ANNUAL REPORT
OF
PROGRAM ACTIVITIES
NATIONAL CANCER INSTITUTE
Division of Cancer Cause and Prevention
Volume II

ANNUAL REPORT
FIELD STUDIES AND STATISTICS PROGRAM
OCTOBER 1, 1981 THROUGH SEPTEMBER 30, 1982

In the Division of Cancer Cause and Prevention (DCCP), the Field Studies and Statistics (FS&S) Program provides the focus for epidemiologic and biostatistical research within the Institute. It conducts intramural and collaborative epidemiologic investigation into the environmental and host determinants of human cancer; coordinates a network of population-based cancer registries for evaluating cancer incidence, mortality, and survival in the U.S.; analyzes the natural history of cancer and efficacy of therapeutic and preventive measures; and designs statistical models for clinical and experimental investigations.

Appointed this year as Associate Director for FS&S was Dr. Joseph F. Fraumeni, Jr. The organizational components of the program are the Biometry Branch (Chief, Dr. Earl S. Pollack), the Clinical Epidemiology Branch (Chief, Dr. Robert W. Miller), and the Environmental Epidemiology Branch (Chief, Dr. Joseph F. Fraumeni, Jr.). In summary reports the Branch Chiefs have outlined the research and other activities taking place this year. In this report the orientation and highlights of the FS&S program are briefly summarized.

Descriptive Epidemiology

A major objective of the FS&S program is to generate national statistics on cancer incidence, mortality, and survival. This provides valuable signals for further epidemiologic study, and for monitoring the progress of the National Cancer Program. This year NCI Monograph No. 57 presented detailed data on cancer incidence and mortality for the period 1973-77, enabling a comparison of cancer incidence and mortality among various ethnic groups and geographic areas. The continued surveillance of cancer patterns is important for identifying emergent problems and changing risks that suggest environmental hazards. Special attention is now being given to the analysis of survival statistics utilizing data available on patients diagnosed from 1973 through 1979, and a summary report is being prepared on survival results for white and black patients by geographic area. At the recommendation of the DCCP Board of Scientific Counselors, efforts were made this year to evaluate alternative mechanisms of achieving follow-up for collection of survival data, and to improve the systematic recording and monitoring of costs in each SEER registry, with the objective of reducing expenses and developing an effective cost-accounting system.

Since non-melanoma skin cancer is not covered by the cancer registries, a special incidence survey was carried out in 1977-78 in various areas of the country, and the results were published this year in a monograph. In the past a county-by-county survey of cancer mortality in the United States (1950-69) identified geographic peculiarities that have provided etiologic clues for further study, and updated maps of cancer mortality for 1970-75 are now being completed for the more common tumors. In addition, a volume of U.S. cancer mortality statistics (1950-75) was published, with emphasis on temporal trends for various cancers, through displays of two- and three-dimensional graphs over time by age, sex, and race. Also published this year was an atlas illustrating

the geographic patterns of non-neoplastic diseases in the U.S. that may share etiologic factors with cancer, and several Institutes are pursuing the leads generated by these maps.

Analytical Epidemiology

Continued emphasis was given this year to case-control and cohort studies aimed at evaluating key hypotheses in cancer etiology. Case-control studies of selected cancers have been undertaken when high-risk communities are identified on the cancer maps, or when major testable hypotheses and special resources become available. Based on leads provided by the U.S. cancer atlases, field studies have implicated shipyard work during World War II as the explanation for the high rates of lung cancer in coastal areas, use of smokeless tobacco for the elevated rates of mouth cancer among women in rural southern areas, and nutritional deficiencies and alcohol consumption for the high rates of esophageal cancer among black men in urban areas.

Occupational studies, a time-tested means of identifying physical and chemical carcinogens, were pursued to assess hazards suspected on the basis of experimental, clinical, and field observations. This year surveys revealed excesses of brain and other cancers in petroleum workers; leukemia and bladder cancer in professional artists; stomach cancer in iron ore miners; and lung cancer in shipyard workers and other groups exposed to asbestos, in steel and foundry workers exposed to hydrocarbons, in copper and zinc smelter workers exposed to inorganic arsenic, and in workers exposed to talc during ceramic plumbing manufacturing. A large follow-up study of workers exposed to formaldehyde during manufacturing and usage is under way. Considerable effort was devoted to working with other Government agencies, to utilize and develop record systems that may help in screening occupational groups at high risk of cancer.

Radiation studies received further emphasis as pressure mounts to clarify the effects of low-level exposure and the shape of the dose-response curve. In an international survey of cervical cancer, the radiation regimens were shown to be less effective in producing leukemia than other radiation exposures that have been studied, perhaps related to the cell-killing potential of high-dose radiation to the pelvis. A survey of breast cancer among atomic bomb survivors revealed elevated risks among women exposed at younger ages, particularly at 10-19 years; and for the first time, women exposed under age 10 showed a dose-related excess risk. A follow-up study of women receiving multiple chest fluoroscopies during pneumothorax treatment of tuberculosis revealed that repeated low radiation doses increased the risk of breast cancer, particularly from exposures during early adult life. No excess of other types of cancer were seen among fluoroscopically examined men or women. In a study of women treated with radioactive iodine for hyperthyroidism, there was a slight excess of cancer of the thyroid and other organs with high 131-I exposure, but the findings are preliminary due to the small numbers of cases.

Drug studies were expanded to evaluate the effects of estrogenic compounds, which now appear to be related to the risk of breast cancer. In several groups of cancer patients treated with alkylating agents, there was a substantially elevated risk of acute non-lymphocytic leukemia.

Nutritional studies were intensified this year to clarify the role of dietary components in cancer etiology. Field studies are taking advantage of geographic

areas in the U.S. (e.g., North/South differentials for large bowel cancer) and migrant groups (e.g., Japanese- and Norwegian-Americans) whose cancer risks may be altered by changing dietary habits. To evaluate the relationship between bladder cancer and saccharin, a case-control interview study of 3,000 cases and 6,000 controls revealed no overall excess risk for persons who had ever used artificial sweeteners, but subgroup analyses provided some evidence consistent with a weak carcinogenic or promoting effect. In case-control studies the role of dietary fat was suggested for breast and colorectal cancers, a broad nutritional deficiency for esophageal cancer, and a deficiency of fruits and vegetables containing vitamin A and C in oral cancer.

Family studies, enhanced by collaborative ties with laboratory investigators and a computer-based data resource, have resulted in the delineation of familial cancer syndromes and leads to mechanisms of host susceptibility. Of special interest has been the identification of the dysplastic nevus syndrome as a marker of susceptibility to melanoma, enabling early detection and treatment of this potentially lethal cancer. Educational videotapes were developed to acquaint high-risk patients and health professionals with the syndrome and opportunities for prevention. Studies of neurofibromatosis have helped clarify the risks of various cancers associated with this hereditary syndrome. Several reports were prepared on familial syndromes associated with sarcoma and other neoplasms, with carotid body tumors, with smoking-related respiratory cancer, with retinoblastoma and other neoplasms including pineal tumors, and with kidney cancer and polymastia. The Inter-Institute Medical Genetics Clinic, co-directed by one staff member, provides a multidisciplinary setting for studying families and patients prone to cancer. The value of alert clinical observations was demonstrated repeatedly by the detection of cancer-prone families and other high-risk groups.

Environmental pollutants were evaluated through epidemiologic studies that integrated appropriate environmental and body measurements, with special emphasis on a case-control study of bladder cancer in relation to the level of halogenated hydrocarbons in the drinking water.

Infectious agents received substantial attention as the program became involved in collaborative studies to evaluate the role of a newly discovered human retrovirus in the origins of T-cell leukemia. In addition, recent clusters of Kaposi's sarcoma and opportunistic infections in homosexual males prompted studies to evaluate the role of sexually transmitted viruses, use of amyl nitrite, and other exposures.

Multidisciplinary projects combining epidemiologic and experimental approaches were emphasized whenever possible to evaluate the influence of oncogenic viruses, dietary and metabolic factors, host susceptibility, air and water pollutants, and other causative factors that may continue to elude detection by traditional observational methods.

Collaborative Activities

Collaborative studies with other Federal agencies have been given high priority to (1) evaluate urgent issues including those of immediate regulatory or public policy concern, and (2) to stimulate the epidemiologic application of technical and data resources that are used by the Government mainly for other purposes. Many research and regulatory agencies are concerned with environmental cancer,

yet few have epidemiologic programs, and require assistance and support on many issues. Particularly at this time of fiscal restraint, it is important to increase initiatives to develop and coordinate national data resources that, with appropriate safeguards, may be tapped by qualified investigators throughout the country.

The bi-national programs offer major epidemiologic opportunities for international study, and this year special emphasis was given to joint studies and exchange programs with Chinese scientists to pursue clues drawn from the recent county-based maps in China and the changing risks among Chinese migrants to the United States. Reports were prepared on the geographic correlations within China between cancers of the cervix and penis, and colorectal cancer and schistosomiasis, and on the patterns of childhood cancer in Chinese populations around the world. Collaborative analytical investigations are being developed in China on cancers of the esophagus and lung, trophoblastic neoplasms, and T-cell leukemia.

Within the Institute, further steps were taken to improve communication and coordination of epidemiology and biometry programs, and to stimulate multidisciplinary activities linking epidemiologists with experimentalists and clinicians. Through the mechanisms of the SEER Program, cancer centers, pre-paid health plans, and other resources, FS&S staff are increasingly involved in coordinating case-control and other studies that involve collective approaches with the pooling and sharing of data. In addition, FS&S staff were involved this year in coordinating the preparation of several comprehensive and critical reviews, including reference volumes on cancer epidemiology and prevention, radiation carcinogenesis, neurofibromatosis, area-wide chemical contaminants, and carcinogenic hazards to children.

Biometric Studies

The development of statistical methodology in FS&S has contributed greatly to several areas, including epidemiology, carcinogenesis research, therapy trials, and screening programs. This year attention was given to the development and testing of multi-cause and multi-stage models of carcinogenesis. For example, an analysis of lung cancer patterns among smelter workers suggested that inorganic arsenic acts in the manner of a promoting agent, perhaps explaining why laboratory studies of arsenic carcinogenicity have been negative in the face of positive epidemiologic results. Issues involved in extrapolating results from animal experiments to man were also addressed. There was substantial involvement in the study design, implementation, and analysis of therapy trials for various forms of cancer. The FS&S program continued to be responsible for statistical support and consultation to intramural scientists throughout the Institute, ranging from basic laboratory research to community activities in cancer control. Further attention was given to the development of new statistical techniques in designing, monitoring, and evaluating programs for the screening and early detection of cancer. With the development of a focus for applied prevention programs in the Division of Resources, Centers and Community Activities (DRCCA), steps were taken to establish working relationships and some collaborative projects. Current emphasis is being given to the detailed analysis of 5-year relative survival rates in the SEER Program, and to analytic studies to identify factors responsible for ethnic differences in survival. To enlarge the population of blacks and Hispanics in the SEER Program, steps are being taken to add another registry from an appropriate section of the country.

