, the RNA of a spontaneous nontransforming segregant of a transforming sarcoma virus was investigated. It lacked the a subunit. Thus, both experiments confirmed the hypothesis that a subunit contains RNA sequences required for transformation of fibroblasts.

The subunit structure of 60-70S RNA of cloned sarcoma viruses was investigated. It was found that cloned sarcoma viruses contain only a subunits and nontransforming viruses only b subunits. This raises the question as to whether 60-70S RNA of cloned virus is haploid consisting of a genome of physically identical but chemically different segments, or whether it is polyploid consisting of identical subunits. The spontaneous emergence of nontransforming virus which is correlated by a simultaneous loss of a subunit argues for a polyploid genome, because it is unlikely that three different a subunits are converted synchronously to b subunits.

Substrate deletion experiments led to the suggestion that the DNA polymerase of RSV transcribes a poly A sequence of the viral RNA. A poly A sequence has since been found to be an integral part of each 30-40S subunit of viral RNA.

Previous studies by Dr. Duesberg and by others suggested that an RNA primer is responsible for the relatively high template activity of 60-70S RSV RNA for RSV DNA polymerase. The role of this primer in viral RNA dependent DNA synthesis has since been investigated and the primer was partially characterized as a 4S subunit species of 60-70S RSV RNA.

It was observed that the RNA of RSV harvested at 3 minute intervals from cells contains mostly 30-40S RNA. Upon incubation at 40°C the 30-40S RNA is converted to 60-70S RNA in the virus. This suggests that 30-40S

is a precursor - and not a breakdown product - of 60-70S RNA.

The glycopeptides of the viral envelope glycoproteins of different RNA tumor viruses were analyzed. It was found, that the glycopeptides of all viruses released from transformed cells are larger than those of all viruses released from phenotypically normal cells. It was concluded that the transformed state of the cell determines the size of viral glycopeptides, because even nontransforming viruses released from virus-transformed cells contain large glycopeptides.

Significance to Biomedical Research and the Program of the Institute:

Basic research on RNA tumor virus structure and replication may provide the basis for understanding viral carcinogenesis which may ultimately lead to the control of human cancer. Studies on the a subunit of 60-70S RNA may pinpoint the localization of the virally transmitted oncogenic information.

Proposed Course: The following goals are set for the coming year: (a) It is planned to investigate whether the RNA of transforming murine sarcoma viruses can be distinguished from that of murine leukemia viruses, analogous to the difference between a and b subunits as demonstrated by Dr. Duesberg in the avian tumor virus system. (b) So far differences between a and b subunits of avian tumor viruses have been demonstrated only in gels containing aqueous buffer systems or gradients. The system will now be examined in gels containing 99% formamide. (c) The chemical basis of the electrophoretic difference between a and b subunits will be investigated by comparative analyses of the oligonucleotide patterns of the RNAs (fingerprints), by cross hybridization of viral RNAs with DNA made by viral DNA polymerase, using viral RNA as template, and by comparative analysis of large RNA-fragments obtained by limited digestion. (d) A search will be made for 30-40S RNA precursor in the murine tumor virus system harvested at short intervals, in analogy with Dr. Duesberg's findings in the avian tumor virus system. (e) Attempts will be made to develop an in vitro Rous RNA or MSV-MLV RNA dependent protein synthesizing system. (f) More extensive purification of the DNA polymerase of MSV-MLV will be undertaken, to test whether RNase H is an integral part of the enzyme. (g) The priming ability of RSV and MSV-MLV RNA primers for RSV and MSV-MLV RNA templates will be compared. (h) End group analysis of viral RNAs will be performed. (i) The x-ray inactivation curve as well as the target size of RSV harvested at short intervals will be investigated to distinguish between polyploidy or haploidy of viral RNA. (j) In collaboration with Dr. Robley Williams, the electronmicroscopic structure of the virion will be studied.

Date Contract Initiated: June 29, 1971

Current Contract Level: $153,000

JEWISH HOSPITAL AND MEDICAL CENTER OF BROOKLYN (NCI-E-72-2034)

Title: Viral Transformation and Chromosome Abnormalities in Human Tumors

Contractor's Project Director: Dr. Harvey Dosik

Project Officer (NCI): Dr. George J. Todaro

Objectives: To conduct systematic clinical, epidemiologic and cytogenetic investigations of patients and relatives of patients with chromosome abnormalities, increased risk of malignancy, and those on chemotherapy for malignancy, and to supply NCI investigators with cell cultures, serum or other specimens from such patients.

Major Findings: By measuring the *in vitro* transformation frequency of human diploid fibroblasts by the oncogenic virus SV40 a number of highly susceptible groups were discovered. These included patients with Fanconi's Anemia, Down's Syndrome and radiation treated cells. Besides increased SV40 transformation these groups have two other features in common: 1) chromosome anomalies; and 2) a high incidence of spontaneous neoplasms. Relatives of patients with Fanconi's Anemia also had increased transformation as well as a high incidence of spontaneous neoplasms and there appeared to be an increase in minor chromosome anomalies.

In studying the SV40 transformation frequency of 83 normal skin fibroblast samples, there was no difference seen as a function of the donor's age or sex. Lines derived from Sapada, an inbred town in Italy, showed increased transformation frequency.

Chromosome studies on 78 normal individuals showed no difference with respect to age groups except for increasing number of cells with 45 chromosomes noted with advancing age. This confirms previously reported studies.

Significance to Biomedical Research and the Program of the Institute:

These studies are enabling the SVCP to determine, on a broader scale, the relationship between chromosome anomalies (particularly those which involve an excess of genetic material), susceptibility to cellular transformation by oncogenic agents and an increased incidence of malignancy.

Proposed Course: Continue to supply and study viral transformability of normal and neoplastic tissues from individuals with chromosome abnormalities.

Date Contract Initiated: October 7, 1970

Current Contract Level: $99,818

ATOMIC ENERGY COMMISSION (NCI-E-72-208)

Title: NCI-AEC Viral Carcinogenesis Program

Contractor's Project Directors: Dr. F. T. Kenney
Dr. R. W. Tennant
Dr. G. D. Novelli
Dr. M. G. Hanna

Project Officer (NCI): Dr. Bernard Talbot

Objectives: In January 1963 an interagency agreement was established between the National Cancer Institute and the Oak Ridge National Laboratory for carcinogenesis studies. During the past 4 years the agreement was funded by both the Carcinogenesis and Viral Oncology (SVCP) Program Areas of NCI. In September 1972, the Viral Oncology funded portion was split out as this separate Interagency Agreement. It is divided into 4 main areas, studying Regulation of Gene Expression (Kenney); RNA Tumor Virus Cell Biology (Tennant); Enzymology of Viral Carcinogenesis (Novelli); and Immunology of Viral Carcinogenesis (Hanna).

Major Findings: A poly(U)-Sepharose chromatography method for fractionation and isolation of specific messenger RNAs was developed, based upon temperature program elution; it can fractionate polymers of (A) differing in chain length by only 4 or 5 nucleotides. Neither ribosomal nor transfer RNA will absorb to the column. Steroid-induced cells showed increases in synthesis of specific mRNA fractions. The technique has been applied to purified viral RNA subunits from various viruses. MuLV 36S RNA subunits are composed of two chromatographically distinct fractions, consisting of poly A containing subunits representing approximately 60% of the viral RNA and non poly A containing subunits comprising 40% of the total RNA.

Poly(G)-Sepharose columns were used to separate viral from cellular polymerases. DNA polymerase I (8S) and II (3S) from a normal spleen extract elute from poly(G)-Sepharose as a single peak much earlier than the RLV-enzyme. When a leukemic spleen extract is chromatographed on poly(G)-Sepharose, cellular polymerase II and the RLV enzyme are almost completely separated, eluting at 0.4 M and 0.6 M NaCl respectively. These two enzymes are easily distinguished by their template preferences. All of the cells examined contained a minor peak of activity that eluted at 0.6-0.7 M NaCl and preferred poly $rA \cdot dT_{10}$ as a template (i.e., like the viral reverse transcriptase). It is not known if this enzyme is a viral polymerase or another cellular polymerase with properties similar to a reverse transcriptase. Acute leukemic cells do not appear to contain more of this enzyme than normal cells.

The RNA-directed DNA polymerase of murine leukemia viruses is competitively inhibited by single-stranded polyribonucleotides, and these polymers especially poly (A), and the relatively RNase resistant derivative 2'-O-methyl poly(A)[poly(Am)], inhibit the replication of leukemia viruses in cultured cells. Concentrations of poly(Am) which do not affect leukemia virus uptake are still effective in blocking replication. Activation of

AKR virus by IdU is not affected by even high concentrations of poly(A) or poly(Am).

The capacity of poly(Am) to inhibit tumor formation by MSV in vivo, in newborn Balb/c mice was tested. At high virus dose there was no inhibition of onset of tumors, but 5 to 10 days after their appearance the tumors regressed in the poly(Am)-treated group. About one month later tumors reappeared in this group and the animals began to die; at 75 days 50% of the poly(Am)-treated animals and all but 1 of the controls are dead. At lower virus concentration no tumors are noted until about 50 days, at which time the control group began to develop tumors. All the controls are dead after 90 days; there is no sign of tumor formation in the poly(Am)-treated animals. With RLV in young adult Balb/c mice, at high virus dose there was little difference in the poly(Am)-treated animals and controls, but at low virus concentration the leukemic process was stimulated by poly(Am).

Mouse cells which are permissive for mouse leukemia virus (3T3) were fused with nonpermissive human cells (Wl-38). Essentially all of the heterokaryons examined were nonpermissive for virus expression, indicating that the nonpermissive state is dominant and implying that the nonpermissive cell is able to repress virus synthesis specifically. Moloney virus is adsorbed with equal efficiency to Wl-38 and 3T3 cells, indicating that restriction is not at the level of virus adsorption. 12 human/mouse hybrids which have lost half or more of their human chromosome complement are permissive for virus synthesis. Restriction of host range of mouse leukemia viruses is not at the adsorption-penetration step, since fusion of nonpermissive (B) cells in the presence of the Gross (N) virus does not overcome the restriction.

Autogenous immunity to endogenous MuLV related antigen(s) in RF mice, a strain with a low natural incidence of lymphoma, but having a high age associated incidence of glomerulonephritis was demonstrated. A marked decrease in detectable MuLV antigen in thymus and spleen at 1.5 months of age correlated with development of humoral immune components of the spleen, antigen localization, and serum immune elimination. This in turn correlated in time with the onset and increasing incidence and severity of glomerulonephritis. Some antibody eluted from kidney of aged mice was determined to be specific for MuLV antigens.

Utilizing serum as well as kidney eluate in immunoelectronmicroscopy (IEM) it could be demonstrated that throughout life B6C3Fl mice possess significant levels of free antibody which is specific for MuLV envelope antigens; this antibody is capable of neutralizing AKR virus as well as Moloney virus. Immunoglobulin and MuLV antigens, presumably in the form of immune complexes, were localized in kidney glomeruli, and type C virus was demonstrated within the glomerular basement membrane matrix of glomerulonephritic kidneys of both B6C3Fl and AKR mice.

A radioimmune precipitation (RIP) test using ^{3}H-leucine labeled AKR virus was developed. A marked difference between mouse strains of age associated levels of free antibody to GVEA was detected by the RIP test,

and the differences were inversely related to the natural incidence of lymphoma.

Immunologic crossreactivity of antigens common to tumor and fetal cells was demonstrated both with humoral and cell-mediated immunity. Suppression of syngeneic fetal cell growth can be obtained by immunization with either syngeneic, allogeneic, or xenogeneic tumor cell systems. Thus it appears that the tumor associated fetal antigens are common crossreactive antigens. The inability to obtain protection within different tumor systems by immunization with tumor cells further suggests that the fetal antigen component of tumor cells is a minor component.

At least 3 peaks of RNA can be resolved when 4S subunits of the 70S viral RNA are analyzed by reversed phase chromatography. They are located in the later portion of the region where mammalian tRNAs are eluted. Narrow rather than diffused appearance of the peaks suggests that they are distinct molecular species (like individual isoaccepting tRNA species). Whether or not these are tRNAs remains to be determined.

A scheme of purification of the viral polymerase from Rauscher murine leukemia virus-infected mouse spleens was developed and used to prepare large quantities of purified RNA-dependent DNA polymerase and bypass the cost and effort of obtaining isolated virus.

The reverse transcriptase from Rauscher murine leukemia virus and from avian myeloblastosis virus are considerably different in: (a) apparent molecular weight; (b) chromatographic behavior on phosphocellulose and hydroxyapatite columns; (c) optimal conditions (divalent metals, pH, temperature) for the utilization of various synthetic template-primers; (d) certain reaction kinetics; and (e) response to the competitive inhibition by single-stranded polyribonucleotides.

Significance to Biomedical Research and the Program of the Institute:

This effort is focused on the phenomenon of viral carcinogenesis; the problem is investigated in terms of enzymology, immunology, cell biology and control of gene expression. This multifaceted approach is focused primarily on the mouse leukemia system, but increasingly the findings are being carried over into work with human tumor cells, in an attempt to understand, and ultimately deal with, the problem of cancer in man.

Proposed Course: Continue to develop a concerted, interdisciplinary research program in the central aspects of viral carcinogenesis.

Date Contract Initiated: July 1, 1963 (Separate Viral Oncology Contract: September 1, 1972)

Current Contract Level: $800,000

UNIVERSITY OF CALIFORNIA AT LOS ANGELES (NOi CP 33283)

Title: Search for Presence and Distribution of Hybridizable Tumor Virus DNA in Tissues from Cancer Patients

Contractor's Project Director: Dr. Marcel Baluda

Project Officer (NCI): Dr. George J. Todaro

Objectives: To determine whether human cancer cells contain putative oncornavirus DNA that is not present in normal cells. Radioactive 70S RNA from candidate human oncornaviruses, and from other oncornaviruses that may be partially homologous to putative human viruses, are used as a probe in hybridization experiments with DNA extracted from normal and neoplastic human cells. If differences between normal and tumor cells are observed, then the distribution of the hybridizable viral DNA among the various tissues, normal and neoplastic, of cancer patients, will be determined.

Major Findings: RNA from RD-114B virus hybridized ten to twenty times more with normal cat DNA than with normal human DNA and hybridized to the same extent with DNA from normal cat and DNA from leukemic feline cells (FL-74) producing Thielen leukemia virus. Also, the 70S RNA from RD-114B virus hybridized to the same extent with DNA from normal human cells and DNA from neoplastic human cells including the RD cells. As expected, however, DNA from RD-114B cells which are producing this virus hybridized two to four times more than the non-virus producing RD cells.

It appears that RNA from RD-114B virus is not suitable as a probe for the search of putative human oncornavirus DNA in human tumors. It also appears that RD-114B virus contains RNA that is not homologous to that of Gardner, or Thielen, FeLV since it cannot differentiate between normal cat DNA and DNA from cat cells that are releasing these two feline leukemia viruses. RD-114 appears to be a feline oncornavirus, different than Gardner or Thielen FeLV.

The same type of experiments were carried out with 70S RNA obtained from purified Gross leukemia virus and, surprisingly, this virus was found to hybridize approximately twice as much with DNA from various human tumors as it did with DNA from normal human tissues.

Significance to Biomedical Research and the Program of the Institute:

This study will tell whether human cancer cells contain putative oncornavirus DNA that is not present in normal cells, and the distribution of such viral DNA among the various tissues. It thus provides important information as to whether oncornaviruses are associated with human cancer.

Proposed Course: To continue hybridization studies with DNA extracted from normal and neoplastic human cells. Hybridization will next be done with 70S RNA from woolly monkey type C virus.

Date Contract Initiated: June 8, 1972

Current Contract Level: $165,000

PUBLIC HEALTH RESEARCH INSTITUTE OF THE CITY OF NEW YORK, INC.
(NCI-E-72-2028)

Title: Study of Cell Surface Alterations Induced by RNA and DNA Viruses

Contractor's Project Director: Dr. Thomas Benjamin

Project Officer (NCI): Dr. George J. Todaro

Objectives: To investigate the relationship between cell surface alterations induced by several oncornaviruses and by mutants of polyoma virus.

Major Findings: In studies on the Balb series of lines, measurements of saturation density and serum concentration dependence of saturation density gave results consistent with expectations based on cell line history and presence or absence of transformed properties. The results of agglutination studies with wheat germ agglutinin and concanavalin A showed agglutinability to be correlated with transformation as expected from previous and extensive studies on DNA virus transformed cells.

Two new independently derived polyoma-transformed Balb/3T3 cell lines were tested for susceptibility to virus infection; they show the same properties as polyoma-transformed Swiss 3T3: namely, they are capable of infection by either wild-type or host range mutant polyoma, both in lysates as well as in direct plaque assays. These lines were obtained from x-irradiated cells, infected by u.v. irradiated wild-type polyoma. Since these lines are positive for polyoma T-antigen, and since the x-irradiated, uninfected controls showed no transformation, they appear to be true polyoma transformants, and not x-ray or spontaneously transformed cells. This result is important in that it further confirms the generalization that the host range mutant can be complemented by any polyoma transformed cell that is permissive for wild-type, by providing a polyoma-specific "transformation function."