Prospects

It is difficult to project activities over time, given the uncertainties related to reorganization plans, available positions, funds, manpower, and especially the direction that new leads and opportunities will take. However, a major objective of FS&S is to attain a comprehensive, flexible, and balanced program that will enhance our capacity at the national level to generate fresh ideas and help settle key questions in cancer epidemiology and biometry. Toward this end, a selective expansion and reorientation of the program seems warranted to augment existing project areas, initiate new lines of research, and make the most efficient use of resources located at NCI and several Federal agencies. Although its primary responsibility is intramural research, FS&S has a clear obligation to provide biometric and epidemiologic support to all parts of the National Cancer Program, to foster parallel efforts throughout the Program, and to promote epidemiology training opportunities at NIH and elsewhere. With mounting interest in environmental cancer and in the contribution to etiology and prevention that can be made through the epidemiologic approach, FS&S is often asked to increase the scope of its work and to help develop Institute and Federal programs and policy in several areas.

Despite substantial growth and support of the intramural epidemiology program over the past five years, there are still insufficient senior staff to keep pace with the opportunities and demands for research and consultation in the field of environmental cancer. It is clear that more vigorous epidemiologic efforts are needed to identify the life-style and other environmental factors that are carcinogenic to man. If additional funds and personnel become available, special priority would be given to research designed to clarify the role of nutritional factors and general environmental (e.g., air and water) pollutants in cancer etiology, with attention to the development of more precise ways of measuring the exposures of concern. In assessing many risk factors, efforts will be made to incorporate biochemical and molecular probes of exposure, response, and mechanisms of action. In addition, more attention will be given to the less common neoplasms, involving coordinated case-control studies in several areas or centers. It should be emphasized that the FS&S program contributes not only to cancer etiology, but also to natural history, end results, clinical trials, preventive measures, and even strategies involved in administrative planning and decision-making. Epidemiologic and biometric approaches permeate many aspects of the National Cancer Program and are fundamental to the design and evaluation of methods to control cancer. The effectiveness of FS&S thus depends greatly upon our success in promoting interaction and coordination with other parts of the Institute.

ANNUAL REPORT
BIOMETRY BRANCH
October 1, 1981 through September 30, 1982

The major functions of the Biometry Branch are: to measure trends in cancer incidence and patient survival over time and to assess differences in these measures among important population subgroups; to conduct research on the etiology of cancer in humans; to develop statistical methodology applicable to clinical trials and other follow-up studies as well as to other problems in cancer research; and to provide statistical and computer science support to other research investigators outside the Branch. This work is accomplished through in-house studies and through field studies, some of which are carried out collaboratively with investigators in this country and abroad. The program as it developed during the year can be summarized briefly as follows:

Surveillance, Epidemiology and End Results (SEER) Program

The SEER Program obtains cancer incidence and patient survival data in the United States through ten population-based cancer registries covering all cancers diagnosed in the populations of five entire states (Connecticut, Hawaii, Iowa, New Mexico and Utah), four metropolitan areas (Atlanta, Detroit, San Francisco and Seattle), and the Commonwealth of Puerto Rico. The program is operated primarily by Dr. John Young and his Demographic Analysis Section. The program began in 1973 and the focus until now has been on the analysis of cancer incidence data. This is due primarily to the fact that insufficient time had elapsed to allow for meaningful analysis of patient survival data. The first full-scale detailed presentation of cancer incidence and mortality data for the SEER Program was released in a large monograph published in October 1981 (NCI Monograph No. 57). Attention has now turned to the analysis of patient survival data with the availability of adequate follow-up information on patients diagnosed from 1973 through 1979. There has been considerable interest in these data during the year because of questions being raised about whether advances had been made, in terms of outcome, following cancer treatment. Up to this point, the only data available to answer that question were those from the former End Results Group, the most recent of which compare survival rates for patients diagnosed in 1970-73 with those for patients diagnosed in 1960-63. The current SEER data are not comparable in that they pertain to a set of large population-based registries as opposed to the group of four primarily hospital-based cancer registries included in the End Results Group. The measure commonly used to assess cancer outcome has been the five-year relative survival rate. This has come under considerable discussion during the year, which resulted in the presentation and discussion of this concept before the Board of Scientific Counselors of the DCCP and the National Cancer Advisory Board. The five-year relative survival rate is the proportion of patients remaining alive five years after diagnosis adjusted for the expected five-year survival for persons in the general population of the same age. During the course of these presentations, it was demonstrated that the proportion of patients diagnosed with cancers of specific sites surviving death from cancer for five years after diagnosis, after persons dying of other causes were dropped from observation at the time they died, was identical to the five-year relative survival rate. This provided additional rationale for considering the five-year relative survival rate as the probabililty of surviving the effects of cancer.

The five-year relative survival rate for patients first diagnosed from 1973-79 was 47%--39% for males and 54% for females. Of course, the rates varied tremendously by primary site from a low of two percent for cancer of the pancreas to a high of 87% for cancer of the uterine corpus. The five-year relative survival rate for female breast cancer was 72%, for bladder cancer--72% for males and 69% for females, but for lung cancer, the five-year relative survival rate was 10% for males and 14% for females. A publication presenting summary survival data for the SEER Program for black and white patients by geographic area will be published this year. Next year a detailed monograph on cancer patient survival from the SEER Program will be published containing data for the various ethnic groups, geographic areas, and taking into account extent of disease at diagnosis and type of treatment.

During the year, the major recommendations of the Board of Scientific Counselors regarding the SEER Program were addressed intensively. Since the Board of Scientific Counselors recommended that consideration be given to discontinuing active follow-up and using passive follow-up as an alternative, two tests of passive follow-up were conducted during the year--one by simply ignoring the results of active follow-up and matching the patient files against the death records obtained from the state offices of vital statistics, and the other involved matching files for two registries against the National Death Index (NDI), operated by the National Center for Health Statistics. The first test indicated that passive follow-up obtained by state death certificate matching leads to gross overestimation of true patient survival. As far as the results of matching against the National Death Index is concerned, the NDI was unable to identify eight percent of the deaths for patients who were known to have died. This seemed to be an unacceptably high proportion of the deaths. Based on the results of these tests, and data from the analysis of costs of the SEER Program, which indicated that follow-up constituted less than 10% of the total cost, it was decided that at least for the time being active follow-up must be continued in order to obtain valid patient survival data.

The other major recommendation of the Board of Scientific Counselors that was addressed during the year was that an analysis of costs of the SEER Program be conducted with a view toward reducing costs and toward developing an ongoing cost accounting system that would make possible monitoring of costs of various aspects of the program. This was accomplished by a team of seven individuals visiting each of the registries in the SEER Program and carrying out an analysis of registry operations, staffing, and costs using a standard protocol. The result of this effort will be a certain amount of reduction in staffing and other costs of the registries, but a good deal of this cost saving will be dissipated due to inflation. A way of establishing budgets for each of the SEER registries and for recording costs by specific registry function was developed, however, and will be implemented as each of the SEER contracts come up for renewal.

Clinical & Diagnostic Trials

Dr. David Byar and his staff provide consultation on the development of large clinical trials and themselves are very active in the development of statistical methodology needed for analysis of data resulting from such trials and also for the analysis of data from epidemiologic studies. In consultations on clinical

trials, members of the section assist investigators in developing detailed study protocols, in determining numbers of patients necessary for the study, and deciding what data should be recorded and at what intervals of time and in developing forms for the reporting of data. In some instances, they provide advice on data analysis and in others they themselves carry out the analyses. During the year, Dr. Byar and his group continued their involvement in major clinical trials for the following cancers: prostate, breast, testis, lung and brain, as well as work in a number of other areas including skin cancer, intraocular melanoma, Burkitt's lymphoma, mycosis fungoides, and others.

The following is a brief summary of some of these activities during the year:

1) During the year a study was completed on the prognostic value of acid phosphatase and its use in cancer staging. It was demonstrated that the level of prostatic acid phosphatase, even in the normal range and in patients whose prostate was removed, was prognostic for progression. This suggested that cancer of the prostate metastasizes early in some patients and that therefore some systemic therapy should be used in addition to, or instead of, surgery.

2) Dr. Green is responsible for the study design and analysis for a nationwide randomized trial comparing adjuvant combination chemotherapy following surgery for resectable stage II cancer of the testis versus using chemotherapy only for relapses. This study is also following stage I patients to determine factors which may predict which tumors will recur. Since this is a relatively rare tumor, cases are accumulating slowly. Among the 30 recurrences that have developed thus far, only one has occurred in a patient who had been treated with cis-platinum as an adjunct to surgery. This is consistent with the findings in the SEER Program that survival rates for non-seminomatous testicular cancer increased dramatically in the mid-1970s following the introduction on a wide scale of cis-platinum as adjuvant therapy.

3) The section is carrying out extensive work on breast cancer and is responsible for obtaining a data file of some 3,600 women with breast cancer, many of whom have had estrogen resector assays performed, and on over 10,000 women from whom sera and background data have been obtained for evaluation of biological markers for breast cancer. The first file is being used to create a natural history data base of stage II patients who received no adjuvant therapy. The data on sera from breast cancer patients are being used for a variety of studies and will continue to be used for a number of years.

4) Two staff members have continued to work with the Lung Cancer Study Group, which comprises six major centers with a capacity to recruit over 150 stage I lung cancer patients per year. Six prospective randomized trials are now in progress.

5) Drs. Byar and Green have been collaborating with Dr. Staquet of the EORTC on the analysis of data to identify clinical activity in drugs. They developed a three-stage strategy for screening drugs that would identify almost all of the potentially active drugs discovered by the present strategy but at a greatly reduced cost.

6) The staff is continuing its work with the Brain Tumor Study Group on several clinical trials. A new phase II trial was begun during the year, patient

accrual was completed on three phase II trials and data analysis was begun, and work on three phase III trials is underway.

This group is involved in a number of additional projects including a large bladder cancer trial with the EORTC, a study of personality factors associated with breast cancer or benign breast disease, studies of the Makari Skin Test, and a study to identify prognostic factors in intraocular malignant melanoma.

This Section has been very productive in developing statistical methodology covering areas related to clinical trials and other associated problems. The following is a list of the areas in which this work was done to indicate the range of this activity: theory of survival analysis, sequential monitoring of clinical trials, methods for direct adjustment of survival curves, development of confidence limits for estimates of median survival times, estimating coefficients in multivariate survival models, study of the impact of secondary treatments in randomized trials on the outcome of interest, prospective matched analyses, logistic regression for serial data, chi-square tests for equality of proportions, subset analysis in epidemiologic studies, a two-sample test for positive random variables, and several others.

Mathematical Statistics

Dr. John Gart and his Mathematical Statistics and Applied Mathematics Section has continued to provide statistical support and consultation to intramural scientists within the Division of Cancer Cause and Prevention, as well as to those in other divisions in NCI. This involves basic study design as well as data analysis. In addition, this section continues to develop basic statistical methodology, and in some instances associated computer software, with applications to the kinds of problems involved in their consultative work but with broad applications as well.

A brief summary of some of the activities of this section during the past year is as follows:

1) Dr. Gart has continued to carry out statistical analysis on two large prospective studies on the relationship between diet and cancer--one in Minnesota among the Lutheran Brotherhood and the other in Norway. A negative association between vitamin A intake and lung cancer was found in both studies--in Minnesota with lung cancer mortality and in Norway with lung cancer incidence. Pancreatic cancer was found to be positively associated with alcohol consumption, chewing tobacco and snuff. There was no relationship between coffee drinking and pancreatic cancer as has been indicated by some other studies.

2) In relation to the Carcinogenesis Intramural Program, collaboration involved design and statistical analysis of experiments investigating factors which influence chromosome damage in human cells induced by fluorescent light or X-ray; an attempt to explain increase of susceptibility to such damage in malignant cell lines; a study of the survivorship of Chinese nasopharyngeal cancer patients in Singapore in relation to the HLA system and Epstein-Barr virus antibody levels; statistical assistance in the study of aryl hydrocarbon hydroxylase induction levels in psoriasis patients and further work on the statistical analyses of bacterial mutagenesis assays of several substances.

3) Collaboration has continued with staff of the Division of Cancer Biology and Diagnosis. This includes collaboration on experiments to study the in vitro survival of lymphoblast and fibroblast cell lines from patients with the cancer-prone disease, xeroderma pigmentosum, and a variety of hereditary primary neuronal degenerations after exposure to DNA-damaging agents; the design and statistical analysis of experiments comparing the monocyte chemotactic response of cancer patients with that of normal controls; statistical assistance on experiments performed to evaluate treatment of MNU-induced rat mammary tumors with intralesionally administered cell walls of Mycobacterium bovis strain Bacillus Calmette-Guerin; and a number of others.

4) Considerable research has been carried out on a number of topics in mathematical statistics, probability and applied mathematics. This includes continuing research on Empirical Bayes methods for analyzing frequency data, a method for using historical control data to test for trend in Poisson means, optimal methods for analyzing survival curves, investigation of distribution-free tests for censored distributions, research on asymptotic efficient estimators of the common relative risk or odds ratio, study of the properties of relative risk for case-control studies with multiple matched controls, study of higher order corrections to the mean in higher order moments of various transformations of binomial proportions, and several others.

Analytic Studies

Dr. Max Myers and his Biometrics Research and Analytic Studies Section conduct research in cancer etiology, screening for early detection, prognosis and in statistical methodology related to each of these areas. Some of this work is carried out through the use of data from the SEER Program, others involve the use of data from other sources, while still others require study design and collection of data from specific populations. Some of the activities of this group over the last year include:

1) A large collaborative study on the long-term effects of cancer and its treatment on patients who reach adulthood after having cancer during childhood years is being carried out with five major institutions. The data collection phase will be completed shortly. Most of the computer software has been completed for data processing and analyses will be carried out during the coming year.

2) A good deal of work has been carried out using the system developed by this section for study of multiple primary cancers. Most of the analyses carried out thus far have used Connecticut data and have focused on specific second cancers following the diagnosis and treatment of specific first cancers. Further study is now being carried out using data from the SEER Program beginning with all leukemias diagnosed as second cancers and analyzing the distribution of first cancers looking for factors that are common among them.

3) Based on the suggestion from the National Bladder Cancer Study of an increased risk of lower urinary cancer among truck drivers, a study was begun of truck drivers and other motor exhaust related occupations from the pooled dataset of the National Bladder Cancer Study.

4) Detailed analyses of trends in cancer incidence and mortality, using five geographic areas for which data were available from the late 1940s to the

present time, have been carried out. These revealed large increases in incidence of lung cancer among both men and women, decreases in stomach and uterine cervix cancer and equivocal findings for both breast and uterine corpus cancer, particularly in view of peaks in incidence for both of these sites in the mid-1970s. Further analyses of these trends are now being carried out.

5) Work is continuing in the area of screening for early detection of cancer for a number of sites. This includes the breast cancer screening project, being carried out among members of the Health Insurance Plan of Greater New York, lung cancer among uranimum workers in Colorado, and a five-country cervical cancer study, being carried out in collaboration with the International Agency for Research on Cancer.

6) A large number of other studies are being carried out, including analysis of time to recurrence with soft-tissue sarcoma, comparison of characteristics of colon cancer patients diagnosed in the SEER Program with those diagnosed through the comprehensive cancer centers, analysis of the cancer experience of the older age population in the U.S., a study comparing rates of carcinoma in situ of the cervix with those of invasive cancer of the cervix for nine geographic areas, development of the theory for evaluation of screening programs using stochastic modeling methods and the use of hospital versus population controls in analyzing coffee-drinking habits.

International Studies

A descriptive analysis of a comparison of cancer incidence data among the Chinese populations in the United States, the Chinese populations in Hong Kong and Singapore with those of Shanghai and perhaps one other area in China is continuing. The focus is on the kinds of inferences that can be made from observed vast differences in incidence rates for specific cancer sites among these populations, given the fact that the Chinese populations in the United States do not originate primarily from the same areas for which incidence data are available in China. Cancer mortality data, which are more extensively available, are being used as a possible link to help explain these differences. Dr. Tu Ji-Tao, an epidemiologist from the Shanghai Cancer Registry, is one of the collaborators in this analysis. He returned to Shanghai in February 1982 after spending a year in the Biometry Branch.

A pilot study of the incidence of stomach, colon and rectal cancer among Puerto Ricans in New York City compared with those in Puerto Rico is now underway. The impetus for this study stemmed from earlier findings from a mortality analysis, which suggested that mortality rates for cancer of the colon among Puerto Ricans in New York City remained close to the level of the low colon cancer rates in Puerto Rico. This is divergent from findings of other migrant studies which suggest that colon cancer rates tend to rise fairly rapidly to the level of those in the host country. Pending the outcome of this pilot study, a case-control study will be initiated to identify possible factors associated with these studies.

Skin Cancer

Extensive studies of the incidence of nonmelanoma skin cancer in the United States is being carried out by Joseph Scotto and Thomas Fears. The focus of

this study has been to clarify a relationship between non-melanoma skin cancers and ultraviolet radiation. Ten geographic areas were selected for study on the basis of the variation among them in the extent of ultraviolet exposure of the population. An increase in the incidence of basal cell carcinoma of the skin among Caucasians in Minneapolis-St. Paul and San Francisco-Oakland was noted but no significant increases in ultraviolet radiation or decreases in stratospheric ozone depletion were detected during the time period under consideration. Models are being developed based on the data generated by this study to determine whether ultraviolet radiation may be acting as a promoter as well as an initiator. This will be carried out using data recently obtained for New Hampshire/Vermont and San Diego.

Computer Science

The Computer Science Section, with Mr. J. Michael Stump as Acting Chief, is primarily responsible for the basic computer support needed to carry out the work of the Biometry Branch. This is carried out with the assistance of a large computer support contract for computer programming and systems analysis. Through a long and involved set of negotiations a new contractor was selected, Operations Research, Inc. (ORI), and the section has had its hands full in bringing the level of competence of this group up to the point where it can provide useful support to the Biometry Branch program. This is now beginning to happen. The software development was completed for the computer system for the Connecticut Tumor Registry. The final testing is expected to take place this summer and the registry should then be ready to begin parallel operation until the new system is fully functional. The section will adapt some parts of this system for use in the California Tumor Registry. The Computer Science Section has also now begun to help explore ways of sharing computer software among the registries in the SEER Program in an attempt to increase efficiency of operations and reduce computer costs. The section is also involved in basic computer support for a number of other activities in the Biometry Branch, elsewhere in Field Studies and Statistics, and in other parts of NCI.

Plans for the Immediate Future

The results of the cost study of the SEER Program and recommendations for cost reduction in a number of registries and the introduction of the systematic uniform cost reporting system will be presented to the DCCP Board of Scientific Counselors in September 1982. Plans are also being made to add another registry to the SEER Program to correct for the deficiency in coverage of the black and Hispanic populations. It is hoped that this can be accomplished through the addition of a single registry in an area that has a large number of both blacks and Hispanics. Prime candidates would be New York City, Los Angeles, Chicago, Miami and some areas of Texas. It is planned to do this through a competitive request for proposals and selection of the area which best meets the criteria established for this registry.

Intensive work is now being carried out to analyze the extent of disease and treatment information in the SEER data. This will then serve as background for the detailed monograph on cancer patient survival which will be produced from the SEER Program during the 1983 fiscal year.

Specific effort will be devoted to developing a better understanding of the differences in survival between black and white patients. Initial studies, for which planning has already begun, will focus on cancers of the bladder and uterine corpus, sites for which differences between the races are the greatest.

The pilot study of cancer of the stomach, colon and rectum among Puerto Ricans in New York City is focusing on whether the low mortality from colon cancer among this group is also supported by the incidence data, and whether this might be explained by possible artifacts. If that is not the case, then a case-control study will be initiated in an attempt to understand reasons why this result differs from that of other migrant studies.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04254-08 B

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)
Cancer Surveillance, Epidemiology, and End Results Reporting (SEER) Program

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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COOPERATING UNITS (if any) U of Calif., San Francisco; Conn. Dept. of Health; Fred Hutchinson Cancer Res. Ctr., Seattle; Res. Corp. of the U. of Hawaii; U of Iowa; Mich. Cancer Found.; N. Calif. Cancer Pgm; U of New Mexico; U of Utah; Yale Univ; Emory Univ; ORI, Inc.; C'wealth of Puerto Rico Dept. of Health

LAB/BRANCH
Biometry Branch

SECTION
Demographic Analysis

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
13	10	3

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Data on cancer patients diagnosed from year of entry into the SEER Program through 1980 (1973-80 for most participants) were submitted to the NCI by the ten participants in December 1980. Analysis of cancer incidence revealed considerable variability across all areas and among the various ethnic groups represented therein. Cancer mortality rates for whites for the total SEER Program are virtually identical with those for the entire U.S. Incidence data from the first five years have been published in NCI Monograph 57, and over 7,000 copies have been distributed throughout the world. Analysis of cancer patient survival experience has begun. The five year relative survival rate based on patients diagnosed 1973-79 was 47% for all cancers combined. There was considerable variation from site to site. Also, in general, females experience greater survival rates than males and whites had better survival rates than blacks. There was also some variation among the geographic areas. A monograph detailing cancer patient survival experience is being developed.

Project Description

Objectives:

To provide descriptive data on cancer incidence and patient survival for defined populations; to investigate variation in risk of specific forms of cancer by region, place of residence, age, sex, race/ethnicity, socioeconomic status. Based on analyses of differences among subgroups, to develop hypotheses concerning etiology for further study; to evaluate patient survival for trends over time, taking into account changes in the distribution of extent of disease and type of treatment, and to compare survival among areas and among population subgroups to identify variables that might be related to variation in survival rates. To promote specialty training in epidemiology, biostatistics, and tumor registry methodology, operation and management.

Methods Employed:

The incidence and survival data are obtained from a group of ten population-based cancer registries covering all cancers diagnosed in the populations of five entire states (Connecticut, Hawaii, Iowa, New Mexico, and Utah), four metropolitan areas (Atlanta, Detroit, San Francisco, and Seattle) and the Commonwealth of Puerto Rico. Data were collected previously from New Orleans, Louisiana but the program has been discontinued in that area. For each case of cancer diagnosed in these areas, information is obtained from hospital records on age, sex, race/ethnicity, place of residence, site of cancer, histologic type, extent of disease, type of treatment. Each alive case is followed at least once a year after diagnosis or date of last contact to determine vital status. A complete updated tape is submitted to NCI each year from each registry; it contains data on all cases diagnosed during the preceding year and follow-up data on all previously reported cases for the latest year.