The host range polyoma mutant, NG18, at low multiplicity, can induce V-antigen in non-permissive cells, although at a somewhat reduced efficiency compared to infection of permissive cells. These results were obtained using a caprine antipolyoma sera, both by indirect and direct immunofluorescence. Previous results using a hyperimmune rabbit anti-viral antiserum gave no evidence of V-antigen synthesis in mutant-infected 3T3 cells. This raises the possibility that the V-antigen synthesized under non-permissive conditions is defective, detectable by the caprine, but not by the hyperimmune rabbit antisera.

NG18 obtained from a lysate of "non-permissive" cells (3T3) was used to reinfect 3T3; in this second cycle of growth, essentially neither virus nor V-antigen was produced, although a reinfection of permissive cells

(PY6) did produce virus and V-antigen. A similar if not quite as dramatic, observation was also made with wild-type polyoma: namely, wild-type also grew and induced V-antigen much more poorly in the second cycle of growth in 3T3, but not in PY6. Since the multiplicity of infection was only 1, it seemed unlikely that this result was solely due to generating defectives in the first cycle; however, this explanation is not ruled out as yet.

With regard to the permissivity of Balb lines to NG18, it appears that the transformed lines are permissive to varying degrees, with the most highly transformed lines providing the best growth, and the least transformed or most contact inhibited lines, providing the worst. If confirmed, these results would indicate that there may be a real permissivity function for the host range polyoma mutant expressed in heterologous transformed cells. This expression correlates with agglutinability properties.

Significance to Biomedical Research and the Program of the Institute:

The studies are aimed at understanding the relationship between the permissive function in polyoma transformed cells - a function necessary for transformation by polyoma, and a general set of cellular and viral functions causing change in the cell surface. The surface changes of transformed cells are undoubtedly involved in the phenomena of unrestrained growth and metastasis of tumors.

Proposed Course: Termination of contract on March 31, 1973 due to Dr. Benjamin's moving to Harvard University.

Date Contract Initiated: December 3, 1971

Current Contract Level: $13,000

UNIVERSITY OF WISCONSIN (NCI-E-72-2022)

Title: Role of RNA Tumor Viruses and Related Genetic Information in Induction of Tumors by Chemicals

Contractor's Project Directors: Dr. Charles Heidelberger
Dr. James Miller

Project Officer (NCI): Dr. George J. Todaro

Objectives: To investigate the extent to which RNA tumor viruses or genetic information related to these viruses are involved in chemical oncogenesis in rodent tissues. Both *in vivo* and *in vitro* oncogenesis systems are studied by a variety of immunological, enzymological (nucleic acid polymerases), nucleic acid hybridization, and genetic technics.

Major Findings: Experiments to determine the optimal experimental conditions for *in vitro* transformation of a contact-sensitive C3H mouse

embryo cell line, 3T1/2, have been completed. These cells are highly sensitive to malignant transformation by the hydrocarbons MCA, DMBA, and dibenz(a,h)anthracene (DBA). The incidence of spontaneous transformation is very low, and the transformation system is highly reproducible and suitable for quantitative comparative studies on chemical transformation in vitro. Many clones of chemically transformed cells have been isolated for inoculation into animals and for studies on the activation of the expression of the MuLV antigens. Tumors have been obtained from transformed clones and have been diagnosed as fibrosarcomas.

18 clones of C3H fibroblastic cell lines (3 non-transformed, 15 transformed by chemicals) were tested for MuLV gsl antigens by immunofluorescence; all were negative. Four of these were tested for infectious virus by XC test and were negative. 6 clones of AKR fibroblastic cell lines (3 non-transformed, 3 transformed by chemicals) were tested for gsl antigens and by XC test; all were positive. This evidence indicates that in this system the genome of the cell determines virus expression, and not the transformed phenotype.

Murine leukemia virus (derived from AKR 3T3 cells) has been fractionated into its six major polypeptides. This was accomplished by two cycles of gel filtration in agarose in the presence of 6M guanidine hydrochloride and reducing agents. Each protein was dialyzed free of the guanidine hydrochloride and used for the immunization of a rabbit. Antisera have been prepared against each polypeptide and are presently being studied for serological specificity.

Six cell-surface antigens associated with infection by MuLV have been identified. Individual mice can be typed for these antigens by cytotoxic and immunofluorescence tests. In genetic crosses between mice of high and low leukemia strains these antigens segregate according to Mendelian ratios. Two of these antigens are of particular interest. They are designated G_L and G_T, and are identified by antisera prepared in inbred rats with progressively growing syngeneic MuLV-induced leukemias. In segregating crosses, these antigens are controlled by two dominant unlinked genes -- the presence of either of these genes will give a positive phenotype. Control of these antigens is analogous to that reported by Rowe for infectious virus.

Significance to Biomedical Research and the Program of the Institute:

Carcinogenic chemicals can cause cancer in humans, mice and other animals. An essential step toward the objective of SVCP is to determine what role, if any, viral genetic information might play in this process. The work in mice under this contract will provide a guide to the solution of this problem in humans.

Proposed Course: Continuation to achieve the objectives described.

Date Contract Initiated: September 1, 1971

Current Contract Level: $203,000

UNIVERSITY OF ILLINOIS MEDICAL CENTER (NCI-E-72-2031)

Title: Studies on the Molecular Mechanism of Carcinogenesis by Oncogenic Viruses

Contractor's Project Director: Dr. Giampiero di Mayorca

Project Officer (NCI): Dr. Bernard Talbot

Objectives: The work continues to focus on 1) temperature sensitive mutants of polyoma virus and 2) structural proteins of polyoma virus. The basic questions being studied are: Which viral protein(s) is responsible for transformation? Is it present in the viral particle or only in the transformed cell? Does it have another function in the virus cycle and/or virus particle other than conferring and maintaining transformation in the cell?

Major Findings: A number of ts mutants of polyoma virus have been examined from the point of view of temperature sensitiveness for transformation and their plating efficiency at two temperatures. A series of new mutants are unable to transform at both the permissive and non-permissive temperatures (some exhibit temperature sensitivity in the lytic cycle while others do not). Another group of mutants are temperature sensitive for the lytic cycle but transform as efficiently as the wild type at both temperatures.

Pulse experiments were consistent with the expected model of polyoma virus being transcribed as a polycistronic message at any time after entry into the cell with only rudimentary control by the cell. The results strongly suggest, as in the case of phage alpha, that the decision of transcribing early or late messages is only a consequence of the molecular status of the virus DNA, with the supercoiled incoming DNA available perhaps only for "early" message transcription, and the replicating uncoiled DNA for total transcription. Since all mutants responded best to a pulse of permissive temperature at a certain time during the vegetative cycle, it appears that what is impaired at the non-permissive temperature, is the utilization of a gene product (a thermally unstable enzyme or structural protein) at a specific time.

Polyoma mutant Ha235 behaves as the wild type in lytic growth, but is completely unable to transform BHK_{21} cells at the permissive or non-permissive temperature at pH 7.2 or pH 6.3. At pH 7.2 the mutant adsorbs poorly to cells, although at pH 6.3 it is as efficient as the wild type. Analysis by gel electrophoresis failed to detect differences in structural proteins between Ha235 and the wild type. To detect possible changes caused by a small deletion or substitution, experiments by bidimensional gel electrophoresis run at two different pH's are currently being carried out.

Significance to Biomedical Research and the Program of the Institute:

Perhaps the most fundamental problem in cancer research is defining the

cellular mechanism involved in cell transformation. Temperature sensitive mutants of oncogenic viruses represent effective tools for probing viral-cell interactions associated with transformation and the mechanisms of cell regulation and control. Sufficient understanding of the transformation phenomenon may provide the basic keys to maintaining or restoring cell normalcy.

Proposed Course: Continuation of characterization of polyoma temperature sensitive mutants, plus attempts to isolate deletion mutants of polyoma, plus initiation of studies on human papova viruses.

Date Contract Initiated: December 9, 1971

Current Contract Level: $162,500

LITTON-BIONETICS (NCI-E-73-3230)

Title: Applications of Animal Virus Model Systems to Human Neoplasia

Contractor's Project Director: Dr. David Valerio

Project Officer (NCI): Dr. Robert H. Bassin

Objectives: To study the nature of MSV defectiveness and the role of helper leukemia virus and to assess the applicability of this model system to human tumors. To establish and characterize new human tumor cell lines and develop methodology to use in the detection of viruses and subviral products. To study the mechanism of SV40 cell transformation by examining virus integration and the turn-on of DNA synthesis with infected cells. To collect, process and characterize human tumor specimens and sera of special interest. To examine cell mediated immunity in human tumor patients. To provide technical support services for the Special Virus Cancer Program.

Major Findings: Additional S+L- cell lines have been developed and characterized. The nature of the RNA and reverse transcriptase in S+L- particles has been described.

A system for rapid quantitative transformation of 3T3 cells with MSV was developed.

Glycolipid transferase activity is reduced following MSV infection in a manner similar to that observed for cells transformed by papovavirus, and it is apparent that these changes occur after cell transformation by MSV.

A human breast tumor cell line, HBT-3, has been isolated and characterized biologically. Virus particles were not seen in HBT-3 cells even after treating with inducing agents such as IdU.

Column chromotography using dT cellulose was used to demonstrate a reverse transcriptase in HBT-3 cells having many of the properties of known viral transcriptases.

Lymphocyte toxicity assays, using the F-265 cell line as a target, demonstrated that this assay is an excellent in vitro correlate of general cell mediated immunity. Membrane preparations from tumors provided by I-E segment contractors were found to contain possible tumor specific antigens in breast cancer, colon cancer and lymphoma.

Significance to Biomedical Research and the Program of the Institute:

An understanding of viral defectiveness and the role "helper" viruses is of value in determining the occurrence and mechanism of viral oncogenesis by type C viruses in man. Identification of viruses or viral products in human breast tumor cells is of value in assessing the role of viruses in human breast cancer and, ultimately, in developing techniques of both diagnostic and therapeutic significance.

Proposed Course: Additional S+L- lines will be isolated from wild-type stocks of MSV and their genetic inter-relationships will be determined. Aspects of quantitative transformation of 3T3 cells by MSV will be studied. The viral-like reverse transcriptase present in HBT-3 cells will be further analyzed, and the applicability of dT cellulose column chromotoraphy to detection of virus-specific polymerase in human cells will be ascertained. Processing of human tumor specimens as well as technical support services will continue.

Date Contract Initiated: June 27, 1969

Current Contract Level: $738,723

MELOY LABORATORIES (NCI-E-72-2006)

Title: Spontaneous and Virus-induced Neoplastic Transformation

Contractor's Project Director: Dr. John E. Verna

Project Officer (NCI): Dr. George J. Todaro

Objectives: To study spontaneous and virus-induced neoplastic transformation, especially focusing on the extent of expression of endogenous type C viral information in normal and transformed cells, and to use this information to find and characterize tumor virus information in human tumors.

Major Findings: Using antisera to the viral reverse transcriptase, it was shown that the Mason-Pfizer monkey virus (MP-MV) was unrelated to either known type C viruses or the primate syncytium-forming ("foamy') viruses. Among the type C viruses, the woolly monkey and gibbon ape viruses were found to be very closely related immunologically, but the two were only distantly related to RD-114.

The major internal protein (gs antigen) from each of the following type C viruses -- mouse, cat, woolly monkey, gibbon ape, and RD-114 -- was purified to homogeneity, and used in species specific radioimmunoassays. The close relationship of gibbon ape and woolly monkey virus, and their difference from RD-114 was demonstrated.

A clonal isolate of a primate sarcoma virus analogous to the S+L- strain of murine sarcoma virus has been obtained. It contains woolly monkey type C gs antigen and woolly polymerase. Primate and murine helper viruses have equal ability to rescue primate or murine sarcoma viruses.

An immunoaffinity chromatography system using the covalent coupling to Sepharose of IgG from an immune serum directed against the gs-3 mammalian intraspecies determinant has been developed. Crude murine antigen containing extracts can be chromatographed on such columns to yield highly purified gs protein.

Radioimmunoassay for mammalian interspecies gs-3 antigen detected reactivity in purified pig type C virus, leukemic bovine cells and rabbit lymphosarcoma tissues. In human tumors the results are still not conclusive, but have generally been negative.

An endogenous virus (CCC) of a single cell clone of feline fibroblasts has been isolated. It has a reverse transcriptase, gs antigen and host range distinct from FeLV and similar to RD-114. CCC and RD-114 both grow well in human and primate cells and poorly on monkey and human cells.

Several type C viruses with properties similar to RD-114 and CCC have been isolated from cat embryo cells and even from stocks of FeLV (Rickard and Theilen strains).

The relatedness of type C viruses in hybridization experiments shows that within one family of viruses there is usually greater than 50% homology (for example, between different mouse leukemia viruses and different cat leukemia viruses). However, there is very little homology between viruses derived from different species. The one exception is in the cat, where the endogenous type C virus (RD-114 or CCC) has little or no homology with various strains of FeLV.

Some spontaneously transformed clones of Balb/3T3 were found to spontaneously and continuously release high titers of the endogenous type C virus. Other transformed subclones were found to be virus-free, but treatment with BrdU induced production of the endogenous virus in

large amounts. Non-transformed Balb/3T3 clones never spontaneously released type C virus, and could be induced to release very small quantities only with BrdU; in contrast, cells transformed by mouse sarcoma virus, radiation, methylcholanthrene and also spontaneously were "superinducible", i.e., BrdU treatment caused virus production within 8 hours and resulted in secretion of very large quantities of virus with exponential kinetics.

All murine cell lines examined so far have been found to contain RNA which is homologous to 35S RNA of murine leukemia virus. After purification of cytoplasmic RNA by dT-cellulose chromatography to remove RNA not containing poly-A sequences, and hybridizing this RNA to the ^{3}H-DNA product, it was found that a "normal" murine cell line, A31, transcribes enough RNA to saturate 2-4% of the ^{3}H-DNA product. Murine cell lines transformed either spontaneously, by radiation, or by SV40 all transcribe an additional amount of RNA so as to saturate about 10% of the ^{3}H-DNA viral probe. Normal rat cell RNA, poly-A, or dT-cellulose purified RNA from an SV40 transformed human cell line do not hybridize to the ^{3}H-DNA murine probe.

Natural expression of the mouse gs antigen was found in all mouse tissues examined, strongly suggesting that a continuous synthesis of this polypeptide is an integral part of the murine cell macromolecular synthesis.

A virus has been isolated in immunosuppressed NIH Swiss mice bearing human rhabdomyosarcoma cells (RD). This virus, AT-124, has mouse virus polymerase and mouse virus gs-1 antigens, yet its host range is primarily human and primate. It grows in none of the known mouse cell systems. A pseudotype with this virus readily transforms human cells.

As a general rule, it has been found that endogenous viruses of several species are unable to reinfect the cells from which the virus is produced. For example, the endogenous Balb/c cell virus does not grow on Balb/c cells, but will grow in NIH Swiss mouse cells. The endogenous cat virus (CCC) does not grow in cat cells, but grows readily in primate cells. Similarily, endogenous viruses have been obtained in the past year from several other species, such as, Chinese hamster, Syrian hamster, rat and pig. In all these cases, the virus does not infect the clonal lines that release it. Further, in all these cases the virus will appear spontaneously in long-term spontaneously transformed cultures. The probability of virus release, however, can be increased with thymidine analogs, such as iododeoxyuridine. It appears that spontaneously transformed cells more readily release their endogenous virus than do untransformed cells. Since the mouse, the chicken, and the cat clearly have recognizably different endogenous and horizontally transmitted type C viruses, the central question becomes which, if either, of these viruses is responsible for naturally occurring tumors in that species. In the cat, CCC and FeLV are easily distinguishable. The endogenous Balb/c virus by immunoelectron microscopy can be distinguished from both the viruses of the FMR group and the Gross leukemia viruses. They are, however, related to the

the viruses found in spontaneously occurring myelomas of Balb/c mice. Thus, methods are available even in the mouse system to distinguish the endogenous virus from some of the laboratory strains.

Immunoelectron microscopy has demonstrated an MSV-associated cell surface antigen on the surface of nonproductively transformed cells infected by two different strains of MSV, Kirsten and Moloney. This antigen is distinct from previously described antigens on the surfaces of cells infected by murine leukemia virus, on the viral envelope, and on the surfaces of cell lines transformed spontaneously or by x-irradiation.

Murine mineral oil induced myeloma-associated virus (MuMAV) carries a specific viral envelope antigen, different from those on MuLV. Endogenous type C viruses released from spontaneously transformed Balb/3T3 cells can be classified into at least two different populations: (a) type C viruses which carry xVEA envelope antigens and (b) uncharacterized type C viruses which have neither xVEA nor the MuLV envelope antigens.

An affinity chromatographic procedure for the avian type C viral polymerase was developed. Using this procedure, viral enzyme can be selectively purified from extracts of virus producing avain cells. Extracts of Rous sarcoma virus transformed rat cells (XC cells) which synthesize no virus but do contain easily detected quantities of the avian gs antigen(s), failed to show any viral polymerase.

Cell strains derived from leukemic patients are generally more susceptible to SV40 transformation than those derived from noraml individuals. This extends the previous observation that cells from patients with Fanconi's anemia or Down's syndrome have increased SV40 transformation susceptibility.

Significance to Biomedical Research and the Program of the Institute:

The ability to identify viruses and/or to detect viral information in transformed and tumor cells is basic to establishment of etiological association, and to an ultimate approach to prevention or treatment of cancer.

Proposed Course: Continuation to achieve the objectives described.

Date Contract Initiated: May 25, 1965

Current Contract Level: $1,778,735

SUMMARY REPORT

Program Resources and Logistics Advisory Group

The Viral Oncology Program Resources and Logistics Advisory Group was established by the Associate Scientific Director in January, 1972. This Group constitutes a standing committee to provide support and make recommendations concerning resources and logistics matters, and to conduct appropriate reviews for those contracts administrated by the Office of Program Resources and Logistics. The Group is chaired by the Chief, Office of Program Resources and Logistics and is responsible to the Office. The membership includes two representatives from each of the three Branches and also representatives from the other major intramural areas of Viral Oncology. The current membership of the Group is listed in a previous section of this report concerning Program management personnel.

In addition to conducting discussions and making recommendations in an advisory capacity, the Group is responsible for formal review of resource type contracts. Such reviews consist primarily of an evaluation of relevance, priority, and need of proposed or ongoing activities in relation to the Program objective of investigating the potential viral etiology of human neoplasia. The types of contracts administrated by the Office of Program Resources and Logistics, and which are reviewed by this Advisory Group represent four general areas of activities. These include:

1. Contracts concerned with production and characterization of purified viruses and viral reagents.

2. Contracts concerned with acquisition, processing, storage, inventory, and distribution of normal and malignant human specimen material.

3. Contracts concerned with production, distribution, and maintenance of various species of experimental animals.

4. Contracts concerned with the provision of specialized testing services for the examination of experimental materials.

The Advisory Group is responsible for reviewing ten separate contracts directed toward virus and reagent production, eleven contracts involving human resource activities, ten contracts concerned with animal resource activities, and six contracts dealing with testing, service, or miscellaneous aspects. The percent of the total resources funding effort devoted to each of these four types of activities are as follows:

1.	Virus and reagent resource contracts	55%
2.	Human resource contracts	5%
3.	Animal resource contracts	30%
4.	Testing, service, and support contracts	10%

Since this Group conducts the initial review for Type I and Type II research-resource contracts, and conducts the only review for Type III exclusively resource contracts, meetings are held at rather frequent intervals, usually monthly. In this respect the Group functions in a manner analagous to the joint Program Segment Chairmen and essentially reviews the relevance of all resource contracts during the course of the calendar year. Type I and Type II research-resource efforts additionally receive a second review by the Viral Oncology Branch and Associate Branch Chiefs for scientific and technical excellence. In keeping with existing procedures, there is no outside review of resource contracts.

OFFICE OF PROGRAM RESOURCES AND LOGISTICS

Dr. Jack Gruber, Chief, OPRL, OASDVO, DCCP, Chairman
Dr. David McB. Howell, OPRL, OASDVO, DCCP, Staff Scientist

AICHI CANCER CENTER (NIH 69-96)

Title: In Vitro Virological Studies of Human Tumor Specimens

Contractor's Project Director: Dr. Yohei Ito

Project Officers (NCI): Dr. Jack Gruber
Dr. Robert Manaker

Objectives: To make available to the Special Virus Cancer Program human embryonic tissues and high-titer anti-herpes virus human antiserum.

Major Findings: The contractor has continued efforts to procure human tumor materials for SVCP distribution and has also established numerous cell lines from a wide variety of neoplastic, hyperplastic, and normal tissues. These cell lines have been made available for Program distribution. Procurement of human embryos and cultivation of embryonic cells continues.

Immunological and seroepidemiological studies were continued on antibody levels against herpes-type virus (HTV) in 822 survivors of the atomic bombings of Hiroshima and Nagasaki. Little abnormality was observed in the anti-HTV titer of these individuals when compared to normal populations.

Significance to Biomedical Research and the Program of the Institute: This project represents a prime source of tissues and sera from a non-Caucasian population in the Far East. These materials, along with the contractor's observations, are of considerable value in obtaining data which are complementary to observations made on Caucasian populations concerning antibody distribution to viral antigens and the possible involvement of viruses in human cancer.

Proposed Course: The contractor will continue procurement of tissues, establishment and cultivation of cell lines, and investigation into the possible involvement of Herpes-type virus in neoplasia in Oriental populations.

Date Contract Initiated: May 2, 1969

Current Annual Level: $40,000

AUERBACH ASSOCIATES, INC. (NIH 72-2023)

Title: Support Services for Preparation of National Cancer Plan

Contractor's Project Director: Mr. Charles Fricker

Project Officer (NCI): Dr. Jack Gruber

Objectives: To perform a feasibility study of alternate systems for program resources and logistical support management.

Major Findings: This pilot study was conducted to determine the acceptability of alternative resource management and logistics systems to the SVCP community. During the study the contractor interviewed 26 Principal Investigators and 19 Contract Administrators from 19 organizations.

The tabulation and analysis of the contractor's interview data indicate an overwhelming preference for an NCI-SVCP operated centralized system. The interviewed participants generally felt that only NCI was qualified to operate SVCP's resource management and logistics system. While some participants believed that a qualified Prime Contractor could be found within the SVCP, they doubted whether such a contractor could be truly objective in placing program interests ahead of company research interests. Very few believed that a qualified Prime Contractor could be found outside the SVCP. No centralized system at all was judged least acceptable on the premise that the SVCP would become unmanageable and result in chaos.

Significance to Biomedical Research and the Program of the Institute: To assess the extent to which various types of Resource and Logistics management systems may apply to the management of the National Cancer Program, the National Cancer Institute was interested in surveying those segments of the scientific community which interface with these systems to obtain value judgments to potential applicability. To provide valid results, a specific NCI Program was utilized as a model in an applicability survey. In keeping with the above need, this Office surveyed Viral Oncology Special Virus Cancer Program participants to obtain value judgments as to the potential applicability and effectiveness of several management approaches to a Resources and Logistics support system. A questionaire was developed and the information obtained will be useful in determining effective management options for Resources & Logistics within the NCI Special Virus Cancer Program, and will provide an aid in determining a future NCI course of action relative to similar anticipated programs within the National Cancer Program.

Proposed Course: Having fulfilled its purpose, this contract terminated during 1973.

Date Contract Initiated: May 18, 1972

Current Annual Level: $24,862

BIOLABS, INC. (NIH 72-2068)

Title: Production of Specified Herpesviruses and the Development of Effective Production and Storage Procedures

Contractor's Project Director: Dr. Clyde R. Goodheart

Project Officers (NCI): Dr. Dharam Ablashi
Dr. Robert J. Goldberg

Objectives: Development and evaluation of methods for large-scale preparation of purified oncogenic herpesviruses, especially Epstein-Barr virus and Herpesvirus saimiri.

Major Findings: Continued efforts to find a suitable method for large-scale production of high-titer Herpesvirus saimiri (HVS) have included testing a variety of cell lines in addition to those previously reported. None tested to date have proved satisfactory. However, mycoplasma in some of the cell lines or in seed virus appeared to enhance viral cytopathic effects. In one contaminated line, adding gentamycin to the medium of parallel cultures after virus infection increased the completion of CPE from about four days (without gentamycin) to about ten days (with gentamycin). Mycoplasma are known to reduce arginine levels in medium. Therefore, medium without arginine was tested in uncontaminated cultures after infection. The results indicate that much higher titers of HVS are obtained when medium without arginine is used. Experiments are underway to find the optimum time after infection for switching to arginine-free medium and to test other pertinent parameters. Both PBl and Vero cells appeared to react similarly to the arginine-free medium. The titers of virus appeared to be higher with the Vero cells.

The biocontainment room that was recommended by the NCI Office of Biohazards and Environmental Control has been completed and became operational in March, 1973.

Significance to Biomedical Research and the Program of the Institute: The major goal of the SVCP is to determine the association of viruses with human neoplasia. The clinical and immunological picture of human lymphoma and leukemia is closely approximated by the malignant disease induced in susceptible non-human primates by H. virus saimiri. Additionally, EBV has been associated with such human malignant neoplasias as Burkitt lymphoma, nasopharyngeal carcinoma, and Hodgkin's disease. Infectious preparations of both H. virus saimiri and EBV are necessary to continue essential studies relevant to elucidating the role of DNA viruses in human malignancies. This contractor has the capability to meet such needs and therefore this project is of high priority.

Proposed Course: Studies indicated above will be continued.

Date Contract Initiated: December 20, 1971

Current Annual Level: $77,293

CALIFORNIA, UNIVERSITY OF (NO1-CP-3-3237 and NCI-FS-8)

Title: Development and Evaluation of Cell Substrates for the Study of Cancer Viruses

Contractor's Project Directors: Dr. Stewart Madin
Dr. Neylan Vedros
Dr. Adeline Hackett
Dr. Walter Nelson-Rees

Project Officers (NCI): Dr. James Duff
Dr. Jack Gruber

Objectives: The Cell Culture Laboratory (CCL) is physically located at the Naval Biomedical Research Laboratory (NBRL), in Oakland. The program of the CCL is funded by a contract (NO1-CP-3-3237) between the University of California and the NCI. In addition, maintenance and operating expenses generated by the CCL are repaid to NBRL by an interagency transfer of funds (FS-8) between NCI and NBRL. The research studies include the development and evaluation of cell substrates for the study of cancer viruses, development of large quantities of specific cell substrates, karyotyping of cell cultures, and performing biophysical, virological, and cytogenetic research.

Major Findings: The contractor has distributed 962 cell culture seed stocks to 118 recipients, primarily within the Special Virus Cancer Program, this year. The contractor's latest catalogue, which was distributed in January 1973, is the first produced from data now in the computer bank and lists human cell substrates initiated or propagated and stored in this laboratory for distribution following antibiotic-free cultivation, characterization and assurance of species specificity, and freedom from microbial contamination.

The contractor has procured tissues and initiated tumor cultures from sources in Los Angeles and the local area. In addition, it has received numerous samples of selected, established tumor cell cultures from various SVCP investigators for partial characterization, experimentation, preservation, and distribution within Program as directed.

Techniques for selective cultivation of epithelial-like cells have been developed and are being used in studies of carcinomatous transformation of normal cells as well as for development of tumor cell lines from carcinomas of colon, rectum, and bladder and from normal human fetuses and mammary tumors of the mouse.

The contractor is continuing work on the UC1-B cell line which transforms after infection with MuLV. Clones vary in their growth pattern and consequently in their efficiency for focus assay. Foci were achieved with MLV, RLV, AKR, and FLV types of leukemia viruses, but not RadLV or KiMLV. A UC1-B related culture, 12-3 clone 8, also derived from Balb/3T3, transforms with MuLV. Both transformants reveal aberrant virus maturation

by electron microscopy, but vary in growth properties and ability to synthesize DNA at confluency. These properties may relate to differential tumorigenic potential of cells in isogeneic mice and induction of splenomegaly.

Interactions between RNA tumor viruses and other viral agents are being studied. Results of these studies indicate that SV40-induced DNA synthesis will not support MSV replication while serum-induced synthesis does. In addition, certain murine cell lines infected with oncogenic viruses are resistant survivors of infection with encepholomyocarditis virus, a member of the picornavirus group. No resistant survivors were found in any normal cell lines.

The contractor has been studying the effect of DMB-rifampicin on cellular transformation by SV40 to compare it with previously observed inhibition of MSV focus formation and of reverse transcriptase by this chemical. It has been found that Amphotericin B (Fungizone) at low levels improves the ability of DMB-rifampicin and rifazacylo-16 to inhibit focus formation, perhaps by altering membrane permeability. However, SV40 transformation is not inhibited by DMB-rifampicin.

Studies have been carried out on surface membrane changes of SV40-infected Balb/3T3 cells which relate to expression of transformation. While ruthenium red stains all transformed cells which continue to divide at confluence whether or not they form multiple layers, agglutination with Concanavallin A occurred and transplantation immunity antigen was present only in lines where multilayers are formed.

Significance to Biomedical Research and the Program of the Institute: The contractor has developed an excellent tissue culture facility and is supplying cell cultures for cancer research studies to NCI investigators and SVCP contract laboratories. These studies are oriented toward a study of the fundamental biology of tumor cells, and the interaction between tumor cells and viruses of oncogenic importance.

Proposed Course: Continue to develop cell reagents as substrates for human carcinogenesis; attempt to isolate and characterize viral agents from human tumor cells; continue a reference laboratory for karyology of cells in culture; study oncogenic viral antigens during embryogenesis and continue basic research in the biology of tumor viruses.

Date Contract Initiated: October 1, 1962

Current Annual Level: $438,000 and $62,700

UNIVERSITY OF CALIFORNIA AT SAN DIEGO (NIH 70-2202)

Title: Development and Operation of a Breeding Colony of Domestic Cats

Contractor's Project Director: Dr. Alexis J. Kniazeff

Project Officers (NCI): Dr. Robert Holdenried
Dr. Robert J. Goldberg

Objectives: To develop and operate a permanent breeding colony of cats which will supply offspring for cancer research.

Major Findings: The cat conditioning and breeding colony supplied pregnant animals to collaborating laboratories in the SVCP, especially the Merck Institute for Therapeutic Research (Contract 71-2059). Cats purchased from pounds after selection for good health and other related criteria through physical examinations were held for a minimum of two months before breeding.

The Merck Institute's weekly requirements for pregnant cats varied throughout the year with regards both to number of animals and age of embryos. In order to meet this variable and occasionally unpredictable demand, at least ten cats were bred per week. The seasonal occurrence of natural oestrus in cats required artificial hormonal stimulation to provide gravid animals throughout the year. The contractor successfully developed a dependable system of artificial stimulation to provide gravid animals throughout the year. The contractor successfully developed a dependable system of artificial stimulation of oestrus which permitted a consistent supply of cats which were usually in the seventh week of gestation.

During the period of March 1972 - February 1973, 179 pregnant cats, 163 of which went to the Merck Institute, were sent to research laboratories.

Significance to Biomedical Research and the Program of the Institute: This colony provided the major source of pregnant cats used in the SVCP for feline leukemia-sarcoma studies, and for viral vaccine developmental studies.

Proposed Course: Because of a decreased emphasis on animal model systems and a reduced need for pregnant cats, this contract was terminated June 22, 1973.

Date Contract Initiated: June 25, 1969

Current Annual Level: $110,000

CHICAGO PARK DISTRICT, LINCOLN PARK ZOO (NO1-CP-3-3271)

Title: Marmoset Breeding Colony

Contractor's Project Director: Dr. Lester E. Fisher

Project Officers (NCI): Dr. Roy Kinard
Dr. Jack Gruber

Objectives: To provide marmosets in a quantity and quality sufficient for the needs of the research on tumor viruses conducted under Contract NIH 73-3219 with Rush-Presbyterian-St. Luke's Hospital, as well as for research conducted in other SVCP laboratories.

Major Findings: There are now 133 animals in the breeding colony, of which 101 are acclimated adults and 32 are offspring which are expected to be second generation reproductive pairs. Production continues at the rate of at least 120 births per year with no problems.

Significance to Biomedical Research and the Program of the Institute: This contract is part of a program utilizing lower primates for testing selected laboratory specimens for oncogenic activity. The marmoset, a small, inexpensive primate, has been shown to be susceptible to several cancer viruses; newborn and young animals are in demand by SVCP investigators.

Proposed Course: The project will be continued to insure the availability of experimental animals of quality.

Date Contract Initiated: June 28, 1965

Current Annual Level: $16,000

UNIVERSITY OF COLORADO MEDICAL CENTER (NIH 69-2080)

Title: Collection of Neoplastic Tumor Specimens

Contractor's Project Director: Dr. William E. Hathaway

Project Officers (NCI): Dr. Jack Gruber
Dr. David McB. Howell

Objectives: To obtain tissues and serum specimens from patients with various types of malignancies for collaborative studies with the SVCP.

Major Findings: During the past year, 845 tumor and control specimens and patient information were obtained and sent to investigators within the SVCP as directed by the project officer. Specimens were available from a wide variety of patients with diagnoses including leukemia, lymphoma, rhabdomyosarcoma, osteosarcoma, various carcinomas, neuroblastoma, and a variety of brain tumors. In addition, sera from close relatives of patients were collected whenever possible and a variety of normal tissues were made available to interested investigators.

Fresh tissue, serum, and blood for HLA typing were procured from a large group of Hodgkin's patients (fifty patients, many samples taken repeatedly). These samples were made available to Program investigators through the Litton-Bionetics Resources Processing Laboratory of the SVCP (69-2160).

Frozen tumor tissue from surgical procedures and autopsies were sent to the Flow Repository for storage and distribution to collaborating investigators.

Under Program direction, the contractor has placed new emphasis on procurement of large amounts of fresh, heparinized blood from new leukemia patients. Relative priorities for a wide variety of tissues have been clearly established and an efficient operation for collection, transmission, and follow-up of all specimens and related information are in progress.

Significance to Biomedical Research and the Program of the Institute: This is a resource contract of major importance to the SVCP, since it is a primary source of diverse cancer specimens for NCI and other SVCP researchers on the East Coast. A continuing supply of such specimens is absolutely necessary to the pursuit of the viral etiology of human cancer.

Proposed Course: Continue to collect serum and tumor specimens as in the past. Materials will be provided to the SVCP at the direction of the project officer for use in investigations by collaborating investigators.

Date Contract Initiated: June 18, 1969

Current Annual Level: $118,000

UNIVERSITY OF CONNECTICUT (NIH 73-3221)

Title: Development and Maintenance of a Specific Pathogen Free Flock of White Leghorn Chickens

Contractor's Project Director: Dr. Roy E. Luginbuhl

Project Officers (NCI): Dr. Robert Holdenried
Dr. Roy Kinard

Objectives: Establish and maintain a flock of chickens free of specified pathogens, including avian leukosis viruses, and to provide eggs for research use.