In order to assure complete, accurate and comparable data among the population-based registries supplying data to the SEER Program, an extensive program of training and quality control procedures is employed. Training is carried out in a variety of ways depending on the need and those being trained. Periodic workshops, attended by representatives from all areas, are conducted to introduce new procedures or to reinforce existing data abstracting and coding rules and conventions. Other workshops are conducted at a local level to train new employees or to upgrade the level of abstracting by hospital tumor registrars who contribute to the central registries. Completeness of reporting audits consists of matching of cases identified from a variety of sources, such as pathology lists, radiotherapy rosters, autopsy reports, oncology department patient files, etc., against the registry file to determine completeness of case finding. Accuracy and comparability are evaluated by reabstracting and/or recoding a sample of the cases, and checking these against the codes on file. Differences are investigated and corrective procedures instituted. Performance reports are given to each participant.

Extensive use of computer edit programs is employed on the data submitted to NCI. This includes a check to ensure that all codes are within the bounds specified. Items within the abstract are compared, such as a date of birth with age, sex with specific site, site with histologic type, etc. For patients who had more than a single cancer, items between extracts are compared to ensure consistency. These edit programs produce printouts, presenting the entire case as well as a message as to the item in question, which are sent back to the registry for resolution.

The registries serve as bases for research projects conducted by the staffs of the individual registries. Once these projects are designed, they are usually carried out through funds obtained from sources other than the basic SEER contract. Since the registries provide complete coverage of all cancers in defined populations, they are an ideal resource for carrying out collaborative case-control studies to test the impact of suspected etiologic factors for specific forms of cancers. NCI-initiated studies are carried out by the Biometry Branch, the Environmental Epidemiology Branch and/or Clinical Epidemiology Branch through contracts with the SEER registries.

In addition, mortality data are obtained from the National Center for Health Statistics (NCHS) so that cancer incidence and mortality rates for the SEER areas can be compared and so that cancer mortality rates for the SEER areas can be compared to those for the United States as a whole. Mortality data for the Commonwealth of Puerto Rico are not available through the NCHS but must be obtained directly from the Department of Health in Puerto Rico. Normally, NCHS releases mortality data for the previous calendar year in late October. However, this year mortality data for 1979 has not yet become available in machine readable form and may not be released until late September. There is every reason to think that similar delays will continue into the future. Thus, incidence data for a given calendar year will be available for analysis one to two years prior to mortality data.

Population estimates of the various geographic areas are obtained from a variety of sources. Estimates by five-year age groups for whites are available for every county in the United States through 1979. However, complicated statistical procedures must be applied to estimate population for Hispanics, blacks, Chinese, Japanese, Filipinos, Hawaiians and American Indians. The accuracy of these procedures diminishes the further the year from the 1970 census. Corrections to population estimates can be applied once data from the 1980 census are released. However, every indication is that these data will not be available until well into the next fiscal year. In the interim, incidence and mortality rates for 1978 forward for races other than whites should be interpreted with extreme caution.

Major Findings:

Data for cases diagnosed between January 1, 1973 and December 31, 1980 were submitted to NCI in December 1981. One participant (Seattle) submitted data only for 1974-1980; one (Atlanta) submitted data only for 1975-1980. While it is felt that all areas have complete reporting for 1979, data for 1980 may be incomplete. In addition, survival data for patients diagnosed between January 1,

1973 and December 31, 1979 are available through at least December 31, 1980. There was considerable variation among the participants with respect to the percent of patients not known to be dead who were actually followed into calendar year 1981 or 1982. Three of the ten participants had rates which were unacceptably low. As a result major efforts were undertaken to improve this deficiency and two of the three submitted additional data in April 1982 to bring their percentages to an acceptable level.

A subcommittee of the Division's Board of Scientific Counselors suggested that passive follow-up measures might be a suitable alternative to collecting cancer patient survival data on an active basis. During this fiscal year two tests were conducted to attempt to determine whether passive follow-up might be suitable.

The first test consisted of comparing survival rates based on active follow-up measures with those calculated based only on deaths obtained through State vital statistics records. All persons not recorded as having died by the State Office of Vital Statistics were assumed to be alive regardless of the date of last patient contact. Survival rates so calculated were dramatically higher than survival rates calculated based on active patient follow-up procedures, especially in those areas with good active follow-up programs in place. In areas with poor active follow-up systems, rates calculated by the two methods were somewhat closer, but rates based solely on passive follow-up techniques still overestimated the true survival rate.

The second test of the acceptability of passive follow-up as an alternative to collecting patient survival data was conducted in cooperation with the NCHS who are developing a National Death Index (NDI). Two SEER participants submitted rosters of patients to be matched to the NDI to determine patient vital status. Results revealed that the NDI could identify a maximum of three percent of patients who were not known to have died by the local area. Conversely, however, the local area was able to identify eight percent of patient deaths which were not picked up by the NDI. This was due primarily to the very rigid matching criteria of the NDI, most notably an exact match on the date of birth of the deceased. Thus, until the matching algorithm of the NDI is changed, cancer patient survival based solely on a passive match to that index would also overestimate cancer patient survival.

Another area of concern to be considered during the year was the appropriate measure of cancer patient survival. The traditional calculation was that of the relative survival rate in which the observed patient survival rate was "corrected" for normal life expectancy. In order to evaluate the relative survival rate, observed survival rates were calculated in which deaths due to causes other than cancer were treated as "withdrawals" rather than "failures." These rates were then compared to the corresponding relative survival rates for five major anatomic cancer sites. In each instance the observed survival rate based solely on cancer deaths was virtually identical to the corresponding relative survival rate. Thus, the relative survival rate does seem to be a good measure of survival corrected for normal life expectancy and will be the survival indicator which the Program will continue to produce and monitor for changes over time.

In calculating patient survival, the percentage of patients lost to follow-up, i.e., those patients whose vital status is not known beyond some previous point in time, must be relatively low in order to yield meaningful results. Since SEER participants are required to actively follow all cancer patients, one might assume that a patient who has been lost to follow-up is more likely to be alive than dead since it is easier to certify that someone is dead rather than alive. If all patients not known to be dead through active follow-up are assumed to be alive at the end of the last calendar year of reporting, an "optimistic" relative survival rate can be calculated. For the SEER participants based on patients diagnosed 1973-78, this rate was 50% versus a relative survival rate of 47% calculated by the traditional method.

For SEER participants, a decision was made that a minimum of 70% of patients not known to be dead must have been contacted during the last calendar year of observation in order for those participants to be included in the calculation of survival rates. As of May 1981 all participants except Puerto Rico have achieved that level as is shown in table 1.

Table 1

Percent of alive cases by year of diagnosis which have been followed into 1980-81, SEER Program, April 1982 submission.

Area	Year of Diagnosis						
	1973	1974	1975	1976	1977	1978	1979
San Francisco	84	81	75	71	71	73	82
Connecticut	71	74	77	79	80	82	85
Detroit	76	78	78	81	83	85	85
Iowa	78	77	81	82	85	88	90
Hawaii	81	86	86	89	89	91	92
New Mexico	76	77	78	79	81	84	84
Seattle	--	71	75	80	81	85	88
Utah	73	78	80	80	84	88	90
Atlanta	--	--	78	81	87	88	92
Puerto Rico	24	23	26	26	20	34	43

In order to calculate relative survival rates, life expectancy in the general population must be known. Life tables are available from the NCHS for whites and other than whites for five-year time periods through 1975. Rates for blacks, Hispanics, Chinese, Japanese, Filipinos, Hawaiians, and American Indians are not available. Therefore, these life tables must be constructed by NCI using mortality tapes available for the total United States and appropriate population (census) data. The unavailability of census data is discussed below. Since appropriate life tables for minorities are not currently available, current analyses of survival data are limited to survival for white patients and a more cautious analysis of data for black patients using as life expectancy the experience of all other-than-white (formerly referred to as non-white) persons.

Five-year relative survival rates for white patients are shown in table 2 for the most common anatomic sites for males and females separately. With the exception of bladder cancer, white females had a higher survival rate than white males for each anatomic site found in both sexes. The much higher overall relative survival rate for females (54% versus 39%) is reflective of the very good survival for breast and corpus cancer versus the very poor survival due to lung cancer which occurs four times more frequently in men than in women. Thus any survival rate based on all cancer sites combined must be interpreted against a knowledge of the relative frequency of the various anatomic sites on which the overall rate was based.

Table 2

5-Year Relative Survival Rates (%)
White Patients, SEER, 1973-79

Site	Males	Females
All Sites	39	54
Stomach	12	14
Colon	47	49
Rectum	44	47
Pancreas	3	2
Lung	10	14
Breast	--	72
Cervix Uteri	--	66
Corpus Uteri	--	87
Prostate	64	--
Bladder	72	69
Non-Hodgkins Lymphoma	42	43

Although the major emphasis of data analysis during the year has been devoted to survival data, the production of incidence and mortality data continues to be an important function and goal of the Program. Incidence rates for the years 1978, 1979 and 1980 are currently available by race, sex and anatomic site for each of the geographic areas participating in the Program. Data for 1978 and 1979 are felt to be relatively complete while that for 1980 could be as much as five percent underreported based on the experience from previous submissions. The major difficulty in producing valid incidence rates at this point in time is the lack of appropriate population denominators from the 1980 census. To date of the ten areas included in the Program census data are available in a usable format for only three areas--Connecticut, Iowa, and Utah. Data for whites and blacks are also available for Hawaii, but data for the other racial groups (Chinese, Japanese, Filipinos, and Hawaiians) are not yet forthcoming. Thus, except for the three areas mentioned, incidence rates for 1978 forward are based on projected populations and are given to the general public with great caution.

Indicative of the problems associated with the calculation of valid incidence rates for racial and ethnic minorities are the problems associated with the census itself. For example, in New Mexico there was a drop of 18% in the white population with a corresponding increase in the other-than-white population between the 1970 and 1980 censuses. This occurred because persons who considered themselves to be Hispanic did not self-classify themselves as whites in the census questionnaire but rather indicated their race as "other." Since many American Indians also have Hispanic names and heritages, a detailed cross tabulation between the race and Hispanic ethnicity questions must be conducted before the appropriate denominators for the white population of New Mexico can be ascertained. These data are not yet forthcoming.

Difficulty has also been experienced in obtaining United States mortality data from the NCHS. Data for the calendar year 1979 will not be released to the general public before September 1982 and for 1980 until September 1983. Thus, mortality data are not available on a timely basis, lagging at least two years behind corresponding incidence data. In order to have some information regarding mortality experience, mortality data for the SEER areas for 1979 were obtained from the Office of Vital Statistics of the various states. These data will be compared with 1979 data eventually obtained from the NCHS to determine whether there are differences between the state and national data for each SEER area. If no significant differences exist, then state data can be obtained in a more timely fashion so that NCI will not be dependent on the NCHS for estimates of the cancer mortalilty experience in SEER areas. However, data for the entire United States must still be obtained directly from NCHS.

Significance to Biomedical Research and the Program of the Institute:

Continuing information on both cancer incidence and patient survival is essential so that the nature and magnitude of the cancer problem can be determined and changes over time can be assessed. As an important step toward prevention, continuing analyses of variations in cancer incidence across subgroups of the population can lead to specific etiologic hypotheses for further testing. This in turn may lead to the identification of risk factors which can be brought under control. Through continuing analyses of survival data, the Program can provide important clues to improved treatment methods. The maintenance of population-based cancer registries provides a data base against which some of the major programs of the Institute can be assessed. For example, the impact of specific cancer control programs can be measured to determine the extent to which these programs are meeting their stated goals. Data from cancer centers can be compared with the population-based data to determine in what ways cancer center patients and the treatment they receive differs from that of the general population.