Major Findings: Approximately 12,000 eggs and additional cell cultures were provided for cancer research. A few of the recipients were Drs. J. Beard, G. S. Beaudreau, F. Deinhardt, P. Sarma, H. Temin, M. Baluda, and H. Morgan.

There is no serological evidence of the following diseases in the SPF flocks: Mycoplasma gallisepticum and synoviae, Salmonella pullorum, Newcastle disease, avian infectious bronchitis, avian adenovirus (Type I), infectious laryngotracheitis, fowl pox, avian encephalomyelitis, avian reovirus (Strain #25), infectious bursal agent, and RSV (serotypes A & B). There have been no clinical cases of Marek's disease or lymphoid leukosis. Several flocks remain negative for antibodies to Marek's disease; all other

flocks contain antibodies, however. Several flocks have been characterized as gs antigen negative or gs antigen positive. The gs status of these flocks' offspring can be reliably predicted, indicating a simple recessive genetic control for gs expression.

Significance to Biomedical Research and the Program of the Institute: The methods being developed indicate that eggs free of specified infectious organisms can be produced. A significant portion of the avian leukosis research is dependent on the continued availability of highly controlled and monitored flocks such as this one.

Proposed Course: Continued maintenance of the flocks, with development of genetic lines of chickens characterized for the susceptibility of their embryos to leukosis virus. Reestablish flocks free of Marek's disease virus and continue work to provide genetically gs-characterized birds.

Date Contract Initiated: June 18, 1962

Current Annual Level: $66,000

NEW YORK STATE VETERINARY COLLEGE AT CORNELL UNIVERSITY (NIH 70-2224)

Title: Feline Tumor Viral Diagnostic Laboratory

Contractor's Project Director: Dr. James H. Gillespie

Project Officers (NCI): Dr. James T. Duff
Dr. David McB. Howell

Objectives: To produce and evaluate cat viral reagents; to monitor cat cell cultures and other materials associated with cat tumors for indigenous cat viruses and other microorganisms.

Major Findings: During the previous year approximately 1500 feline tissue and swab samples were examined for the presence of indigenous and/or contaminating feline viral agents in cooperation with the NCI participating laboratory at Merck, Sharp & Dohme (71-2059). Samples from naturally-born offspring of Merck's caesarian-derived SPF breeding colony have been free of cytopathogenic feline viral agents to date. Two instances of feline syncytium-forming virus infection in caesarian-derived "gnotobiotic" kittens were studied. These two animals constituted the only cases of virus isolation in the caesarian-derived offspring of 39 cats. The kittens were from different litters, leading to the conclusion that congenital infection of the cat with feline syncytium-forming virus is an isolated event and occurs infrequently.

Serologic screening has been and is currently being carried out for the presence of antibody to four feline viruses in samples from the Merck SPF cat colony. These viruses include feline panleukopenia, feline herpesvirus, feline syncytium-forming virus, and a representative feline picornavirus

(calicivirus). No samples from the SPF colony have shown antibody with the assay system used, while control kittens and cats raised under conventional conditions had at least low levels of neutralizing antibody to both rhinotracheitis (herpes) virus and representative picornaviruses.

The contractor has found in preliminary evaluation that the hemagglutination-inhibition test appears to be a more rapid and sensitive measure of antibody to the feline herpesvirus than the neutralization test. In addition, adolescent cats that were apparently "runted" after inoculation with RD-114 cells failed to show any abnormality on pathologic examination.

The contractor has filled a number of requests for the Crandall feline kidney cell line from SVCP laboratories, and a limited number of samples were screened for the presence of feline viruses for SVCP contractors other than Merck, Sharp & Dohme.

Significance to Biomedical Research and the Program of the Institute: This laboratory is a major source of reagents and expertise concerning the feline tumor viruses, and makes both available to scientists within the SVCP. The contract provides a central laboratory where materials isolated from normal cats and cats suffering from cancer can be sent to determine if they contain indigenous feline agents, as well as for viral identification.

Proposed Course: This laboratory will continue to examine material from SVCP laboratories for the presence of feline viruses as well as to provide such feline virus stocks or cell line as might be required by these laboratories. Selected feline cell cultures initiated from diagnostic samples will be treated with IUDR and/or BUDR to determine if this method has application in promoting the activation of indigenous feline viruses from cultures of cells thought previously to be negative. Further characterization of SVCP-produced feline viral reagents will be assisted by additional data in the feline herpesvirus hemagglutination inhibition test as well as additional study of the serologic variation of the feline picornaviruses.

Date Contract Initiated: June 25, 1970

Current Annual Level: $42,000

DUKE UNIVERSITY (NIH 71-2132)

Title: Study and Production of Avian Leukosis Virus

Contractor's Project Director: Dr. Joseph W. Beard

Project Officers (NCI): Dr. Michael A. Chirigos
Dr. John W. Pearson

Objectives: The objectives of this project are: (1) to continue quantity and quality production of BAI strain A avian tumor virus; (2) to continue investigations on RNA avian leukosis viruses. The contractor provides an average monthly production of 55 gms wet weight of plasma and 45 gms wet weight of tissue culture grown BAI strain A avian tumor virus. Additionally, the contractor employed a multidisciplinary approach to the study of avian tumor viruses, including: biochemistry, tissue culture, virology, electron microscopy, pathology, and immunology.

Major Findings: The major work has been the production and distribution of BAI Strain A (myeloblastosis) virus, and leukemia myeloblast cells. Among the major recipients of virus are: D. Allen, D. Baltimore, D. Bolognesi, R. Gallo, M. Green, R. Huebner, J. Hurwitz, S. Spiegelman, G. Todaro, G. Vande Woude, P. Zamecnik, and other international investigators.

In addition to producing large quantities of routine avian myeloblastosis virus (AMV) for over 75 investigators in the United States and abroad, the contractor has prepared radio-labeled AMV and has contributed to the preparation of specific products of AMV, e.g., purified gs antigen, labeled RNA, intact un-nicked RNA for end-group analysis, 62S viral RNA, etc., in response to requests from SVCP researchers.

The contractor has continued research on the immunological and biochemical characterization of the major avian and mammalian virus polypeptides. Five avian myeloblastosis virus polypeptides are immunologically distinguishable, but three of these contain determinants in common. Polypeptides of Rous sarcoma virus (RSV), Prague strain, a helper free member of a distinct subgroup, behave similarly, and a close interrelationship between AMV and RSV (Prague) polypeptides shows the existence of multiple group specific antigens.

A major portion of the contract consists of a detailed analysis of RNA tumor virus particles and their interactions with the host cell. End group analyses and amino acid composition of individual polypeptides, including the avian glycoproteins, are being studied, and tryptic peptide maps have been obtained for the virus proteins.

Studies on localization of polypeptides in the virus indicated that mammalian tumor virus cores are very similar to cores of avian tumor agents in terms of structure, physical properties, and polypeptide composition. Study of the biological activity of the virus core substructure is continuing.

Significance to Biomedical Research and the Program of the Institute: One of the major objectives of the SVCP is to explore fully all important animal model systems for the determination of the possible viral etiology of cancer in man. Avian tumor viruses induce a variety of diseases similar to those which occur in man (erythroblastosis, myeloblastosis, myelocytomatosis, reticuloendotheliosis, and sarcomas); the causative viruses have been isolated and the disease can be induced *in vivo* under controlled conditions which permit the study of the immunology, virology, biochemistry and therapy of the tumor virus complex. Moreover, BAI Strain A avian tumor

virus is the only RNA C-type virus which is at present available in large enough quantities to permit exhaustive investigation into the biochemical makeup and behavior of both the virus and its components. As such, it represents an important model for the C-type viruses of higher animals and is an essential tool in the search for cancer viruses in man. Future studies will depend upon large quantities of concentrated virus, which the contractor is uniquely in a position to supply.

Proposed Course: Since the contract's Project Director has retired from the faculty of Duke University, this aspect of the contract terminated on April 18, 1973. The production staff and equipment were moved to Life Sciences, Inc. under the leadership of the current Project Director to function under a new contract. It is anticipated that the new contractor will continue to meet requests for virus and viral components, and will continue the activities outlined above.

Date Contract Initiated: April 19, 1971

Current Annual Level: $674,000

ELECTRO-NUCLEONICS LABORATORIES, INC. (NIH 71-2253)

Title: Development of Propagation Procedures, Purification, and Characterization of Viruses

Contractor's Project Director: Mr. John Lemp

Project Officers (NCI): Dr. George Todaro
Dr. Jack Gruber

Objectives: To develop propagation procedures to produce high virus yields from cell cultures, and to purify, determine particle count per ml., and otherwise characterize the produced virus.

Major Findings: A total of 38 cell lines, shedding C-type virus particles, were propagated and the viruses purified and characterized in the contractor's laboratory. All of these cell lines were new to the contractor's operation. Two additional cell lines were not infected, but packed cells were provided to the Project Officer.

The contractor has concentrated, partially purified, and characterized 4,110 liters of tissue culture-virus fluid in 179 runs which comprised double sucrose density gradient and pelletizing centrifugations.

Significance to Biomedical Research and the Program of the Institute: The search for evidence of the viral etiology of human cancer must include studies on viruses present in cell cultures established from animal tumors as well as on those candidate human cancer viruses growing in either animal or human cell cultures. Large volumes of these well-characterized and concentrated viruses are essential for the preparation of specific

antisera and for the biochemical, immunological, and epidemiological investigations necessary in cancer virus research.

Proposed Course: To continue the propagation of cell lines and the harvest of virus as directed by the Project Officer.

Date Contract Initiated: May 28, 1971

Current Annual Level: $350,000

ELECTRO-NUCLEONICS, INC. (NIH 72-3249)

Title: Large-Scale Production of Oncogenic Viruses

Contractor's Project Director: Mr. John Lemp

Project Officer (NCI): Dr. Jack Gruber

Objectives: To provide research and services related to the isolation, large-scale production, concentration, and assay of oncogenic viruses of animals and potentially oncogenic viruses of humans; production and quality control will involve tissue culture, electron microscopy, immunology, and various biochemical/biophysical techniques.

Major Findings: This contract, which was initiated in March, 1972, provides the Special Virus Cancer Program with approximately 135 liters per week of tissue culture-grown oncogenic and suspected oncogenic viruses which are concentrated and prepared for distribution. Agents which have been propagated in large quantities since the inception of the contract include Rauscher leukemia virus, Gross leukemia virus, Moloney leukemia virus, and AKR virus.

Distribution of these agents has been as directed by the Office of Program Resources and Logistics both to intramural investigators in Viral Oncology and to collaborating investigators within the Special Virus Cancer Program.

Significance to Biomedical Research and the Program of the Institute: In order to carry out important research on the biochemistry and biophysics of oncogenic animal viruses, it is imperative that large quantities of concentrated virus be available for analysis. This contract helps meet this need with oncogenic animal viruses that have been produced under rigidly controlled conditions, and also serves to find the best means of producing and concentrating large quantities of new candidate human cancer viruses as they are discovered.

Proposed Course: To continue the large-scale propagation and concentration of C-type oncogenic viruses as directed by the Office of Program Resources & Logistics. Additional viruses will be produced as program needs dictate.

Date Contract Initiated: March 27, 1972

Current Annual Level: $850,000

EMORY UNIVERSITY, YERKES PRIMATE CENTER (NIH 71-2256)

Title: Maintenance of a Colony of Irradiated, Aging Rhesus Monkeys

Contractor's Project Director: Dr. Harold McClure

Project Officer (NCI): Dr. Roy Kinard

Objectives: To determine the incidence of tumors in a unique group of irradiated, aging rhesus monkeys and to supply tissue from tumors to SVCP collaborators for transplantation, tissue culture and virus isolation studies.

Major Findings: A group of 72 rhesus monkeys with adults ranging in age from 17-22 years remain from an earlier study on the effects of irradiation. Forty-seven animals received irradiation in 1956-1958, and 16 are non-irradiated controls. The remainder are non-irradiated offspring born in the last four years.

The incidence of cancer in animals of this colony is much higher than is found in similar animals in a wild population. There are presently two monkeys in the colony, one with a siminoma and the other with an adeno-carcinoma, which are periodically operated upon for removal of tumor tissue. During the past year, specimens have been shipped to SVCP investigators, including Drs. Rabin, Kawakami, Deinhardt, and Wolfe. These investigators also received specimens from an animal which died of myelogenous leukemia.

Significance to Biomedical Research and the Program of the Institute: The SVCP conducts collaborative projects for the study of relationships between the etiologies of tumors of various primates. This project provides tumor tissues and other important specimens from aging non-human primates which have been subjected to irradiation to researchers within the SVCP. At the same time the contractor conducts a screening operation for the appearance of virus-like particles or viral antigens in the monkeys. Malignant changes in these primates may provide useful information which might be applied to humans, who are also subjected to various forms of radiation as well as to the natural aging process.

Proposed Course: The entire group of monkeys will continue to be monitored for neoplasia by physical and hematologic examinations. All tumors which develop will be evaluated by the contractor by light and electron microscopy. Specimens of these tumors will also be made available to SVCP investigators. In addition, a breeding program is underway to evaluate the incidence of leukemia or other tumors in infants with aging and irradiated parents.

Date Contract Initiated: May 1, 1971

Current Annual Level: $25,330

FLOW LABORATORIES, INC. (NIH 73-3201)

Title: Maintenance of a Repository for Storage and Distribution of Reagents and Tissue Specimens

Contractor's Project Directors: Mr. Harry F. Adkins (Serum Repository)
Dr. Fang-tsun Kuo (Tissue Repository)

Project Officers (NCI): Dr. Jack Gruber
Dr. David McB. Howell

Objectives: To provide for the SVCP a centrally located low temperature storage and distribution center for viral reagents and tissues.

Major Findings: In 1972, the contractor made 512 shipments of viruses, viral reagents, sera, and tissues which comprised a total of 4,938 vials of material. He received 319 shipments of similar materials which comprised 22,400 vials. All incoming shipments were carefully checked for damage in transit and were cataloged before being placed in the low temperature repository.

During the period of October 1972 - January 1973, the frozen tissue repository portion of the contract received 500 tissue and fluid specimens, most of which were from patients with neoplastic disease. All specimens were examined by Dr. Kuo, a pathologist, classified as to tumor or tissue type, and either cataloged and stored or sent to SVCP investigators.

Significance to Biomedical Research and the Program of the Institute: An efficient research program must have readily accessible adequate characterized resource materials. The laboratory, storage, and shipping facilities operated under this contract enable collaborating investigators to have access to a large inventory of special research materials without the burden of procurement, storage, inventory, and distribution.

Proposed Course: It is anticipated that the activities of this contract will continue to provide rapid and flexible support to changing needs of the SVCP and its collaborating investigators.

Date Contract Initiated: June 22, 1965

Current Annual Level: $161,472

FLOW LABORATORIES, INC. (NIH 71-2341)

Title: Animal Holding Facility to Support Intramural Research on RNA Viruses and Autoimmune Diseases

Contractor's Project Director: Dr. William A. Knapp

Project Officers (NCI): Dr. John W. Pearson
Dr. Adi Gazdar

Objectives: The objective of this contract is to support ongoing activities in the Virus and Disease Modification Section, VBB, NCI and the Viral Pathology Section, VLLB, NCI. The nature of the above activities require supportive services which this contract provides. In general, the contractor receives and maintains mice, rats, hamsters, and other small animal species as required for the purpose of observation and experimentation during the aging process for the following research studies: (a) Development of autoimmune disease, (b) Relationship of autoimmune disease to oncogenic viruses, (c) Development of spontaneous cancers and their modification to chemo-immunotherapeutic agents, and (d) Immunologic responsiveness to immuno-enhancers and/or suppressors.

Major Findings: A transplantable Nova leukemia maintained in aged Fisher rats has been effectively controlled by cytoxan therapy with a remission period of approximately 10 to 12 days. The disease has been found to be a lymphoblastic leukemia. Administration of 8 x 10 viable BCG organisms at different time intervals during the period of total tumor remission resulted in no significant increase in MST or long-term survivors when compared to the group that received drug treatment alone. Preliminary studies have failed to show any antigens on the Nova tumor. Based on findings involving the Nova leukemia, it is apparent that in order for immunostimulation therapy to be advantageous to the host there must first exist an immune response against antigen(s) on the tumor.

A transplantable virus associated Gross leukemia has been maintained in aged W/Fu rats. This leukemia kills animals in approximately 12 to 15 days. The contractor has been unable to effectively control this leukemia. However, a regimen of therapy using prednisone, cytoxan and vincristine over a period of a week appears to be giving a remission period of 5 to 6 days.

For over a year, combination drug therapy studies against the spontaneous leukemia in AKR mice for the purpose of induction of remission have been in progress. Of the various regimens of drug therapy tested for the induction of remission, treatment with 30 mg/kg. of prednisone and 0.50 mg/kg. of vincristine once a week for two weeks appears to be the best.

Recently, a mouse myeloma, MOPC-104E, has been used to hyperimmunize rabbits. The antiplasma cell serum is currently being absorbed against normal mouse tissues and characterized for specificity against B cells.