Since the SEER Program identifies all cases of cancer diagnosed in defined populations, it is a valuable resource for case-control studies to identify possible etiologic agents. A major advantage is that case-finding techniques are already in place, and it is possible to modify them to permit very early identification of the cancers of interest so that patients, rather than proxies for the patients, can be interviewed. Furthermore, population controls can be used, thus avoiding a number of problems posed by the use of non-cancer hospital controls. Two major studies of this type have been carried out in most of the

SEER locations. One was aimed at studying the relationship of bladder cancer and the use of artificial sweeteners and the other was attempting to assess the impact of ozone depletion on the occurrence of non-melanoma skin cancer. The use of the SEER mechanism to study the relationship between the use of oral contraceptives and cancers of the breast, ovary and endometrium is now underway.

The Program serves as the major data base for cancer incidence and mortality data in the United States. Data are given to other government agencies, the American Cancer Society, the World Health Organization, the International Agency for Cancer Research, Congressional Offices and the general public. In addition to those requests received by the NCI Office of Cancer Communication, the Demographic Analysis Section received 181 telephone requests for data during the period October 1, 1981 through May 31, 1982 for an average of six telephone requests for data per week. In general, these requests were from the news media and the general public and could be answered with data already available. Without the SEER data base, it would be impossible to respond to these telephone inquiries. It is estimated that 15% of professional staff time is devoted to responding to either verbal or written requests for data. In addition, 7,000 copies of NCI Monograph 57 have been distributed to medical institutions and researchers throughout the world.

Proposed Course:

1. Currently a detailed analysis of the cost of the SEER Program is being conducted. Each contractor is being visited by the same group of individuals to determine both the cost of the program to NCI as well as the total cost of the Program based on costs contributed by the local contractor, local hospitals, other federal and state programs, etc.

After detailed cost data have been gathered and analyzed, standards of performance and costs will be developed. Already areas of cost reduction have been identified, and individual contractors have been directed to reduce the scope of their operation as well as the size of their staff. It is anticipated that the cost of the Program may be reduced by as much as 10%. However, this reduction will almost assuredly be offset by inflation. Ultimately, new cost reporting procedures will be initiated so that yearly costs of the Program can be more closely monitered by NCI staff as well as contract staff.

2. A detailed analysis of the cost of various procedures such as active follow-up, collection of detailed extent of disease data, etc., will be conducted by NCI to determine which areas of the Program could be reduced to bring about cost savings. Already, new abstracting and coding procedures are being developed to reduce the amount of data to be encrypted regarding cancer stage. Every effort is being made to ensure that enough data are being collected to allow comparability with the past as well as compatability with staging schemes of the American Joint Committee. New coding schemes will be introduced for the 1983 diagnosed cases.

3. An examination of data processing costs has lead to the purchase by several participants of dedicated computer hardware. An analysis of the need for such equipment for other contractors is being considered in order to keep the ultimate costs of data management at an acceptable level. NCI will continue to work with the participants in upgrading and maintaining data management systems appropriate to the local operating environment of the individual participant.

4. In order to supplement the coverage of the SEER Program with respect to the cancer experience of all minority groups, the Program will be expanded to include one additional participant. This expansion will most likely take place through a competitive procurement. Populations eligible for consideration must consist of large numbers of black and Hispanic individuals. It is not anticipated that such an expansion of the Program can take place within existing budget limitations. However, the need for greater coverage of the black and Hispanic populations is apparent.

5. A publication detailing the cancer patient survival experience of white and black patients diagnosed 1973-79 is underway. A detailed monograph containing the survival experience of all racial and ethnic groups and containing data on stage of disease at diagnosis and treatment modality is planned for fiscal year 1983. As appropriate mortality data and appropriate census data become available, 1979 and 1980 incidence data will be released to the public. Incidence data for 1981 should be available in March 1983.

6. An exhibit of available data along with handouts of various SEER publications will be displayed at the XIII International Cancer Congress in Seattle.

7. To assist in the tumor registry training program and to provide guidance to SEER Program participants and others concerned with cancer registration, a number of publications have been developed and distributed. These include manuals that set out clear guidelines for collecting and coding the data to be submitted to NCI, as well as books that can be used for on-the-job training of personnel responsible for carrying out the functions required for data abstracting and coding. The demand for these publications has been large and mailings have been made to a variety of medical personnel and facilities. A new book documenting antineoplastic drugs has been at the Government Printing Office since November 1981 but has not yet been printed because of a government-wide moratorium on the printing of new materials. Earlier books on anatomy and extent of disease have been revised. Additional books on statistical and epidemiological methodology and computer assistance for the tumor registrar are being readied for the printer.

Publications:

Berg, J.W., Percy, C., and Horm, J.W.: Recent Changes in the Pattern of Occurrence of Oat Cell Carcinoma of the Lung. In Magnus, K. (Ed.): Trends in Cancer Incidence. Washington, D.C., Hemisphere Publishing Corporation., 1982, Part 4, 215.

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Swanson, G.M., Belle, S.H. and Young, J.L.,Jr.: U.S. Trends in Carcinoma of the Cervix - Incidence, Mortality and Survival. In Hatez, E.S.E. and Smith, J. (Eds.): Carcinoma of the Cervix. Martinus-Nijhoff, The Hague, Netherlands. In Press.

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Young, J.L.,Jr. and Ries, L.A.: The Use of Descriptive Epidemiology in Cancer Control. In Mortenson, L., Anderson, P., and Engstrom, P. (Eds.): Proceedings of the Progress in Cancer Control Conference. Alan R. Liss, Inc., New York. In Press.

CONTRACTS IN SUPPORT OF THIS PROJECT:

CALIFORNIA, UNIVERSITY OF, SAN FRANCISCO (NO1-CP-11004)
COMMONWEALTH OF PUERTO RICO (NO1-CP-43386)
CONNECTICUT STATE DEPARTMENT OF HEALTH (NO1-CP-61002)
EMORY UNIVERSITY (NO1-CP-61027)
FRED HUTCHINSON CANCER RESEARCH CENTER, SEATTLE (NO1-CP-61059)
HAWAII, RESEARCH CORP. OF THE UNIVERSITY OF (NO1-CP-53511)
IOWA, UNIVERSITY OF NO1-CP-43200)
MICHIGAN CANCER FOUNDATION (NO1-CP-61028)
NEW MEXICO, UNIVERSITY OF NO1-CP-33344)
NORTHERN CALIFORNIA CANCER PROGRAM (NO1-CP-21025)
UTAH, UNIVERSITY OF NO1-CP-43382)
YALE UNIVERSITY (NO1-CP-33235)

Title: Surveillance, Epidemiology & End Results (SEER) Program

Project Officer (NCI): John L. Young, Jr., Dr.P.H.

Objectives: To obtain and analyze data on cancer morbidity, treatment, extent of disease, and patient survival from population-based registries; to identify areas for epidemiologic investigations; to initiate preliminary investigations needed to develop epidemiologic study protocols; and to promote specialty training in tumor registry methodology.

Methods Employed: The National Cancer Institute is sponsoring a collaborative program for Cancer Surveillance, Epidemiology and End Results (SEER). Participants in this program are all population-based registries, covering entire states or geographically specified areas, such as Standard Metropolitan Statistical Areas. The Demographic Analysis Section has professional and technical responsibility for supervising these contracts. Basic information is collected on all patients treated for cancer in the area, area residents treated outside the area, and anyone dying in the area whose death certificate mentions cancer--or area residents dying outside the area, whose death certificate mentions cancer. Periodic follow-up information is obtained on the vital status of all those patients in the registries. Data are submitted semiannually to NCI on magnetic tape according to a specified format. These data are available for analyses as well as for providing an extensive resource for special studies.

Core epidemiologic staff are present in most of the SEER Programs. These staff members review the morbidity data to identify epidemiologic research leads that should be investigated. These staff then develop study protocols and field test the study schedules. Funding for such proposals are either through other contract or grant support or financial assistance from other sources.

Significance to Biomedical Research and the Program of the Institute: Information on incidence, survival and mortality from a 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 mechanisms, should lead to the identification of controllable

risk factors. Such research is an integral part of the ultimate goal of the national program, i.e., to reduce the occurrence of and mortality due to cancer. Continued recording of cases will also provide a data base that could be useful in the evaluation of cancer control activities. These data could provide the base-line against which changes resulting from specified cancer control programs could be measured to determine the effectiveness of these programs in reaching their stated goals. A system of record linkage between the cancer registry and employees of specific industries or specific types of occupations would provide early warning of possible occupational carcinogenicity.

Major Findings: Data for cases diagnosed between January 1, 1973 and December 31, 1980 were submitted to NCI in December 1981. One participant (Seattle) submitted data only for 1974-1980; one (Atlanta) submitted data only for 1975-1980. While it is felt that all areas have complete reporting for 1979, data for 1980 may be incomplete. In addition, survival data for patients diagnosed between January 1, 1973 and December 31, 1979 are available through at least December 31, 1980.

Five-year relative survival rates for white patients are shown below for the most common anatomic sites for males and females separately. With the exception of bladder cancer, white females had a higher survival rate than white males for each anatomic site found in both sexes. The much higher overall relative survival rate for females (53% versus 38%) is reflective of the very good survival for breast and corpus cancer versus the very poor survival due to lung cancer which occurs four times more frequently in men than in women. Thus any survival rate based on all cancer sites combined must be interpreted against a knowledge of the relative frequency of the various anatomic sites on which the overall rate was based.

5-Year Relative Survival Rates (%)
White Patients, SEER, 1973-79

Site	Males	Females
All Sites	38	53
Stomach	12	14
Colon	46	48
Rectum	43	46
Pancreas	2	2
Lung	10	15
Breast	--	71
Cervix Uteri	--	65
Corpus Uteri	--	86
Prostate	62	--
Bladder	69	67
Hodgkins Disease	65	70
Non-Hodgkins Lymphoma	43	45
Leukemia	26	29

Although the major emphasis of data analysis during the year has been devoted to survival data, the production of incidence and mortality data continues to be an important function and goal of the Program. Incidence rates for the years 1978, 1979 and 1980 are currently available by race, sex and anatomic site for each of the geographic areas participating in the Program. Data for 1978 and 1979 are felt to be relatively complete while that for 1980 could be as much as five percent underreported based on the experience from previous submissions. The major difficulty in producing valid incidence rates at this point in time is the lack of appropriate population denominators from the 1980 census. To date, of the ten areas included in the Program, census data are available in a usable format for only three areas--Connecticut, Iowa, and Utah. Data for whites and blacks are also available for Hawaii, but data for the other racial groups (Chinese, Japanese, Filipinos, and Hawaiians) are not yet forthcoming. Thus, except for the three areas mentioned, incidence rates for 1978 forward are based on projected populations and are given to the general public with great caution.

Difficulty has also been experienced in obtaining United States mortality data from the NCHS. Data for the calendar year 1979 will not be released to the general public before September 1982 and for 1980 until September 1983. Thus, mortality data are not available on a timely basis, lagging at least two years behind correponding incidence data. In order to have some information regarding mortality experience, mortality data for the SEER areas for 1979 were obtained from the Office of Vital Statistics of the various states. These data will be compared with 1979 data eventually otained from the NCHS to determine whether there are differences between the state and national data for each SEER area. If no significant differences exist, then state data can be obtained in a more timely fashion so that NCI will not be dependent on the NCHS for estimates of the cancer mortalilty experience in SEER areas. However, data for the entire United States must still be obtained directly from NCHS.