The effects of continuous treatment with interferon inducers on the appearance and severity of immune complex nephritis and antibodies to nucleic acids in B/W mice remains the major long-term project. It appears that IF inducers have little or no protection effect in B/W mice and may even accelerate their disease.

Studies are under way attempting to understand the immunologic basis of regressions induced by MSV tumors. Mice with regressing or regressed MSV tumors are immune to further MSV challenge for several months.

Significance to Biomedical Research and the Program of the Institute: The major goals of SVCP program are: (1) to detect and isolate tumor viruses, (2) the prevention and control of virus-induced malignancies in man as well as in animals associated with man. Current in-house efforts are underway to investigate the relationship of the aging process and autoimmune disease states to spontaneous and virus-induced malignancies. In addition, ongoing investigation utilizing chemo-immunotherapeutic approaches are being studied in both rat and mouse leukemia and tumor model systems. It is expected that the information resulting from this research will have considerable application in studies on similar diseases in man.

Proposed Course: The application of chemoimmunostimulation studies against the spontaneous AKR leukemia as well as the described rat leukemia systems will continue. Immunization studies involving the various rat leukemias cell lines will continue. Likewise, long-term studies involving the effects of interferon inducers on the appearance and severity of immune complex nephritis and antibodies to nucleic acids in B/W mice will be continued.

Date Contract Initiated: June 15, 1971

Current Annual Level: $90,000

GEORGETOWN UNIVERSITY (NIH 72-3248)

Title: Supply of Blood and Tissue Specimens from Patients with Malignancies

Contractor's Project Director: Dr. Richard A. Binder

Project Officers (NCI): Dr. Rogert J. Goldberg
Dr. David McB. Howell

Objectives: To collect fresh blood and tissue specimens from patients suffering from various neoplasias.

Major Findings: During the period of August 1972 - February 1973 a total of 94 tissue specimens, 113 sera, and 3 specimens of whole blood were collected and sent, either fresh or frozen, to the Flow Laboratories serum and tissue repository, Litton Bionetics Processing Laboratory, and specified investigators. Working arrangements have been made with the Departments of Pathology and Medicine and the Division of Hematology for obtaining specimens of fresh operative tissue, post-mortem tissue, and serum from patients with neoplastic disease.

Significance to Biomedical Research and the Program of the Institute: It is vitally important that a continuing supply of specimens from patients suffering from neoplasias be available to researchers seeking the viral etiology of cancer. This contract is particularly advantageous because the contractor is located within a few miles of NCI. This proximity allows the formulation and alternation as necessary of particularly detailed protocols, and also allows for the availability of very fresh specimens to SVCP researchers in the Washington area.

Proposed Course: It is anticipated that increasing numbers of fresh tissues will be procured and provided to the Special Virus Cancer Program.

Date Contract Initiated: June 28, 1972

Current Annual Level: $18,000

HEALTH RESEARCH INC. (RPMI) (NIH 72-3247)

Title: Procurement of Leucocytes and Tissue Specimens for the SVCP

Contractor's Project Director: Dr. Joseph Sokal

Project Officers (NCI): Dr. David McB. Howell
Dr. Charles Boone

Objectives: To collect tissues and blood samples from adults suffering from neoplastic hematologic disorders, particularly Hodgkin's disease and leukemia, for use by researchers within the SVCP.

Major Findings: From July 1972 - February 1973, the contractor shipped a total of 264 autopsy specimens ranging in weight from 0.4 g. to 1.0 kg. to the Flow Laboratories Repository of the SVCP for storage and/or dissemination to SVCP investigators. As of February, 1973, the rate of shipment was 40-60 specimens per month. In addition, the contractor completed arrangements to ship blood specimens from patients with malignant lymphoma, chronic leukemia, and other selected diseases to Litton Bionetics and the Flow Repository for distribution to program scientists.

Significance to Biomedical Research and the Program of the Institute: A major goal of the SVCP is to identify, isolate, propagate, and characterize candidate human cancer viruses. Of paramount importance in the efforts to reach this goal is the continued availability of clinical specimens and histories from patients suffering from cancer. This contractor, who sees a large number of these patients annually, is in an excellent position to help meet increasing program needs for large quantities of human neoplastic specimens.

Proposed Course: Procurement as described.

Date Contract Initiated: June 22, 1972

Current Annual Level: $15,000

HOSPITAL FOR SICK CHILDREN (NIH 72-3266)

Title: Human leukemic and Normal Tissue Collection and Preservation

Contractor's Project Director: Dr. Peter D. McClure

Project Officers (NCI): Dr. David McB. Howell
Dr. Charles Boone

Objectives: To obtain serum and plasma specimens for a wide variety of research purposes from pediatric leukemics, relatives of such patients, and non-leukemic controls.

Major Findings: During the past year, the contractor has continued to collect serum samples from new patients with leukemia and from patients on long-term follow-up. Less emphasis has been placed on obtaining sera from family members. The contractor is presently devoting more energy to the collection of fresh tumor cells and normal tissues from surgical patients and autopsy specimens in the same categories from autopsy cases. Fresh samples have been shipped to the Litton Bionetics Processing Laboratory for distribution to SVCP investigators while sera and autopsy specimens are shipped to the Flow Laboratories Repository for storage and later distribution. During 1972 the contractor collected 375 serum specimens and 23 solid tissue specimens from patients with a wide variety of neoplasias, as well as from normal controls and family members.

Significance to Biomedical Research and the Program of the Institute: As the largest pediatric hospital in North America, this contractor supplies many vital serum specimens to SVCP not readily available elsewhere. The contractor also provides an important service to Program by collecting numerous samples of tissue for uses in which the unavoidable delay in passage through customs is not critical. In addition, the contractor consistently refers patients to NCI for study whose cases are felt to be particularly relevant to the research needs of Program.

Proposed Course: The contractor will continue the procurement activities outlined above.

Date Contract Initiated: February 3, 1965

Current Annual Level: $30,000

HUNTINGDON RESEARCH CENTER (NIH 73-3223)

Title: Development of Oncogenic Virus Diagnostic Reagents and Services

Contractor's Project Director: Dr. Roger E. Wilsnack

Project Officers (NCI): Dr. Robert Holdenried
Dr. Wallace Rowe
Dr. Robert J. Goldberg

Objectives: To develop, produce, and characterize special diagnostic reagents for use in the SVCP, primarily antisera and antisera conjugates to viruses, gs antigens, globulins of various animal species, and to T-antigens of polyoma and SV40 in tumored hamsters.

Major Findings: This year, as well as last, tumored Fischer rat MSV(M) antiserum was the most heavily and widely used of the reagents produced by the contractor. However, the contractor also continued to produce and characterize antisera against a very wide array of antigens encountered in cancer research, and added a number of antisera to his inventory during the year. These included new antisera against Woolly Monkey Fibrosarcoma virus, RD-114 virus, RD-114 gs antigen, avian myeloblastosis virus, and guanidine-purified Rauscher and feline leukemia viruses. He also made large volumes of donkey antisera against species immunoglobulins for use in the double antibody radioimmunoassay system.

In February 1973, the contractor installed a computer terminal which permitted access to the NIH Computer Center. Data concerning the contractor's inventory of antisera were entered in the computer memory, thereby allowing the contractor to query the computer to determine the proper lot of antiserum to fit a large number of subtle biological parameters. Moreover, access to the computer has greatly facilitated inventory control.

Goats, swine, donkeys, rats, and rabbits are being employed as hosts for antigens used in the continued production of oncogenic virus antisera.

Significance to Biomedical Research and the Program of the Institute: The reagents and test systems developed and produced by the contract are vital tools in cancer research. The project functions in close collaboration with SVCP research projects and is felt to be of very significant usefulness to the needs of the program.

Proposed Course: To continue development, characterization, and production of antisera and serological test systems.

Date Contract Initiated: June 2, 1963

Current Annual Level: $308,240

JOHNS HOPKINS UNIVERSITY (NO1-CP-33245)

Title: Pediatric Tumor Resource

Contractor's Project Director: Dr. Herbert Kaiser

Project Officers (NCI): Dr. Paul Peebles
Dr. Jack Gruber

Objectives: To provide fresh tumor specimens from pediatric patients to collaborating laboratories within the SVCP for biochemical and virological investigations.

Major Findings: This is a new contract, initiated March 1, 1973.

Significance to Biomedical Research and the Program of the Institute: Detection, treatment, and prevention of human cancer require an accurate

determination of its etiology. Viruses have been implicated as possible causative agents in human cancer. Unfortunately, a lack of sufficient tumor material from patients in the pediatric age group limits investigations for oncogenic viruses that may have been vertically transmitted from mother to child. Materials from this contract will help make possible these imperative human studies.

Date Contract Initiated: March 1, 1973

Current Annual Level: $29,355

LEO GOODWIN INSTITUTE FOR CANCER RESEARCH (NIH 72-3261)

Title: Germfree Research and Operation of a Collaborative Germfree Tumor Virus Laboratory

Contractor's Project Director: Dr. Joel Warren

Project Officer (NCI): Dr. David McB. Howell

Objectives: This contract is intended to provide germfree and specific pathogen-free animals in an environmentally controlled facility to support SVCP and intramural research investigations requiring clean, well defined animals and viral reagents.

Major Findings: The contractor has provided the well-defined animals and reagents indicated above. He has also completed research investigations on tobacco tar carcinogenesis initiated under an earlier work scope.

Significance to Biomedical Research and the Program of the Institute: The special animals, reagents, and facilities provided by this contractor are also provided by other contractors in the SVCP. In order to avoid unnecessary duplication of effort, this contract is to be terminated October 31, 1973.

Proposed Course: Termination

Current Annual Level: $84,000

LIFE SCIENCES, INC. (NIH 73-3210)

Title: Production of Germfree and Reagent Grade Specific-Pathogen-Free Animals

Contractor's Project Director: Dr. Wendall M. Farrow

Project Officers (NCI): Mr. John P. Kvedar
Dr. David McB. Howell

Objectives: To produce both germfree and specific-pathogen-free (SPF) animals for research. SPF animals are maintained under environmentally controlled conditions which preclude intercurrent infection by pathogenic microorganisms or infestation by parasites and are referred to as "reagent grade" hosts.

Major Findings: The contractor's supply flock of SPF leukosis-negative White Leghorn chickens, housed in a 2600-square-foot area protected by a shower lock and recirculated, filtered air, now produces about 750 fertile eggs per week.

Selective breeding has produced two flocks of barrier contained, reagent grade chickens with 75-80% fertility and 85-90% hatchability. The results of these efforts are reflected in the quality and quantity of the eggs and chicks from the production flock which are provided to numerous investigators within the SVCP.

The contractor is presently characterizing the SPF White Leghorn chicken flock for composition of phenotypes. All of the production flock will eventually be derived from phenotypically identifiable, pedigreed birds.

A production flock of Japanese quail consisting of 235 birds continues to supply fertile eggs to SVCP users at a rate of about 900/week. In addition, it provides a steady input of 21 to 28-day-old quail to program. Representative sampling of aged quail in this production flock indicated that these birds were freed of the usual avian pathogens.

An inbred pedigreed foundation colony of Balb/c mice free of all laterally transmitted viruses tested for continues to be maintained. Two random bred production colonies derived therefrom continue to supply certified SPF mice to certain SVCP investigators. In addition, a foundation colony of certified SPF NIH Swiss mice is being maintained as insurance against loss to program of this valuable stock.

Significance to Biomedical Research and the Program of the Institute: This contract serves as an essential supply of embryonated eggs and day-old chicks to contract NIH 69-93, which involves studies on Marek's disease as a model for herpesvirus-associated oncogenesis. It also provides other SVCP investigators with genetically and microbiologically well-defined laboratory animals. The advantage of having such animals is that oncogenic and suspected oncogenic viruses can be administered to them with a minimal danger of interference from other contaminating, adventitious microorganisms. Therefore, research can be carried out upon animals with a known and controlled viral flora, and cell lines can be derived from these animals which share this same advantage.

Proposed Course: This service-type contract for the production of germfree and reagent grade SPF animals will be continued, with the flexibility of being reoriented as rapidly as possible to meet changing needs of SVCP activities as they occur.

Date Contract Initiated: February 8, 1968

Current Annual Level: $416,811

LITTON BIONETICS, INC. (NIH 71-2025)

Title: Investigations of Viral Carcinogenesis in Primates

Contractor's Project Director: Dr. Harvey Rabin

Project Officers (NCI): Dr. Roy Kinard
Dr. Jack Gruber
Dr. Gary Pearson

Objectives: (1) Evaluation of long-term oncogenic effects of human and animal viral inocula in primates of various species; (2) maintenance of monkey breeding colonies and laboratories necessary for inoculation, care and monitoring of monkeys; and (3) collaborative research studies of primate virus isolates of demonstrated oncogenic potential which may be involved in the causation of human cancer.

Major Findings: At the end of January, 1973 the total inventory of primates in the Bionetics colony was 1,239. Of these, there were 490 animals (macaques of four species, including a number of replacement breeders and a small number of Galago crassicaudatus) in the Old World breeding colony. There were 138 New World primates in breeding comprising 6 species. The juvenile and experimental oncology section housed 524 animals, of which 57 were New World species. The nursery admitted 59 newborn animals during the 5 months since the last report, including four New World animals. Fifteen Galagos were sent to another research center. Forty-five procedures were performed in the surgery section. Resource activities included the shipment of monkey milk, mammary tissue biopsy material, and chimpanzee serum to three research groups.

Myelogenous leukemia occurred in a female, rhesus monkey at Yerkes Regional Primate Research Center and bone marrow and other tissues were made available to Bionetics for inoculation into immunosuppressed macaques. Lethal irradiation with autologous bone marrow reconstitution and ALS, and ALS alone have been employed in attempts to ensure survival of the allografted leukemic cells. Initially, numerous immature cells of the granulocytic series were seen in peripheral blood but their presence did not extend beyond 30 days. Efforts to define the origin of these cells in peripheral blood by cytogenetic analysis were not successful due to low numbers of dividing cells. Five monkeys from the ALS alone treated series have shown marked elevation in peripheral blood lymphocyte counts one to two months after inoculation. In three of these monkeys Trypanosoma cruzi has been noted in peripheral blood smears. Attempts at culturing cells from peripheral blood of four allografted macaques are in progress. Cultures from each of the animals show large numbers of undifferentiated leukocytic cells.

An African green monkey (Cercopithecus aethiops) with a squamous cell carcinoma was made available to Bionetics from the Akron Zoo and serial

passage of neoplastic cells to immunosuppressed (ALS) recipients has been attempted. Presently, two recipients (59 and 79 days post-inoculation) are surviving but with no clinical evidence of neoplastic disease. Cultures were prepared from the tumor which show two general types of cells; a densely packed cell type which grows in discrete clusters and is very sensitive to trypsin, and a fibroblastic cell type which grows as a loosely arranged monolayer.

In collaboration with Dr. M. A. Epstein of Bristol University, England, a series of owl monkeys had been inoculated with a Burkitt Lymphoma preparation. Lymphoid cells from one of these animals were cultured and found to be positive for surface immunoglobulin, negative for non-specific rosette formation with sheep rbc, and lacking a complement receptor as determined by the rosette formation test in the presence of antibody and complement. Two other owl monkeys from this series died, and tissues were sent to Dr. Epstein.

Herpesvirus saimiri (HVS)-infected owl monkeys (Aotus trivirgatus) were tested for the immunocompetence of the host during development of lymphoma. Lymphocytes were tested biweekly for response to the mitogens phyto-hemagglutinin (PHA), pokeweed mitogen (PWM), and concanavalin A (Con A). The response of peripheral lymphocytes to these mitogens, as measured by the incorporation of tritiated thymidine (^{3}H-TdR) into acid insoluble material, showed a marked suppressive effect with Con A and PHA as opposed to PWM with the degree of suppression falling as low as 4% of the control rate. Increased ^{3}H-TdR incorporation rates into non-mitogen stimulated lymphocytes occurred and was coincident with depressed response to the general mitogens. Lymphocytes from these same owl monkeys were tested for HVS genome rescue by cocultivation with vero cells at the same biweekly intervals. Virus was recovered at 28 days and, subsequently, genome-carrying lymphocytes were found to be as numerous as one in 12. In collaboration with Dr. Gary Pearson, fluorescent antibody patterns to both late antigen (LA) and early antigen (EA) were followed on these same owl monkeys. Antibody to LA was demonstrable at 14 days and remained evident thereafter. Antibody titers to EA, however, were directly correlated with disease as measured by numbers of peripheral blood lymphocytes and enlarged lymph nodes. Lymphocyte DNA synthesis, mitogen response, and virus rescue also correlated directly with the presence of disease and, therefore, with anti-EA antibody titers.

Two lymphoblastoid cell strains initiated from HVS-infected owl monkeys were examined for their ability to form rosettes with heterologous erythrocytes (rbc). Greater than 88% of viable cells from these cultures formed rosettes in a non-specific fashion with sheep rbc. Rosettes also formed with mouse (85%), African green monkey (84%), goat (66%), rabbit (25%), and rhesus monkey (2.5%) rbc. Rosettes did not form with human A, B, & O, goose, chicken, guinea pig, bovine, burro, capuchin monkey, or owl monkey rbc. Complement receptors (indicated by rosette formation in the presence of antibody, complement, and rbc negative in non-specific binding) were not detected. Rosette formation was not inhibited by ALS, anti-human heavy and light chain sera, and anti-HVS sera.

To determine their functional characteristics, cultured cells derived from HVS-induced lymphomas were examined for a series of immunologic functions. Tissue culture fluids produced 8 to 20 units per ml. of interfering activity against vesicular stomatitis virus. Globulin was found on surfaces of viable cells as determined by immunofluorescent staining with anti-human gamma globulin. When inoculated onto rhesus monkey fibroblasts, the lymphoid cells adsorbed and induced release of ^{3}H-proline labeled protein. PHA-stimulated normal allogeneic lymphocytes failed to cause cytotoxic release of ^{3}H-proline labeled protein from lymphoid tumor cells used as target cells, but stimulated their growth as measured by increased colony counts in soft agar.

Significance to Biomedical Research and to the Program of the Institute: Inasmuch as tests for the biological activity of candidate human viruses will not be tested in the human species, it is imperative that another system be developed for these determinations and subsequently for the evaluation of vaccines or other measures of control. The close phylogenetic relationship of the lower primates to man justifies utilization of these animals for these purposes.

Proposed Course: Work will continue on HVS-induced owl monkey lymphoma. Attempts will be made to determine the possible presence of type C virus in virus preparations and tumor tissues. Further work on the characteristics of the target cells will be attempted. Isolation and characterization of the antigens of the tumor cells will be initiated. A pilot series of inoculations in owl monkeys and marmosets with Herpesvirus ateles will be made if the prototype strain of this virus becomes available. Continued efforts on the rhesus monkey myelogenous leukemia will be made in terms of cell culture and transplantation.

Further reductions in the Old World breeding colony will be made as infertile animals are culled, and culling will continue in those inoculated animals in which nothing develops. Additional numbers of New World animals will be obtained for experimentation and to increase the breeding effort.

Date Contract Initiated: February 12, 1962

Current Annual Level: $1,933,845

UNIVERSITY OF LOUISVILLE (PH 43-66-902)

Title: Preparation of Simian Foamy Virus Reagents and Antisera

Contractor's Project Director: Dr. Paul B. Johnston

Project Officers (NCI): Dr. Robert Holdenried
Dr. James Duff

Objectives: To prepare and test reference reagents (virus and corresponding antisera) for the simian foamy viruses, types 1-7, and foamy virus from other laboratory species.

Major Findings: The seven types of simian foamy viruses and antisera against them have been prepared, packaged, and tested for homogeneity, potency, and purity. During this reporting period, foamy viruses and antisera have been provided to a number of SVCP investigators, including Drs. Green, Manning, Morris, Epstein, and Heberling.

A zonal rotor (Beckman AL-14) has been standardized by the contractor with control culture fluids for the purpose of purifying foamy viruses through 650 ml. of sucrose gradient (3% to 15%) at 91,300 Xg. It is anticipated that this rotor will be of considerable value in providing highly purified foamy virus reagents.

Consultation and/or foamy virus reagents are being provided to other SVCP investigators on a regular basis.

Significance to Biomedical Research and the Program of the Institute: The simian foamy virus reagents will be used in the identification of viruses and viral antibodies in primates used for cancer research. The indigenous viruses of laboratory primates pose husbandry problems, in addition to contaminating test systems and complicating the attempts to recover oncogenic virus from tissues and tissue extracts. The specific antisera may also be useful in suppressing the growth of these adventitious viruses in primate tissue cultures.

Proposed Course: This laboratory will continue to provide foamy viruses and antisera to SVCP investigators, as well as assisting in the detection of foamy virus contaminates on a referral basis.

Date Contract Initiated: June 13, 1966

Current Annual Level: $18,000

MELOY LABORATORIES (NIH 72-3202)

Title: Murine Mammary Tumor Virus Studies

Contractor's Project Director: Dr. John E. Verna

Project Officers (NCI): Dr. Jack Gruber
Dr. Louis R. Sibal
Dr. Wade Parks

Objectives: To propagate, concentrate, and distribute murine mammary tumor virus (MTV) for collaborating SVCP investigators; to perform immunological and biological assays for the detection and quantitation of MTV; to develop improved methods for the propagation and detection of MTV and MTV antigens; to conduct studies on the control of neoplasia in the susceptible murine host by vaccination with inactivated virus.

Major Findings: The primary purpose of this contract is the production of quality reagents for the study of the mouse mammary tumor virus system as a model for the further examination of the human breast cancer problem. The contractor is purifying MTV from the milk of C3H and RIII mice by the combination of rate zonal and isopycnic centrifugation. Purified virus is employed in the following ways: (a) as a source of supply to various SVCP investigators; (b) as a reagent in the HA, HAI, CF, and ID tests; (c) employed to produce MTV antiserum in goats, swine, rabbits, and guinea pigs; and (d) used in cell culture experiments.

Purified virus and/or viral antisera and skim C3H or RIII mouse milk have been sent during the past year to the following investigators: Drs. W. Holder, E. Kurstak, B. Gerwin, M. Rich, E. Dowdle, H. Chopra, J. Keydar, A. Andrese, I. Irlin, D. Fine, P. Myers, H. Hoffman, D. Martin, R. Huebner, J. Schlom, and others.

Some additional studies that are associated with the contract include the serological testing of human milk and sera as well as human and mouse tumor extracts and cloned cell culture lines that are received through the Project Officer or that are generated through the developmental phase of this contract. These samples are examined for the presence of MTV antigens or antibodies. Experiments are currently in progress which attempt to demonstrate the specificity of the reactions that have detected common or cross-reacting antigens or antibodies in human milk and sera. Complement fixation, protein analysis, and assay for reverse transcriptase have been added to the capabilities of the MTV assay laboratory and serve to supplement the present assays.

A developmental phase of the contract is concerned with the establishment of an in vitro cell culture source of MTV. Preliminary data indicate that MTV synthesis does occur in mammary tumor cell cultures. Current studies suggest that the regulation of MTV expression is very different from that previously described for other viruses of this type. Specifically, virus expression is associated with differentiated cell functions.

Significance to Biomedical Research and the Program of the Institute: Breast cancer is a leading cause of death from cancer among women. The finding of a virus, resembling a Type B RNA oncogenic virus of mice, in the milk of a significant number of women from high-risk breast cancer families strongly suggests a possible viral etiology for this disease. A major effort of the Special Virus Cancer Program is directed toward determining the relationship of viruses to human breast cancer. This contract was established for the purpose of obtaining correlative information on the detection, isolation, and propagation of a murine mammary tumor virus, because this is the only available animal model system in which approaches to the study of viruses as a cause of breast cancer in humans may be developed.

Proposed Course: Purified MTV, viral reagents, and mouse milk will continue to be supplied as needed by SVCP investigators. In addition, the biological and immunological methods developed in this laboratory will be used in

systematic studies to develop further the mouse MTV system as a laboratory model for breast cancer virus studies in man. The contractor will attempt to establish possible recipient epithelial cultures from normal mouse mammary glands as substrates for in vitro mammary tumor virus infection. It is anticipated that the information gained from these studies will be applied to human breast cancer studies.

Date Contract Initiated: December 30, 1965

Current Annual Level: $671,309

MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES (NIH 71-2116)

Title: Acquisition of Human Materials for Use in the Search for Transmissible Agents in Human Tumors

Contractor's Project Director: Dr. Yashar Hirshaut

Project Officer (NCI): Dr. Jack Gruber

Objectives: To gather sera and tissues from patients with tumors to be used in the search for tumor-specific antigens and human oncogenic viruses.

Major Findings: The contractor operates within one of the largest hospital centers in the nation devoted solely to the care of the patient with cancer. During the past year, over 7000 sera were collected, along with over 500 surgical specimens. These materials were either supplied directly to participating cancer research investigators in the SVCP or frozen and stored at the contractor's own regional repository for later Program distribution.

Clinical information for each specimen is entered into the SVCP computer bank, under the direction of the Office of Program Analysis and Communication, and a computer printout of the contractor's entire inventory of frozen specimens is prepared on a monthly basis.

Numerous samples of materials have been distributed to collaborating investigators in the SVCP, including Drs. Hanafusa, Huebner, Herberman, Spiegelman, Yohn, Girardi, Gallo, Terry, Eagle, Nonoyama, and others.

Significance to Biomedical Research and the Program of the Institute: In the last ten years, rapid progress has been made in the study of oncogenic animal viruses. Unfortunately, human studies have frequently been limited by the lack of suitable materials to be used in virus isolation and detection attempts. The procurement program at Memorial Hospital for Cancer and Allied Diseases in New York City provides cooperating investigators with sufficient numbers of specimens from tumor-bearing patients to permit them to undertake intensive studies of the possible viral etiology of human cancer.

Proposed Course: It is anticipated that this contractor will maintain the present high level of material supplied to the program.

Date Contract Initiated: March 1, 1971

Current Annual Level: $115,000

UNIVERSITY OF MICHIGAN (NIH 73-3224)

Title: Collection of Leukemia-Lymphoma Specimens

Contractor's Project Director: Dr. Chris J. D. Zarafonetis

Project Officers (NCI) Dr. Robert J. Goldberg
Dr. Deward Waggoner

Objectives: To collect and distribute specimens and information from patients with leukemia or lymphoma.

Major Findings: The major efforts of the contractor have been divided between procurement of specimens and evaluation of special clinical situations. During the past year, the contractor added over 4,000 sera to the serum bank, and all pertinent information on these sera was keypunched for computer retrieval. In addition, solid tissue specimens from patients having a variety of neoplasias were shipped to the Flow Tissue Repository.

Recipients of tissue specimens from this contractor via direct distribution have included Drs. Spiegelman, August, G. Henle, Aaronson, Levine, Huebner, Herberman, Verna, Gallo, Todaro, Terasaki, and others.

Significance to Biomedical Research and the Program of the Institute: Availability of clinical specimens and pertinent information on the cases is paramount in the achievement of a major goal of the SVCP, i.e., to identify, rescue, characterize, and propagate a candidate human cancer virus. Large volumes of leukemic cells are necessary for the biochemical characterization of the polymerases present in these cells, and a large number of tissues will be necessary to keep up with the biochemical demand. In addition to helping meet this need, the contractor is also collecting a large number of sera for the SVCP serum bank and has been able to meet new requests for a variety of specimens for SVCP investigators.

Proposed Course: Continuation as described.

Date Contract Initiated: June 21, 1965

Current Annual Level: $98,318

MICROBIOLOGICAL ASSOCIATES, INC. (PH 43-66-914)

Title: Establish and Operate a BALB/c Mouse Colony

Contractor's Project Director: Mr. Wilbur Athey

Project Officers (NCI): Mr. Samuel Poiley
Dr. Michael Chirigos
Mr. Clarence Reeder

Objectives: To provide BALB/c mice for laboratory investigations supported by the SVCP, primarily for virus bioassays on Contract 43-67-697.

Major Findings: The contractor has provided the maximum numbers of mice that can be produced in 2,500 cages. All regulations of The Institute for Laboratory Animal Resources are followed carefully. Requests for animals based on age, sex, weight, suckling litters, or breeders, etc. have been consistently met.

Production under this colony has been increased to fully utilize the capabilities of half an animal building (1050 square feet) at Walkersville, Maryland. During the past year, over 60,000 weanling mice have been provided to the SVCP, along with over 3,000 pregnant mice, 800 newborns, and 400 litters. All of these have been well-characterized mice, free from the usual murine viruses, including Sendai virus.

Significance to Biomedical Research and the Program of the Institute: The murine tumor viruses are being extensively studied as models for human cancer viruses. The availability of high quality BALB/c mice is important for assay of these viruses as well as for other studies in viral oncogenesis.

Proposed Course: Mouse production will be continued at the current level.

Date Contract Initiated: June 16, 1966

Current Annual Level: $64,000

MICROBIOLOGICAL ASSOCIATES, INC. (NO1-CP-3-3288)

Title: Development of Laboratory Animal Virus Diagnostic Reagents and Services

Contractor's Project Director: Dr. John C. Parker

Project Officers: Dr. Robert Holdenried (NCI)
Dr. Wallace P. Rowe (NIAID)
Dr. David McB. Howell (NCI)

Objectives: To develop reagents and tests for the detection of murine and other laboratory rodent and cat viruses; to apply these and other tools in the determination of the importance of the indigenous viruses in experimental systems; to study means for elimination of viruses from laboratory populations. An additional collaborative study to assist in the characterization of the gene-dependent expression of murine leukemia was initiated in 1971 and continues.

Major Findings: The contract continued to provide murine virus resources and services through the serodiagnostic and virus diagnostic laboratories. During the past year, 3,627 sera were submitted to the serodiagnostic laboratory where 32,947 tests were performed. The following types of specimens were received and tested: 1,649 mouse sera (15,026 tests), 145 rat sera (948 tests), 138 hamster sera (1,180 tests), 13 guinea pig sera (195 tests), 157 cat sera (2,669 tests), 1,140 MAP sera (11,818 tests), and 385 coded sera (1,111 tests). The virus diagnostic laboratory received 96 requests for virus testing by antibody induction tests (MAP, RAP). Twenty-four of these specimens were found to be contaminated by one or more viruses. Viruses found as contaminants in decreasing order of prevalence were: minute virus of mice, lactic dehydrogenase virus, Sendai virus, polyoma virus, and lymphocytic choriomeningitis virus.

In addition to the diagnostic testing services, the contract prepared where necessary and maintained an inventory of monotypic, certified viral antigens and antisera for 20 murine and 17 feline viruses. Antigens are available to investigators in either the infectious or inactivated form.

Research on the thymic virus has been directed towards gaining a basic understanding of the course of infection in its natural host, the mouse. Also, the contractor is studying how thymic virus infection might alter the course of diseases of the lymphatic system such as lymphoma and immunopathologic mediated diseases such as lymphocytic choriomeningitis. Sensitive assays have been developed, and reference reagent pools prepared and certified. Thymic virus has been identified as a herpesvirus measuring 135 nm which has physical properties of heat, ether, and chloroform sensitivity. Infection in the neonatal mouse occurs primarily in the thymus; however, after 14 days this tropism shifts to the salivary glands where a chronic infection is established which lasts indefinitely. Natural infections in colony reared mice are common as are infections in wild mouse populations.

In a joint study with Dr. R. J. Huebner, NIH, and Dr. M. Gardner, U.S.C., the contractor has been attempting to isolate and characterize an agent responsible for a paralytic central nervous system disease which is epizootic in a wild mouse geriatric colony in California. The contractor has been successful in producing an identical disease in swiss mice by passaging extracts prepared from the brains and spinal cords of diseased wild mice. It may be noteworthy that lymphoma without paresis has been observed in a few mice and also that infectious leukemia virus is found in association with the induced paresis syndrome.

Numerous other service and research projects were completed or are continuing. Some of these projects are directed towards unravelling the genetic complexities of MLV infection in AKR mice, while others involve elimination of virus contaminants from reference reagent pools of the SVCP.

Significance to Biomedical Research and the Program of the Institute: The virus diagnostic capabilities provide the NCI with the ability to monitor laboratory rodent and cat colonies and laboratory animal-produced

viral reagents and tumors which have resulted in the production of highly characterized systems for cancer research. This contract provides assistance and guidance of particular importance for the detection of LCM in rodent systems. LCM virus, in addition to being infectious for humans, is difficult to detect. Significant contributions are being made to the knowledge of the natural history of several indigenous viruses of laboratory animals.

Proposed Course: To continue the serodiagnostic services outlined above and to improve the sensitivity of the tests. To apply the information developed to reduce and control viral infections in laboratory animal colonies and materials derived from animals.

Date Contract Initiated: April 10, 1961

Current Annual Level: $475,000

MONTREAL CHILDREN'S HOSPITAL (NIH 72-3277)

Title: Procurement of Normal and Leukemic Sera from Children

Contractor's Project Director: Dr. Ronald L. Denton

Project Officers (NCI): Dr. David McB. Howell
Dr. Charles Boone

Objectives: To obtain sera from a variety of pediatric oncology patients, family members, and controls for virologic study; to identify special cases for more extended workup.

Major Findings: Serum procurement during the past year included collections from 30 new cases of acute leukemia and tumor, 72 family members, and 70 controls. One hundred one followup samples were obtained from previously studied patients. Among the 82 other specimens collected, special attention was given an American Burkitt's Lymphoma patient, a new case of naso-pharyngeal carcinoma from Bermuda, several cases of complicated infectious mononucleosis, a patient with malignant histiocytosis, and an unusual case of chronic myelocytic leukemia. In addition to the 1,730 vials of serum and plasma that were transferred to the Flow Laboratory Serum Bank, a number of specimens were sent to Litton-Bionetics Resources Processing Laboratory, Dr. Paul Terasaki, Dr. Sol Spiegelman, Dr. Paul Levine, Dr. Robert Ting, and Dr. John Verna.

Significance to Biomedical Research and the Program of the Institute: This contract is one of the program's primary sources of serum from leukemic children and from suitable, normal controls. The increasing need within the SVCP for samples of this kind makes it essential that the supply be continued to satisfy research requirements.

Proposed Course: Continue to collect serum specimens for distribution to collaborating investigators within the SVCP.

Date Contract Initiated: September 24, 1965

Current Annual Level: $35,000

UNIVERSITY OF PADUA (PH 43-68-1389)

Title: Collection of Human Tissue Specimens

Contractor's Project Director: Professor Giovanni Dogo

Project Officers (NCI): Dr. Robert H. Depue, Jr.
Dr. Charles W. Boone

Objectives: To establish fibroblast cultures from skin biopsies taken from inbred and isolated human donors and to provide these cultures to NCI for use in research programs.