Proposed Course: Intensive effort has been expended to assure that the data being submitted to NCI are of the quality and completeness required for an effective cancer surveillance network. Incidence data are now of high quality. Effort will be expended to improve the quality of data collected for patient survival so that the percentage of alive patients under active surveillance will reach at least 85%. The quality control staff, in cooperation with the training staff of the University of California Medical School in San Francisco, will continue to conduct a program of quality control visits, as well as national and regional workshops, and intensive formal training programs.

To assist in tumor registry training programs and to provide guidance to SEER Program participants and others concerned with cancer registration, a number of publications have been developed and distributed. These include manuals that set out clear guidelines for collecting and coding the data to be submitted to NCI, as well as books that can be used for on-the-job training of personnel responsible for carrying out the functions required for data extraction and coding. The demand for these publications has been large and mailings have been made to a variety of medical personnel and facilities. A new book documenting antineoplastic drugs is awaiting printing. Additional books on statistical and epidemiological methodology and computer assistance for the tumor registrar are being developed. Earlier books on anatomy and extent of disease have been revised.

Incidence and mortality data covering the first five years of the Program were published in the NCI Monograph series and 7,000 copies have been distributed throughout the world. A companion monograph containing data on cancer patient survival classified by race, sex, age, and residence of the patient and by site, type, extent of disease, and treatment is being developed.

	Date Initiated	FY 82 Negotiated	Man Years
California, University of, San Francisco (NO1-CP-11004)	1974	$ 252,984	5.15
Commonwealth of Puerto Rico (NO1-CP-43386)	1968	130,665	14.6
Connecticut State Department of Health (NO1-CP-61002)	1963	850,000	29.5
Emory University (NO1-CP-61027)	1976	841,133	24.4
Fred Hutchinson Cancer Research Center (NO1-CP-61059)	1973	681,185	27.4
Hawaii, Research Corp. of the University of (NO1-CP-53511)	1971	421,084	15.7
Iowa, University of (NO1-CP-43200)	1973	1,405,591	47.0
Michigan Cancer Foundation (NO1-CP-61028)	1973	1,693,166	63.9
New Mexico, University of (NO1-CP-33344)	1973	892,976	33.6
Northern California Cancer Program (NO1-CP-21025)	1982	1,850,000	48.0
Utah, University of (NO1-CP-43382)	1973	497,270	14.9
Yale University (NO1-CP-33235)	1973	359,914	6.0

ISRAEL CENTER FOR REGISTRATION OF CANCER AND ALLIED DISEASES (NO1-CP-33351)

Title: Continued Cancer Registration and Selected In-Depth Analyses

Contractor's Project Director: Dr. Leah A. Katz

Project Officers: Dr. John L. Young, Jr.
Mr. William I. Lourie, Jr.

Objectives: With primary focus upon the effective functioning of the Israel Cancer Registry (ICR) in the collection of high-quality data of interest to NCI's Surveillance, Epidemiology and End Results (SEER) Program; (1) to continue the country-wide registration of cancer patients; (2) to utilize the data in a variety of statistical and epidemiological studies; (3) to acquire additional information from other pertinent record sources for use in formulating or testing hypotheses and in planning for collaborative studies; (4) to aid in implementation of field epidemiological studies including case-control studies which involve cooperation with medical specialists and other organizations; (5) to increase quality control of collected data and its classification by paralleling the methods of the SEER Program; (6) to submit original data to the SEER staff for NCI analysis in addition to local analyses; (7) to attempt recruitment of professional epidemiological personnel and to continue the technological upgrading of processing data in a confidential manner so that ICR's information may be used expeditiously by the health, medical and educational professionals of Israel, as well as epidemiologists throughout the world.

Major Findings: Using other national record sources, the ICR obtains information on country of origin, date of immigration, date and cause of death. Precise therapy details, identification of the cancer patient's demographic characteristics and the primary site, histology, and extent of disease of the tumor are obtained from medical records. An important recent advance has been a more effective liaison with the Population Register so that periodically an updated set of microfiche by name and by register number is received by ICR. This permits a more accurate determination of vital status and of the date of death and, as a result, a reduction in the number of patients "lost to follow-up" and, thus, the more timely production of survival data. In addition, the Population Register is the major source of confirmatory data concerning the origins of the various ethnic or cultural subgroups of the Jewish population as well as other immigrants. Differences in these subgroups in cancer incidence and/or mortality constitute the major epidemiological value of the ICR to NCI.

The present cycle of 14,972 new cases and of 15,956 updates was completed on April 30, 1982. This was the first cycle worked upon on the PDT 11/151 microcomputer. The primary search for known cases and the updating was carried out on disquettes. New cases were entered first on code sheets and keyed in onto separate disquettes. For the first time full use was made of the population register on microfiches. Ascertaining the correct names and adding missing identifying information hopefully prevented a large proportion of duplicate registrations, later corrections and cancellations, and unnecessary correspondence. Unfortunately, the date of death was mistakenly deleted from the first set of microfiches provided by the Population Registry.

Data on epidemiology of cancer in Israel were repeatedly provided to the Division of Public Health, to the Ministry's Speaker, to the Department of Foreign Relations and to the Director General. Part of this was due to the growing interest of the public and of the media in cancer prevention, and to several current issues, such as geographic differences in the incidence of cancer and certain occupational exposures.

Significance to Biomedical Research and the Program of the Institute: The Israel Tumor Registry has incidence data available from 1961 forward. In December 1980, a data tape containing information on 102,582 cases diagnosed between January 1, 1961 and December 31, 1978 was submitted to NCI. These cases are being converted to the SEER Program codes and format for analysis and comparison purposes. Of immediate interest are comparisons of incidence rates for various ethnic groups residing in Israel (including migrants from the United States) with rates for ethnic groups in the United States; a comparison of U.S. and Israel histologic patterns of cancer incidence within primary site groups; and a comparison of trends in cancer incidence in the two countries. While these analyses are being conducted by NCI, contract staff in Israel (in addition to maintaining the day-to-day operations of the registry) will devote themselves to several detailed analyses: cancer incidence in Israel by geographic area (district), cancer incidence in second generation immigrants, and the analysis of multiple primary tumors. It is now anticipated that these and other analyses will be published as an NCI or an IARC publication (monograph).

Proposed Course: Funding is being planned through March 1984. This should allow sufficient time to complete the above analyses and to prepare a monograph. Currently, 65% of the costs of the Israel Tumor Registry are funded through NCI. During this funding period, the Registry is being encouraged to find other sources of funding.

Date Contract Initiated: June 28, 1973

Current Annual Level: $137,500

Man Years: 10.3

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04257-25 B

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)
Studies of Etiology, Prognosis and Screening for Early Detection of Cancer

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	M.H. Myers, Chief,	Biometric Research & Analytic Studies Section,	BB NCI
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COOPERATING UNITS (if any)
Health Insurance Plan of Greater New York;
ORI, Inc.; Memorial Hospital for Cancer and Allied Diseases

LAB/BRANCH
Biometry Branch

SECTION
Biometric Research and Analytic Studies Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
14.5	10.5	4

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Continued surveillance of the occurrence of malignant mesothelioma using SEER data for 1980 substantiated an earlier finding of increased incidence during the 1970s. Elevated risk of bladder cancer has been observed in truck drivers, taxicab drivers, bus drivers, deliverymen and tool and die makers. Breast cancer patients treated with adjunctive radiotherapy were at increased risk of developing a second breast cancer after 10 years of survival. Excess risk of leukemia was observed among uterine corpus patients who received radiotherapy. The five-year survival rate for acute lymphocytic leukemia (ALL) among children diagnosed during 1973-75 in SEER areas was 50 percent. The increase in 3-year survival rates (59% to 71%) for childhood ALL from 1973-75 to 1976-77 was statistically significant. The survival advantage of white over black patients with cancer of the uterine corpus was sustained even after adjustment for histologic type (sarcoma vs. adenocarcinoma), age and stage. Parity was found to be an important prognostic factor in young (less than 36) breast cancer patients with survival inversely related to number of children. Research in theoretical statistical modeling has led to a procedure for determining the appropriate frequency of screening for use in trials of methods for early detection of cancer.

Project Description

Objectives:

To conduct research in cancer etiology, screening for early detection, prognosis and in statistical methodology.

To provide consultation to other divisions of NCI, to other institutes of NIH and to nongovernmental groups on collection and analysis of data on human populations, on design and analysis of special studies.

Methods Employed:

A wide variety of statistical procedures and principals are used for designing studies and analyzing results. Some of these are standard techniques, while others are developed for handling specific conditions dictated by the subject matter.

Major Findings:

1. Previous analyses of malignant mesothelioma cases ascertained during the Third National Cancer Survey (1969-71) and by the SEER Program (1973-79) indicated that the incidence of this disease had increased significantly over time among white males. Continued surveillance (SEER data for 1980) adds support to the earlier finding. However, the magnitude of the apparent increase is difficult to establish. The histopathology of mesothelioma can resemble benign lesions as well as other cancers, and the effect of misclassification on population-based incidence data and on projections of the future burden of mesothelioma cases is unclear. A large epidemiologic study of mesothelioma cases is now underway. This study will provide additional data on the secular trend in mesothelioma incidence in two large population-based registries outside of the SEER Program. Inherent in assessing this trend is confirmation of the diagnosis by pathology review. The role of asbestos and other industrial materials in the etiology of the disease will also be studied.

2. Analysis of the Detroit occupational data from the National Bladder Cancer Study has indicated a significant increased risk of lower-urinary-tract cancer among truck drivers. A significant trend in risk with increasing duration of employment as a truck driver was also apparent. These findings, in conjunction with increased risks observed among taxicab drivers, bus drivers, and deliverymen, warranted further investigation. Therefore, a study of truck drivers and other motor exhaust-related occupations from the pooled dataset of the national study was begun in collaboration with Dr. Robert Hoover and Dr. Thomas Mason of NCI and Dr. Marie Swanson of the Michigan Cancer Foundation. Also noted was a suggested elevated risk of bladder cancer among tool & die makers. This finding was provocative and merited further investigation in the pooled dataset from the national study. A study of the tool & die makers, other metal machinists, metalworkers, as well as the industries in which these occupations are prevalent (e.g., metalworking machinery industry) was begun in collaboration with Dr. Robert Hoover of NCI, Dr. Marie Swanson of the Michigan Cancer Foundation and Dr. Elaine Smith of the University of Iowa.

3. Data from Atlanta, Detroit, San Francisco, Iowa and Connecticut were used to assess the trends in cancer incidence and mortality among white men and women from the late 1940s to the late 1970s. Large increases occurred in lung cancer incidence among men and women. Smaller increases were observed for lymphomas. Cancers of the bladder, kidney, and prostate exhibited increasing incidence rates. Breast and uterine corpus cancer incidence peaked in the mid-1970s, but the mortality rates did not show such fluctuations. Large decreases in rates for cancer of the uterine cervix were observed and the decreases for stomach cancer were the largest of any site. The data suggest that declines in stomach cancer incidence and increases in pancreatic cancer may be diminishing in recent years.

4. Work has continued on the study of multiple primary cancers utilizing historical data from the Connecticut Tumor Registry (CTR) and SEER data. The development of computer software has been completed. During the past year the focus has been on studying the multiple primary experience of cancer patients having first primaries of the breast, uterus, cervix, salivary gland, and thyroid utilizing the Connecticut historical data. Findings from these studies include the following. For patients with an initial breast cancer, second breast cancer risk was found to be inversely related to age and directly related to stage and calendar period of diagnosis of the first breast cancer. For patients diagnosed with a first breast cancer during 1960-73 there was suggestive evidence that the risk of second breast cancers 10+ years subsequent to diagnosis of the first breast cancer was somewhat elevated (relative risk of 1.4) for those patients who had both surgery and adjunctive radiotherapy for their first breast cancer as compared to those patients who had only surgery. Patients with a first cancer of the uterus who were treated with radiation were found to have a significant excess of second leukemias relative to those patients treated with surgery alone. Virtually all of the excess occurred for patients diagnosed since 1965. Patients with a first cancer of the cervix who were treated with radiation were found to have an excess of second leukemias as well as an excess of cancers of the lung, bladder, kidney, and genital organs. The second primary experience of patients with salivary gland cancer was studied in an attempt to verify the previously reported high excess risk of second breast cancers; however, no excess of second breast cancers was found. The second primary experience of patients with a first thyroid cancer was studied in an attempt to uncover etiologic clues for this disease. Significantly elevated relative risks were found for second cancers of the breast, pancreas, and kidney. A study of second leukemias following the occurrence of selected first cancers generally treated by radiation therapy and/or chemotherapy is currently underway utilizing the SEER data.

5. Further improvements in survival were observed among white children under age 15 years diagnosed with acute lymphocytic leukemia (ALL) in the nine SEER areas between 1973 and 1977. Statistically significant differences at the 0.05 level were noted between the 3-year survival rates for children diagnosed in 1973-75, 59%, as compared to those diagnosed in 1976-77, 71%. The 5-year survival rate for those diagnosed with ALL in 1973-75 was approximately 50%, which exceeded the rate reported previously for those children diagnosed with ALL in End Results Group hospitals during the period 1970-73 (34%). As previously observed, children under age five years diagnosed with ALL had better

prognosis than children aged 5 through 14 years. There were too few black children diagnosed with ALL in the SEER areas in 1973-77 to permit the analysis of racial differences. Improvements in survival between 1973-75 and 1976-77 were not observed for children diagnosed with acute non-lymphocytic leukemia (ANLL) in the SEER areas, with rates of 39% and 38%, respectively, at three years. These rates did exceed the rate reported previously for the End Results Group hospitals in 1970-73 of 21%. Survival at five years for those diagnosed in 1973-75 with ANLL was 32%.

6. The survival advantage of white over black females with uterine corpus cancer was demonstrated with two similar patient series diagnosed in 1950-73 under the End Results Group Program and 1973-75 under the SEER Program. In both series, the distribution of age at diagnosis, stage of disease, and histologic type favored the whites. Higher percentages of white females were diagnosed under age 55 years, with localized disease, and had tumors classified as adenocarcinomas. Statistically significant differences in the survival experience of white to black females were found for both age groups and at various stages, with the localized patients having better prognosis than those with more advanced disease. Within each racial group those diagnosed with adenocarcinomas had better prognosis than those with sarcomas or other histologic types but, in general, whites with sarcomas and other types had better survival than blacks with adenocarcinomas. Trends were examined for the first patient series, comparing 1950-64 and 1965-73. Statistically significant increases in 5-year relative survival rates were observed for both races in both age groups, for all histologic types, and for all stages combined and localized disease. Adjustment of survival rates for these factors, plus geographic area, narrowed the gap, but did not greatly diminish the significant differences between the two groups. Socioeconomic data for the 1973-75 group did not appear to have a significant effect on length of survival.

7. Study of long-term follow-up of cancer patients has shown that for some forms of cancer normal survival expectation is virtually achieved for some subgroups of patients. The rate of approach to normal expectation was rapid for patients with cancers of the uterine cervix, uterine corpus or colon. For breast cancer, a much more gradual increase was observed. Furthermore, even after 20 years, breast cancer patients with localized disease seemed to only achieve 90 percent of expected survival. For lung and prostate patients, the maximum levels seemed to be of the order of 80 and 75 percent, respectively, of normal. Thus, the chronic nature of cancer has been again observed and it is clear that there is no single number of years survived that can be referred to as the break point for "cure". The maximum level and rate of approach to normal survival expectation is a function of cancer site, age, stage at diagnosis and perhaps other factors.

8. Findings to date in the Health Insurance Plan Study strongly suggest the usefulness of the annual screening for breast cancer using clinical examination plus mammography. Over a 14-year period of follow-up, the study group has about 30 percent less mortality from breast cancer than the control group. This reduction appears to be concentrated among women 50 years of age and older. The differential between study and control cases in fatality from breast cancer is due almost entirely to exceptionally better survival among cases detected

through screening. Both the clinical examination and mammography contributed to this favorable situation, but the magnitude of the independent contribution of each modality is difficult to determine.

9. A study of lung cancer among uranium miners was continued in collaboration with Dr. Thomas Mason of NCI, Dr. Geno Saccomanno of Grand Junction, Colorado, and Dr. Victor Archer of Salt Lake City, Utah. The natural history of lung cancer as defined by sputum cytology, and the influence of smoking, radiation and demographic variables on etiology are being investigated using a descriptive epidemiologic approach and stochastic modeling concepts. An analysis of the sojourn times revealed variation among cytologic states in both distributional shape and mean duration with the marked atypia and malignant state durations having exponential-like shapes and the shortest mean durations. There was also variation in distributional shape within a given cytologic state depending upon the previous or subsequent state occupied. When duration and smoking data were jointly analyzed, the mild atypia state exhibited a consistent trend in shape and mean duration suggestive of a sensitivity to increased exposure to cigarette smoke. Attempts have been initiated to create a more complete data set for the miner population by combining work history, cytology, clinical and mortality information. The results of this project have potential implications for lung cancer prevention programs and industrial safety regulation legislation.

10. The WHO/IARC Working Group on the Evaluation of Screening Programs for Cancer of the Uterine Cervix met again in Oslo, Norway to discuss further joint efforts to analyze the pap smear screening programs in Iceland, Norway, Sweden, Finland, Denmark, Aberdeen and Canada. An attempt is underway to perform a common analysis of these programs to better evaluate screening impact and implications for optimal screening frequency. Progress was made toward the collection and editing of data from each country on cases of cervical cancer, screening histories of the populations and incidence rates. The initial analytic approach will involve use of incidence rates after one, two or three negative screens to estimate the preclinical state sojourn time density and false negative rate. Work on an initial report of the Working Group was begun.

11. A descriptive study of stomach and colorectal cancer incidence and mortality among Puerto Ricans in New York City was begun in collaboration with Dr. Earl Pollack and Ms. Evelyn Shambaugh of NCI and Dr. David Schottenfeld and Ms. Ellen Warshauer of Memorial Sloan-Kettering Cancer Center. An earlier study comparing stomach and colon cancer mortality between Puerto Rico and Puerto Ricans in New York City from 1958 through 1971 indicated that the stomach cancer rates among Puerto Ricans in New York City were midway between the rates for Puerto Rico and those for white residents of New York City. This finding is consistent with those of previous migrant studies. For colon cancer, in contrast, the rates among Puerto Ricans in New York City remained closer to those of Puerto Rico. This finding merited further investigation. The purpose of the present study is (1) to provide further clarification of the mortality data and (2) to compare stomach and colorectal cancer incidence among Puerto Ricans in New York City with those in Puerto Rico. Mortality data will be obtained from New York City death certificates from 1975 through 1979. Incidence data for this time period will be obtained from the New York State Cancer Registry and will be supplemented by data collected from hospital record reviews.

12. Data from randomized clinical trials carried out by the Cooperative Breast Cancer Group are being reviewed. The first trial to be analyzed involved an assessment of the additive effect of Vincristine in the Cooper five drug regimen for the treatment of advanced breast cancer. The survival of 219 patients who were treated with Cyclophosphamide, Methotrexate, 5-Fluorouracil, Prednisone, and Vincristine, i.e. CMFVP, are compared with 208 patients who received CMFP. The use of Vincristine was found to have virtually no effect on survival. Furthermore, the patient group receiving Vincristine was found to have significantly more central nervous system side effects. Multivariate analysis indicated that important prognostic factors for advanced breast cancer patients were performance status, alkaline phosphatase, calcium, weight, respiratory system impairment, liver impairment, and "other" impairment.

13. An analysis of data including that from a slide review for all female breast cancer patients <36 years old diagnosed in the Province of Saskatchewan, Canada, during 1945-71 suggested that parity was an important prognostic factor. Nulliparous patients were found to have an observed five-year survival rate of 0.85 with the corresponding rates for parous patients with 1-3 children being 0.62 and for parous patients with 4+ children 0.59. These differences in survival could be explained in large part by differences in distribution among the three parity groups with respect to the nuclear grade of the tumor and axillary lymph node status. Because these factors were found to have a statistically significant association with parity and because they either characterize or are manifestations of the aggressiveness of the disease, it is concluded that parity may well influence the biological behavior of breast cancer in young women.

14. Analysis of time to recurrence for patients with soft tissue sarcoma demonstrated the significance of histologic grade, tumor location, direct extension and symptoms. Histologic grade is one of several factors in a soft tissue sarcoma staging system published by the American Joint Committee on Cancer. However, multivariate analysis using the Cox proportional hazard model indicated that the other factors contribute significantly in addition to grade in assessment of risk of recurrence.

15. Biostatistical support to investigators in the Laboratory of Viral Carcinogenesis has continued. Antibody titers to Epstein-Barr virus (EBV) were studied in Greenland Eskimos, Danes living in Greenland, and Danes living in Denmark. Both Eskimos and Danes living in Greenland had significantly higher titers of EBV antibodies than Danes living in Denmark, suggesting that environment was more important than genetics or socio-economic factors in determining the antibody response to EBV. Over 400 cases of Burkitt's lymphoma (BL) reported to the American Burkitt's Lymphoma Registry were evaluated to obtain information about the cause and control of this disease. Although EBV was less frequently associated with American BL than African BL, a high antibody titer to EBV viral capsid antigen was associated with a more favorable prognosis. American BL resembled African BL with regard to time-space clustering, male predominance, and excellent response to chemotherapy. Antibody titers to EBV antigens were also investigated in nasopharyngeal cancer patients from intermediate incidence areas (Algeria, Tunisia, and Malaysia) and from low incidence areas (West Germany and the U.S.A.). The remarkable predominance of

IgA antibodies to EBV found in nasopharyngeal carcinoma patients from populations at high, intermediate and low risk suggest that studies of these antibodies should be useful in the early detection of this disease in all areas of the world.