Major Findings: Skin biopsies have been obtained from inbred and isolated populations in Sappada and Sauris, two isolated communities in the Dolomite Mountains of Italy, whose inhabitants are highly inbred. Sauris is particularly interesting, since the frequency of both cancer and non-cancer related suicides is very high. Moreover, these frequencies still affect a group of former Sauris inhabitants who descended into a plain community near Tolmezzo at the beginning of this century.

A sample of each skin biopsy has been sent to NCI: in addition, blood samples have been procured from each donor and genealogies are being established for donors from Sauris. Family trees have already been worked out for the Sappada population.

Significance to Biomedical Research and the Program of the Institute: The cell lines established from these skin biopsies will be used in a project to detect human oncogenic viruses in vitro and to determine the significance of the transformation test to oncogenesis.

Proposed Course: To collect and culture human skin biopsies and blood samples as before, and to establish genealogies of the Sauris population.

Date Contract Initiated: October 27, 1964

Current Annual Level: $15,938

CHARLES PFIZER & CO., INC. (NO1-CP-3-3234)

Title: Tumor Virus Research

Contractor's Project Director: Dr. Sami Mayyasi

Project Officers (NCI): Dr. Jack Gruber
Dr. Ray Bryan
Dr. Robert Manaker

Objectives: Provides a service facility for the production of large volumes of selected oncogenic and suspected oncogenic viruses, cellular antigens, tissue culture cell lines, and specific antisera to various oncogenic viruses. Production of these materials is supported by appropriate laboratory groups whose activities include process improvement, product standardization, quality control testing, and applied developmental research.

Major Findings: During the past year, the contractor has processed over 20,000 liters of harvest fluids from tissue culture systems and distributed purified virus concentrates or cells to over 50 different laboratories throughout the world. This year there was a marked reduction in the production of Mason-Pfizer monkey virus (M-PMV), while Gibbon ape lymphoma (GALV) and Woolly monkey sarcoma (SSV-1) viruses came into full scale production. New production and process improvement measures studied by the contractor included the use of roller bottle apparatus, new cores for zonal centrifuges, and UV monitoring of gradients.

Production of RD-114 virus was continued at a relatively high level. Attempts to develop an infectivity assay led to findings that this virus will cause transformation of some human embryonic cells. These transformed cell lines have karyological changes and will produce tumors in mice.

Human tumor cell lines received from Dr. S. Stewart have been treated with IUDR in efforts to enhance virus production. Current emphasis is on the Gomez line (osteosarcoma patient) which contains both type A and type C particles.

A major effort has been directed toward the development of production methods with new simian agents. The SSV-1 is now in production (200 l/week) in two cell lines: a marmoset line (HF-Deinhardt) and a human lymphoblastoid line (NC-37) infected in the contractor's laboratories. Both lines yield abundant virus which has similar biological and biochemical properties. The infectivity of SSV-1 preparations is monitored on normal rat kidney cells (RNK). The Gibbon ape lymphoma virus is produced in the original tumor line (Kawakami) at low yield, but human lymphoblastoid cells (NC-37) infected with this agent yield 100 times as much virus. The GALV-NC-37 is in production at 100 l/week, and the GALV (Kawakami) is produced on request.

A virus has been isolated from the mammary tissue of a lactating rhesus monkey. Morphological, serological, biochemical and biological studies indicate that this virus (X-381) is closely related to the prototype Mason-Pfizer monkey virus. However, the breast culture producing X-381 virus shows characteristics of transformed cells; evidently this virus is worthy of further study and will be of Program interest.

Quality control has been aided by the development of a rapid and sensitive method for analysis of viral RNA by gel electrophoresis of detergent-disrupted virus and spectrophotometric scanning of the gel. The RNA from as few as 10^9 virus particles can be detected by this assay, which is 10-20 times more sensitive than sucrose gradient procedures.

Development of a prototype sub-micron particle analyzer is underway, in collaboration with General Electric Co. Efforts are being made to increase the sensitivity (currently at 10^8 particles/ml) and reproducibility of the instrument and to compare results obtained by conventional electron microscopic enumerations.

Significance to Biomedical Research and the Program of the Institute: Since its inception in November, 1961, this contract has been an invaluable back-up to intramural and collaborating investigators involved in virus-cancer research. The staff and facilities have been consistently responsive to changing needs of the Special Virus Cancer Program. They have provided support to a wide variety of collaborating investigators making possible studies on viruses in cancer that could not otherwise be conducted.

Recent research developments to determine the association of viruses with human neoplasia have involved activities concerned with the molecular biology, biochemistry and immunology of oncogenic viruses. These types of investigations may provide clues to the mechanism whereby viruses mediate the transformation process. Such knowledge could indicate methods by which neoplastic transformation can be averted or inhibited and provide appropriate control measures applicable to the human cancer situation.

Studies of this type have created a substantial demand for large quantities of concentrated and purified oncogenic and suspected oncogenic viruses. This contractor has both the capability to help meet such needs and the flexibility to quickly accommodate shifts in Program requirements.

Proposed Course: The production of virus and cell materials in support of pertinent research will continue.

Date Contract Initiated: November 6, 1961

Current Annual Level: $1,650,000

ST. JOSEPH'S HOSPITAL (NIH 69-2074)

Title: Study of Human Sarcomas and Their Possible Viral Etiology

Contractor's Project Director: Dr. Jeno E. Szakacs

Project Officers (NCI): Dr. Albert J. Dalton
Dr. David McB. Howell

Objectives: To find and supply fresh human sarcomas or other tumors which contain EM evidence of virus particles, and to attempt to establish cell cultures from these tumors.

Major Findings: During the past year, the contractor continued collection of human sarcomas. Of 49 sarcomas collected, 33 active cell cultures were derived and screened for virus particles. Negative cultures were terminated or frozen after the twelfth passage. Twenty-three tumors with initiated tissue cultures were shipped to NCI or the Litton-Bionetics Resources Processing Laboratory, and twenty-six tumors were shipped to the Flow Labs. Tissue Repository. Shipments of sera from sarcoma and breast cancer patients continued.

Studies were completed on line R323, a human fibrosarcoma cell line carrying C-type particles which were lost after the eleventh passage. All efforts to induce viral reappearance were fruitless. The contractor is continuing studies on a human lymphoblastic suspension culture from Rhabdomyosarcoma which was found to contain EB virus. Both chemical analysis of viral enzymes and morphologic studies of virus produced in IUdR stimulated cells from this line are being carried out.

Significance to Biomedical Research and the Program of the Institute: This is an important project concerned with the search for viruses in human tumors. Extensive and careful examination, by electron microscopy, of a large number of human tumors and cell cultures established from these tumors is essential in determining the viral etiology of cancer.

Proposed Course: Collection of specimens, shipping to assigned laboratories and screening of cultures by EM and by indirect immunofluorescent technique for EB Virus will continue. Screening for RNA tumor viruses will be extended by the inoculation of specific pathogen-free chicken embryo fibroblasts with human tumor cell homogenates and culture filtrate.

Date Contract Initiated: June 24, 1969

Current Annual Level: $75,000

SOUTHWEST FOUNDATION FOR RESEARCH AND EDUCATION (NIH 69-2001)

Title: Housing and Maintenance of a Chimpanzee Colony

Contractor's Project Director: Dr. Seymour S. Kalter

Project Officer (NCI): Dr. Roy Kinard

Objectives: To supply young chimpanzees to SVCP investigators.

Major Findings: The animals maintained under this contract have been in generally good health during the past year. Four infant chimpanzees were born during the year; one of these was inoculated with material from a human

leukemia, another with Gibbon ape lymphoma virus (both of these under the direction of Dr. Joseph Melnick, contract 68-678), a third is being readied for experimentation, and a fourth died of unrelated natural causes.

The chimpanzee colony now comprises the following animals: six breeding age females, two breeding age males, one juvenile male, two juvenile females, and four infants.

Significance to Biomedical Research and the Program of the Institute: The chimpanzee now appears to be the laboratory animal most similar to humans, biochemically and immunologically. Newborn chimpanzees are particularly useful in determining susceptibility to suspected human cancer viruses because their resistance to virus infection is very low. This is the only source of newborn chimpanzees for the SVCP.

Proposed Course: The chimpanzee colony will be maintained as before and newborn animals will continue to be supplied to investigators within the SVCP.

Date Contract Initiated: April 25, 1969

Current Annual Level: $25,000

UNIVERSITY LABORATORIES, INC. (PH 43-66-1133)

Title: Production of Oncogenic Viruses and Antisera

Contractor's Project Director: Dr. Eugene H. Bernstein

Project Officers (NCI): Dr. Robert Holdenried
Dr. Jack Gruber

Objectives: Production of leukemia and sarcoma viruses and antisera.

Major Findings: The production of Rous sarcoma virus (Prague strain) in tissue culture roller bottles continues at a very high level. This year production level was increased to approximately 50 liters per week, and the production of Rous-associated viruses (RAV-2, RAV-7) was terminated and replaced by the additional production of Rous (Prague) virus. This accounts for the marked increase in Prague production. The virus is pelleted, resuspended and issued to various collaborating laboratories at 100X or more of its original concentration.

Feline leukemia virus production was continued utilizing the Rickard F-422 continuous thymus cell line. The production rate is in excess of 5,000 ml. of culture fluid per month which is concentrated tenfold before being placed in ampoules. The electron microscope particle count for a twofold concentrated virus has averaged about 3×10^{10} particles per ml.

The production of Rauscher leukemia virus harvested from BALB/c mouse plasma was also continued. The production rate has been increased to an average of 400-500 ml. per month. The plasma virus is highly infective to BALB/c mice, assays an average of 4.5 log focus forming units per ml., and exhibits about 10^7 particles per ml. by electron microscope particle count. Requests for this virus continue to be in excess of production capability. A program to produce a high titer, highly specific BALB/c mouse antiserum to Rauscher leukemia virus is now also underway.

Significance to Biomedical Research and the Program of the Institute: The supply of highly standardized oncogenic viruses and antisera produced by this contractor has been extensively used by SVCP researchers and is essential to the continuation of many important research projects presently being carried out in the program.

Proposed Course: Production of needed strains of oncogenic viruses and their antisera will continue in volumes necessary to meet SVCP needs.

Date Contract Initiated: June 4, 1962

Current Annual Level: $347,980

WOLF RESEARCH AND DEVELOPMENT CORP. (NO1-CP-3-3256)

Title: Computer Services in Support of Cancer Research

Contractor's Project Director: Dr. William H. Lake

Project Officer (NCI): Dr. Deward E. Waggoner

Objectives: The major objective of this contract is the implementation of a computerized central inventory system for the various resources of the SVCP. The system now being installed embraces a number of institutional repository subsystems contributing to the central base currently in the VO Program Analysis and Communications Office (PAC). Each computerized subsystem is used at a given resource repository to inventory the items produced or stored at that location. Since the types of resources at the various resource banks differ in kind, the subsystems all differ to some extent. They are, however, all designed to contribute compatible information to the central system in PAC, thus forming the base for a program-wide central inventory and control.

The resource bank subsystems are also used at storage sites for controlling materials in functions unique to each institution, and this contributes to the detailed differences. Some of these secondary functions include catalog production, local inventory, and retrieval of clinical information.

Major Findings: Due to the new growth and current size of the PAC specimen inventory data bank (now approximately 100,000 specimens), a random access computer disc pack was assigned to PAC. The PAC programs (PAC II) were

modified to make the most effective use of this memory device with direct access as opposed to a sequential search normally used with magnetic tapes. The resultant new programs (PAC III) have proved reliable and efficient; a significant decrease in operating costs was thus effected.

Programs were completed for production of management information reports and catalogs of data in the Central Inventory System.

Wolf Research & Development Corp. also completed in collaboration with the SVCP personnel, the following work in repository subsystems:

1. Naval Biomedical Research Laboratory. Automation of the cell culture repository was completed. The system is operational at the University of California Medical School, via a remote terminal at NBRL. Provision was made for summary reporting of NBRL data to the Central Inventory System at PAC.

2. Special African Specimen Collection. Work was begun on the conversion of the data on specimens to PAC format. Work was also started on the maintenance and retrieval of associated clinical data from lymphoma treatment centers.

3. Huntingdon Research Center. Automation of the antiserum/reagent retrieval system was completed. The system is operational at the National Institutes of Health via a remote terminal at HRC.

4. Others. Data from Memorial Institute, M. D. Anderson, Hospital for Sick Children in Toronto, Montreal Children's Hospital and the Center for Disease Control was captured and merged into the PAC system.

Significance to Biomedical Research and the Program of the Institute: The necessary expansion of the inventory of viruses, sera, tissue cultures, human specimens, and other materials used in cancer research makes it vital that close control be exercised over these resources. Computerization of the inventory will eventually make it possible for the NCI Office of Program Resources and Logistics to rapidly obtain information necessary to determine availability, location, quantity, etc. of all resources within its jurisdiction, thereby permitting rapid response to the needs of the program while avoiding resource excesses or shortages.

Proposed Course: The contractor will continue to extend and refine the Central Inventory System. Additional management reports will be generated for SVCP resources control. Additional work on implemented subsystems will include: regular collection of new data; maintenance of automated subsystems; addition of interactive updates of test and bleeding data in Huntingdon Research Center's production of antisera. Work on new subsystems will be scheduled by PAC.

Date Contract Initiated: May 3, 1971

Current Annual Level: $170,000

DIVISION OF CANCER CAUSE AND PREVENTION

NCI

Table of Ratios of Contracts to
Intramural Effort Based on
Estimated Current Level of Expenditure

JUNE 30, 1973

	ODD	DEMOGRAPHY	CARCINOGENESIS	VIRAL ONCOLOGY	TASK FORCES	TOTAL
Total	1,812,000	9,297,000	26,233,000	49,366,000	5,874,000	92,582,000
Contracts	615,000	6,891,000	22,649,000	42,511,000	5,509,000[1]	78,175,000
Intramural*	1,197,000	2,406,000	3,584,000	6,855,000	365,000[2]	14,407,000
Ratios: Contract to Intramural	--	2.9:1	6.3:1	6.2:1	15.1:1	5.4:1

*Includes costs of managing contract activities.

[1]Includes $2,903,000 for Lung Cancer, $1,000,000 for Large Bowel, $600,000 for Pancreas (Carcinogenesis area); and $1,006,000 for Breast Cancer (Viral Oncology area).

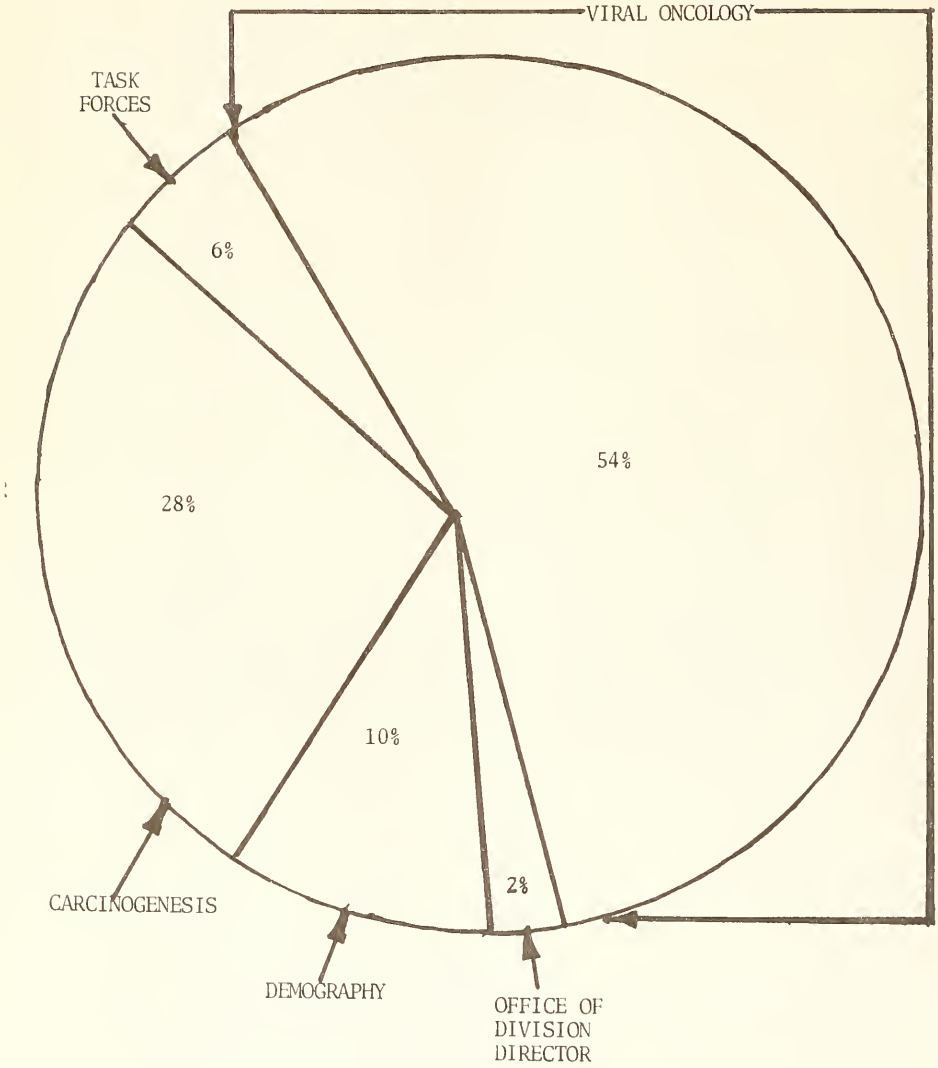
[2]Direct operations for Lung Cancer (Carcinogenesis area).

Note: Construction not included.

NATIONAL CANCER INSTITUTE

Division of Cancer Cause and Prevention

Current Distribution of Funds-Fiscal Year 1973



(Division reorganization effective April 16, 1973.)

OFFICE OF DIVISION DIRECTOR

Peters, James A.	Acting Director
O'Connor, Timothy E.	Coordinator for Molecular Control
Depue, Robert H., Jr.	Scientist Director
Bourland, Orley R., Jr.	Biochemical Engineer (FCRC)

Administrative Management Branch

Miller, John M.	Administrative Officer, Chief
Olimpio, Nicholas A.	Program Analyst
Velthuis, Robert, Jr.	Administrative Officer, Viral Oncology
Hull, Robert	Acting Admin. Officer, Carcinogenesis
Doyle, James D.	Admin. Officer, Field Studies & Stat.

Office of Associate Director for Program

Gori, Gio B.	Associate Director
Sberro, Joseph E.	Program Analysis Officer

Office of Associate Director for Field Studies & Statistics
(formerly Demography)

Schneiderman, Marvin A.	Associate Director
Fox, Bernard H.	Research Psychologist
Levin, David L.	Surgeon
Fears, Thomas R.	Staff Fellow

Biometry Branch

Office of the Chief

Haenszel, William M.	Chief
Cutler, Sidney J.	Associate Chief
Mantel, Nathan	Sr. Res. Math. Statistician
Correa, Pelayo	Visiting Scientist
Glober, Gary A.	Sr. Staff Fellow (Hawaii)
Murray, James L.	Sr. Veterinary Officer
Moore, Joseph O.	Surgeon (Hawaii)
Conlon, Nancy M.	Statistician (Health)

Automatic Data Processing Management Section

Weiss, Theodore	Digital Comp. Sys. Admin., Head
Kuch, Terrence D.	Computer Systems Analyst
Larson, James E.	Computer Systems Analyst
Oslik, Norman	Computer Systems Analyst (Hawaii)
Stump, James M.	Computer Specialist
Gales, Altemease A.	Computer Programmer

Biometry Branch (continued)

Clinical & Diagnostic Trials Section

Byar, David P.	Medical Officer, Head
Gail, Mitchell	Surgeon
Sedransk, Nell	Sr. Staff Fellow
Muenz, Larry R.	Staff Fellow

Demography Section

Young, John L., Jr.	Health Services Officer, Head
King, Haitung	Research Sociologist
Propok, Philip C.	Staff Fellow
Locke, Frances B.	Statistician
Devesa, Susan S.	Statistician

End Results Section

Myers, Max H.	Res. Math. Statistician, Head
Lourie, William I., Jr.	Statistician (Health)
Axtell, Lillian M.	Statistician (Health)
Brown, Charles	Res. Math. Statistician
Connelly, Roger R.	Statistician (Health)
Baylor, Stephen M.	Surgeon
Cramer, Daniel W.	Surgeon
Hankey, Benjamin F.	Res. Math. Stat.
Baylis, Paula H.	Supv. Stat. Officer
Baranovsky, Anne	Statistician (Health)
Heise, Herman W.	Statistician (Health)
Shambaugh, Evelyn M.	Statistician (Health)
Asire, Ardyce J.	Statistician (Health)

Mathematical Statistics & Applied Mathematics Section

Gart, John J.	Res. Math., Head
Connor, Robert J.	Res. Math. Statistician
Pettigrew, Hugh M.	Res. Mathematician
Thomas, Donald G.	Res. Math. Statistician
Layard, M.W.	Sr. Staff Fellow
Nam, Jun-Mo	Math. Statistician
Smith, Alroy M.	Statistician (Health)

Special Cancer Survey Section

Geller, Harvey	Statistician (Health), Head
Percy, Constance L.	Statistician (Health)
Scotto, Joseph	Health Services Officer
Godwin, Joseph D., II	Surgeon

Epidemiology Branch

Office of the Chief

Miller, Robert W.	*Chief*
Fraumeni, Joseph F., Jr.	*Associate Chief*

Ecology Studies Section

Fraumeni, Joseph F., Jr.	*Medical Director, Head*
Li, Frederick P.	*Surgeon (Boston)*
Mulvihill, John J.	*Surgeon*
Creagan, Edward T.	*Surgeon*
Grundy, Gordon W.	*Surgeon*
Chabalko, John J.	*Surgeon*
Hoover, Robert N.	*Surgeon*
Mason, Thomas J.	*Staff Fellow*
McKay, Frank W., Jr.	*Computer Specialist*

Epizoology Section

Priester, William A., Jr.	*Vet. Officer Dir., Head*
Hayes, Howard M.	*Vet. Med. Officer*

Special Cancer Studies Section

Miller, Robert W.	*Acting Head*

Office of Associate Director for Carcinogenesis

Saffiotti, Umberto	*Associate Director*
Kraybill, Herman F.	*Sci. Coordinator for Environ. Carc.*
Baldschwieler, John D.	*Expert*
Cooper, John A., II	*Chemist (Sci. Admin.)*
Barrett, Margaret D.	*Biologist*

Epidemiologic Pathology Unit

Berg, John W.	*Medical Officer, Head*
Lingeman, Carolyn H.	*Medical Officer*
Silverman, Sidney J.	*Res. Microbiologist*
Howell, Margaret A.	*Scientist Director*

Office of Coordinator for Collaborative Research

Heim, Allen H.	*Res. Microbiologist, Head*
Owen, Thomas B.	*Microbiologist*
Pledger, Richard A.	*Res. Microbiologist*
Kaplan, Ann E.	*Chemist*
Litwack, Marcia D.	*Staff Fellow*
Sontag, James M.	*Staff Fellow*
Smith, Carl E.	*Chemist*

Biology Branch

Office of the Chief

Rapp, Herbert J.	Chemist, Chief
Borsos, Tibor	Associate Chief

Cellular Immunity Section

Zbar, Berton	Sr. Surgeon, Head
Bekierkunst, Adam	Visiting Scientist
Bast, Robert C., Jr.	Surgeon
Littman, Bruce H.	Surgeon

Cytogenetics and Cytology Section

DiPaolo, Joseph A.	Res. Pharmacologist, Head
Asher, Stephen W.	Surgeon
Evans, Charles H., Jr.	Surgeon
Myhr, Brian C.	Sr. Staff Fellow
Popescu, Nicholae C.	Visiting Associate
Donovan, Paul J.	Chemist

Tumor Antigen Section

Leonard, Edward J.	Medical Director, Head
Meltzer, Monte S.	Surgeon
Boetcher, David A.	Surgeon
Smith, Howard G.	Surgeon
Stewart, Laura C.	Res. Chemist

Immunochemistry Section

Borsos, Tibor	Res. Chemist, Head
Dunkel, Virginia C.	Sr. Staff Fellow
Ohanian, S. H.	Sr. Staff Fellow

Chemistry Branch

Office of the Chief

Gelboin, Harry V.	Biochemist, Chief
Wiebel, Friedrich J.	Sr. Staff Fellow

Protein Section

Peacock, Andrew C.	Res. Chemist, Head
Ness, Arthur T.	Res. Chemist
Cates, Robert J.	Surgeon
Green, Marie R.	Res. Chemist

Chemistry Branch (continued)

Nucleic Acids Section

Dingman, C. Wesley, II	*Medical Officer, Head*
Kakefuda, Tsuyoshi	*Medical Officer (Res.)*
Day, Rufus S., III	*Sr. Staff Fellow*

Cell Growth Regulation Section

Bader, John P.	*Res. Microbiologist, Head*
Hatfield, Dolph	*Res. Biologist*
Lew, Michael A.	*Surgeon*
Kitano, Yoshiyuki	*Visiting Associate*

Molecular Carcinogenesis Section

Gelboin, Harry V.	*Acting Head*
Whitlock, James P.	*Surgeon*
Selkirk, James K.	*Staff Fellow*

Experimental Pathology Branch

Office of the Chief

Bates, Richard R.	*Medical Director, Chief*

Digestive System Carcinogenesis Section

Bates, Richard R.	*Acting Head*
Lieberman, Michael W.	*Surgeon*
Yamamoto, Richard S.	*Sr. Staff Fellow*

Endocrine Carcinogenesis Section

Bates, Richard R.	*Acting Head*
Janss, Douglas H.	*Staff Fellow*

In Vitro Pathogenesis Section

Yuspa, Stuart H.	*Surgeon, Head*
Dermer, Paul E.	*Surgeon*
Hennings, Henry	*Sr. Staff Fellow*
Morgan, David L.	*Biologist*

Perinatal Carcinogenesis Section

Rice, Jerry M.	*Scientist, Head*
Joshi, Sewa R.	*Sr. Staff Fellow*
Cotton, William G.	*Visiting Fellow*
Edwards, Winston D.	*Visiting Fellow*

Lung Cancer Branch

Office of the Chief

Sporn, Michael B.	*Medical Director, Chief*
Barr, Robert	*Visiting Fellow*
Genta, Valerio	*Visiting Fellow*

Differentiation Control Section

De Luca, Luigi	*Chemist, Head*

Lung Cancer Pathogenesis Section

Sporn, Michael B.	*Acting Head*
Harris, Curtis C.	*Surgeon*
Kaufman, David G.	*Surgeon*
Clamon, Gerald H.	*Surgeon*

Carcinogen Bioassay and Program Resources Branch

Office of the Chief

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Cameron, Thomas P.	*Vet. Officer Dir.*
Siegel, Sidney	*Biologist*
Linhart, Mary S.	*Computer Programmer*

Carcinogen Metabolism and Toxicology Branch

Office of the Chief

Weisburger, Elizabeth K.	*Scientist Dir., Chief*

Analytical Chemistry Unit

Keefer, Larry K.	*Sr. Staff Fellow*
Roller, Peter P.	*Staff Fellow*

Metabolic Studies Section

Weisburger, Elizabeth K.	*Acting Head*
Poirier, Lionel A.	*Sr. Staff Fellow*
Ward, Jerrold M.	*Vet. Med. Officer*
Grantham, Preston H.	*Res. Chemist*
Idione, Jane B.	*Res. Chemist*

Office of Associate Director for Viral Oncology

Moloney, J. B.	*Associate Director*
Hearn, Henry J.	*Microbiologist (FCRC)*
Sibal, Louis R.	*Microbiologist*
Zeve, Victor H.	*Res. Biologist*
Bryan, W. Ray	*Expert*
Perk, Kalman	*Visiting Scientist*
Goldberg, Robert J.	*Staff Fellow*
Kvedar, John P.	*Bio. Technologist*

Office of Biohazards & Environmental Control

Hellman, Alfred	*Scientist Dir., Head*
Keefer, Garrett V.	*Microbiologist*

Biohazards Research Section

Hellman, Alfred	*Scientist Dir., Head*
Fowler, Arnold K.	*Sr. Scientist*
Steinman, Harry G.	*Supv. Chemist*
Kind, Phyllis	*Res. Microbiologist*
Strickland, James E.	*Sr. Staff Fellow*

Environmental Control Section

Barkley, W. Emmett	*San. Engineer, Head*
West, David L.	*San. Engineer*
Barbeito, Manuel S.	*Microbiologist*

Office of the Coordinator of Ultrastructural Studies

Dalton, Albert J.	*Res. Biologist, Coordinator*

Virus Studies Section

Heine, Ursula I.	*Res. Microbiologist, Head*
Suskind, R. Gerald	*Medical Director*
Bader, Artrice V.	*Res. Biologist*
Dahlberg, John E.	*Sr. Staff Fellow*
Weber, George H.	*Staff Fellow*
Elliott, Benjamin F., Jr.	*Bio. Lab. Tech.*

Office of Program Analysis & Communications

Waggoner, Deward E.	*Statistician, Head*
Greenberg, Louis P.	*Tech. Information Specialist*
Varrato, Wilma L.	*Statistical Assistant*
Wilson, Frank	*Computer Programmer*

Office of Program Resources and Logistics

Gruber, Jack	*Microbiologist, Head*
Howell, David M.	*Microbiologist*

Viral Biology Branch

Office of the Chief

Manaker, Robert A.	*Microbiologist, Chief*
Chirigos, Michael A.	*Associate Chief*
Reisinger, Robert C.	*Veterinarian*
Guss, Maurice L.	*Microbiologist*
Harada, Minoru	*Visiting Scientist*
Gissebrecht, Sylviane	*Visiting Fellow*
Stansly, Pauline	*Biologist*

Cell Biology Section

Boone, Charles W.	*Medical Officer, Head*
Orme, Thomas W.	*Sr. Staff Fellow*
Paranjpe, Meera S.	*Staff Fellow*
Koegel, Robert J.	*Res. Chemist*

Electron Microscopy Section

Chopra, Harish C.	*Res. Microbiologist, Head*
Holder, Walter G.	*Surgeon*
Andrese, Angelo P.	*Staff Fellow*

Experimental Pathology Section

Manaker, Robert A.	*Acting Head*
Pearson, Gary R.	*Sr. Staff Fellow*

Human Tumor Studies Section

Vande Woude, George F.	*Res. Chemist, Head*
Ascione, Richard	*Res. Chemist*
Ebert, Paul S.	*Res. Chemist*
Robey, William G.	*Staff Fellow*

Microbiology Section

Manaker, Robert A.	*Acting Head*

Virus & Disease Modification Section

Chirigos, Michael A.	*Res. Chemist, Head*
Woods, Wilna A.	*Res. Microbiologist*
Pearson, John W.	*Sr. Staff Fellow*
Papas, Takis	*Sr. Staff Fellow*

Viral Carcinogenesis Branch

Office of the Chief

Huebner, Robert J.	Medical Director, Chief
Duff, James T.	Associate Chief
Kelloff, Gary J.	Sr. Staff Fellow
Rand, Kenneth H.	Surgeon
Streicher, Harriet L.	Program Assistant
Capps, Worth I.	Bio. Lab. Tech.
Shiflett, Shirley B.	Statistical Assistant

Ecology and Epizoology Section

Sarma, Padman S.	Res. Microbiologist, Head

Field Studies Unit (California)

Arnstein, Paul	Vet. Officer Dir.
Estes, John D.	Bio. Lab. Tech.

Molecular Biology Section

Aaronson, Stuart A.	Medical Officer, Head
Stephenson, J. R.	Staff Fellow
Tronick, Steven R.	Staff Fellow

Solid Tumor Virus Section

Duff, James T.	Res. Microbiologist, Head
Hampar, Berge	Sr. Dental Surgeon

Viral Genetics Section

Huebner, Robert J.	Acting Head
Portugal, Franklin H.	Sr. Staff Fellow
Parks, Wade P.	Surgeon
Greenberger, Joel S.	Surgeon
Lowy, Douglas R.	Surgeon
Simonds, Josephine A.	Staff Fellow
Teich, Natalie M.	Staff Fellow
Twardzik, Daniel R.	Staff Fellow

Serology Unit

Hill, Paul R.	Supv. Bio. Lab. Tech.

Trailer Unit (Poolesville)

Lane, William T.	Bio. Lab. Tech.

Viral Leukemia and Lymphoma Branch

Office of the Chief

Todaro, George J.	Medical Officer, Chief
Holdenried, Robert	Scientist Dir.
Leiseca, Sergio A.	Res. Biologist
Talbot, Bernard	Medical Officer
Kinard, Roy F., Jr.	Vet. Officer Dir.
Benveniste, Raoul E.	Staff Fellow
Chieco-Bianchi, Luigi	Visiting Scientist
Ikawa, Yoji	Visiting Scientist

Genetics Section

Scolnick, Edward M.	Medical Officer, Head
Livingston, David M.	Sr. Staff Fellow
Henderson, I. Craig	Surgeon
Vogel, Kivity Tikvah	Visiting Fellow

Immunology Section

Aoki, Tadao	Medical Officer, Head
Plata, E. J.	Microbiologist
Hollis, Vincent W., Jr.	Research Chemist
Sendo, Fujiro	Visiting Scientist

Tumor Virus Section

Fischinger, Peter J.	Medical Officer, Head
Haapala, Daniel K.	Sr. Staff Fellow
Phillips, Leo A.	Sr. Staff Fellow
Peebles, Paul T.	Surgeon
Nomura, Shigeko	Visiting Scientist

Viral Pathology Section

Gazdar, Adi F.	Medical Officer, Head
Merwin, Ruth M.	Research Biologist
Buswell, Richard S.	Surgeon
Lieber, Michael M.	Surgeon
Oie, Herbert K.	Staff Fellow

Clinical Studies Section

Levine, Paul H.	Medical Officer, Head
Burton, George J.	Scientist Dir.
Gaylord, Clarice E.	Health Scientist Admin.
Perkins, Ida Virginia	Microbiologist (Ghana)

Viral Leukemia & Lymphoma Branch (continued)

Primate Virus Section

Ablashi, Dharam V.	*Res. Microbiologist, Head*
Easton, John M.	*Sr. Surgeon*

Viral Biochemistry Section

Bassin, Robert H.	*Microbiologist, Head*
Gerwin, Brenda I.	*Sr. Staff Fellow*