16. The characteristics of colon cancer tumors diagnosed in patients seen at hospitals participating in the National Cancer Institute's Surveillance, Epidemiology, and End Results (SEER) Program and at Comprehensive Cancer Centers belonging to the Centralized Cancer Patient Data System (CCPDS) were compared. There were 10,998 SEER and 3,146 CCPDS colon tumors identified among cases diagnosed between July 1, 1977, and December 31, 1978, the first eighteen months of registration for the CCPDS centers. A higher proportion of CCPDS colon tumors were diagnosed in black patients, 15.4 percent versus 6.8 percent for SEER, reflecting the urban location of many Comprehensive Centers. The CCPDS patients were slightly younger, with a median age of 67.5 years versus 70 years for the SEER cases. Higher percentages of CCPDS patients were treated by chemotherapy alone or by modalities other than surgery, chemotherapy, and radiotherapy, particuarly those with later stages of the disease. Few disagreements existed between the two groups in distribution by stage, segment of the colon, and histologic type.

17. The analysis of the cancer experience of the older-aged population in this country has continued. Persons 65 years of age and older comprise 11 percent of the population, one-half of all cancers diagnosed and 60 percent of all cancer deaths. Lung and bronchus leads as a cause of elderly male cancer deaths, followed by prostate and colon. For elderly females, lung and bronchus is third, with breast and colon cancers first and second as causes of cancer mortality. Five-year relative survival rates were higher for white elderly females than for males for lung and bronchus and colon and about the same for stomach, rectum, pancreas, urinary bladder, and kidney. This was also observed for the black elderly, but their survival rates were poorer than those of the whites, except for cancers of the stomach and the pancreas, for which they were about the same. In general, 5-year relative survival rates decreased with increasing age group, when comparing patients aged 65-74 with those 75 and older, with the immediately preceding age group, 55-64. The survival rates were about the same for stomach, pancreas, female breast, and ovary in the three age groups, decreased slightly for colon, lung and bronchus, and decreased more sharply for rectum, uterine cervix, corpus, and prostate.

18. A study comparing the reported rates of carcinoma in situ of the uterine cervix with those of invasive cancer of the same site for the nine geographic areas covered by the SEER Program is underway. It was observed that areas with high in situ rates tended to have high invasive rates and those with low rates for one also tended to have low rates for the other, for cases both under 50 years of age and 50 years and older at time of diagnosis. This positive association was also seen in a comparison of invasive incidence rates with mortality rates. Some measures of the intensity of cytologic screening, the level of hysterectomy experience, and the ratio of preclinical to clinical localized invasive disease are potential correlates to assist with understanding the observed geographic patterns.

19. Work has continued on the development of theory for the evaluation of screening programs using stochastic modeling methods. Two approaches have been used to investigate age dependence, lead time, length bias and natural history relationships. Using renewal theory concepts, new distribution results for bounded recurrence times and lead time were derived. These led to a procedure for determining the number of screens to include in a screening evaluation trial based upon changes in lead time at successive screens. An alternative model was also developed based upon the age at entering the preclinical state, the age at screening and the duration in the preclinical state. The joint density functions of these three variables were derived for individuals detected by screening, and for those with clinically surfacing disease. Based upon this model, a method was developed for estimation of an approximate lead time distribution at a prevalence screen. A detailed numerical investigation of relationships between length bias, screening parameters and disease natural history has been undertaken. A new bivariate lifetime density was developed for this purpose.

20. In a case-control study conducted in Detroit as part of the National Bladder Cancer Study, we compared the proportion of coffee drinkers between hospital and population control series. The comparison was based on interviews with 262 hospital controls and 427 population controls. The proportion of coffee drinkers in the total hospital control group was similar to that in the population control group. However, the proportion of moderate-to-heavy drinkers (i.e., drinkers of two or more cups of coffee per day) in the total hospital control group was slightly lower than that in the population control group. Controls hospitalized for conditions that may have altered diet had a lower proportion of moderate-to-heavy coffee drinkers than population controls. The lowest proportion of moderate-to-heavy drinkers was observed among controls hospitalized for digestive disorders. Controls hospitalized for chronic conditions that may have altered diet (e.g., cardiovascular disease) also had a lower proportion of moderate-to-heavy drinkers than population controls. In contrast, the proportion of moderate-to-heavy coffee drinkers among controls hospitalized for conditions that probably did not alter diet (e.g., fractures) was almost identical to that among population controls. These results suggest that, in selecting a control group in hospital-based studies of the effects of coffee drinking, it would be prudent to restrict the referent group to those patients hospitalized for conditions that probably did not alter diet.

21. In an attempt to answer recent questions concerning the use of the five-year relative survival rate as a measure of "curability" and whether the relative survival rate is an appropriate measure of cancer patient survival, an investigation was begun to determine how much of the mortality of cancer patients was in fact due to the diagnosed cancer and the amount that could be attributed to other causes, including other cancers. In a preliminary analysis, five major sites were chosen: three with fairly good prognosis (female breast, uterine corpus, colon and rectum combined), and two with poor prognosis (stomach and male lung and bronchus). For these sites, it appeared that the majority of these patients died of either the diagnosed cancer or another cancer as follows: uterine corpus, 65.7%; female breast, 70.0%; colon and rectum combined, 75.5%; stomach, 79.6%; and male lung and bronchus, 84.7%. The major non-cancer cause of death category was cardiovascular disease, followed by "unknown" cause of

death. For all of the sites examined the relative survival rate appeared to give a reasonably good estimate of the overall effects of cancer, including the possible excess risk of dying of other causes.

Significance to Biomedical Research and the Program of the Institute:

Studies of long-term trends in cancer incidence and mortality provide insight into the changing impact of cancer on the U.S. Population. This information is essential to development of plans for future emphases of the National Cancer Program. This research also produces clues regarding hypotheses of cancer etiology that should be pursued by more definitive analytic studies. Development of cancer preventive measures depends upon studies which establish cancer risk in relation to determinants such as environmental and occupational exposures and lifestyle characteristics. Research on methods of screening for early detection of cancer or precancerous conditions affords the opportunity to distinguish between effective methods and those which should be abandoned. This array of different but related research areas combine with research on the epidemiology of cancer patient survival to provide a solid base of information regarding cancer etiology, diagnosis and treatment outcome to advance our understanding of the natural history of cancer.

Proposed Course:

The emphasis of this project has been on examining patterns of cancer patient survival in relation to therapy, extent of disease, histologic type, etc. This kind of activity will continue as new studies of factors related to patient survival are conducted. Effort will be devoted to developing a better understanding of the differences in survival between white and black patients. Initial studies will focus on cancers of the bladder and uterine corpus, sites for which the racial differences are largest.

Study of the risk of developing a subsequent primary cancer has been based on data from the Connecticut Tumor Registry. Further exploration of the Connecticut historical resource will continue although attention will be focused on assessing the subsequent primary cancer experience among patients identified through the SEER Program. These patients now have sufficient follow-up for identifying cancers which may have resulted from chemotherapy for the initial cancer.

Appropriate data resources are being compiled for assessing long-term (30+ year) trends in cancer incidence and mortality among whites. Analysis will include age-specific and area-specific as well as age-adjusted trends.

Analytic studies for investigating factors related to cancer etiology will be developed. Special emphasis will be placed on environmental or occupational determinants that can lead to preventive measures.

Activities will continue on the development of methodology for the design and analysis of screening programs. New models for the analysis and comparison of screening designs and measures of effectiveness will be developed. The role of lead time and length-biased sampling in the analysis and interpretation of

screening data will be studied. It is anticipated that the HIP breast cancer screening study, a contract of the Biometry Branch, will continue through at least 15 years of follow-up of the entire study population, and that the results of modeling research will be applicable to the HIP data as well as to data from screening programs for other cancers.

Publications:

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Chen R., Mantel, N., Connelly, R.R. and Isacson, P.: A monitoring system for chronic diseases. Methods Inf. Med. 21: 86-90, 1982.

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CONTRACTS IN SUPPORT OF THIS PROJECT:

HEALTH INSURANCE PLAN OF GREATER NEW YORK (NO1-CP-43278)

Title: Evaluation of Periodic Breast Cancer Screening with Mammography and Clinical Examination

Contractor's Project Director: Mrs. Wanda Venet

Project Officer (NCI): Dr. Philip C. Prorok

Objectives: A. To determine the frequency with which a screening technique using mammography and clinical examination can detect early breast cancer.

B. To establish whether such screening holds substantial promise for improving survival rates among women with newly diagnosed breast cancer and for lowering breast cancer mortality in the female population.

C. To investigate, through a prospective study of women screened, the relationships 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 clinical examination plus mammography at annual intervals and a control group of approximately 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. The study is now in the long-term follow-up phase.

Major Findings: Findings to date strongly suggest the usefulness of annual screening which includes clinical examination plus mammography. Over a 14-year period of follow-up, the study group of women has about thirty percent less mortality from breast cancer than those in the control group. This reduction in mortality appears to be concentrated among women over 50 years of age at entry into the study. The differential between control and study cases in fatality from breast cancer is almost entirely due to the exceptionally low rate among the cases detected through screening. Both the clinical examination and mammography contributed to this favorable situation, but the magnitude of the independent contribution of each modality is difficult to determine. Recent results based on long-term follow-up of the breast cancer cases found in the study suggest that the benefit of screening may have been concentrated among the positive node cases.

Significance to Biomedical Research and the Program of the Institute: Early diagnostic procedures offer considerable promise for the secondary prevention and potential cure of cancer. It appears that the combination of clinical examination and mammography is such a procedure and is being extended to other populations. The role of mammography alone in this process is being investigated, as is the long-term impact of screening on breast cancer mortality.

Proposed Course: The screening part of the study is complete and the emphasis from now on will be on follow-up and data analysis. The women will be followed for as long a time period as is necessary to determine the long-term impact on mortality from breast cancer. All cases of breast cancer will be followed for at least 15 years. The current contract year is the second of a five-year period for the long-term follow-up of all 60,000 women in the study. Such follow-up will allow quantification of the long-term reduction in breast cancer mortality as a result of screening.

Date Contract Initiated: December 1, 1973; Modified on June 29, 1981

Current Annual Level: $300,000

MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES (N01-CP-11024)

Title: Stomach and Colon Cancer Incidence and Mortality Among Puerto Ricans in New York City

Contractor's Project Director: Dr. David Schottenfeld

Project Officer (NCI): Dr. Debra T. Silverman

Objectives: To compare stomach and colorectal cancer mortality rates for Puerto Ricans in New York City to those in Puerto Rico and to those for whites and blacks in New York City.

To compare stomach and colorectal cancer incidence rates for Puerto Ricans in New York City to those in Puerto Rico.

Methods Employed: Mortality data will be obtained from New York City death certificates from January 1, 1975 through December 31, 1979. Incidence data for this time period will be obtained from the New York State Cancer Registry and supplemented with data collected from hospital record reviews. These reviews will be conducted at approximately 30 hospitals that serve Puerto Rican cancer patients in New York City.

Major Findings: This project has just entered the data collection phase so there are no findings to report.

Significance to Biomedical Research and the Program of the Institute: A previous study comparing stomach and colon cancer mortality between Puerto Rico and Puerto Ricans in New York City from 1958 through 1971 indicated that the stomach cancer rates among Puerto Ricans in New York City were midway between the rates for Puerto Rico and those for white residents of New York City. This finding is consistent with those of previous migrant studies. For colon cancer, in contrast, the rates among Puerto Ricans in New York City remained closer to those of Puerto Rico. This observation merited further investigation. If incidence data and more recent mortality data confirm this finding, a case-control study in New York and Puerto Rico will be initiated to identify risk factors for stomach and colorectal cancer that might explain this atypical pattern of cancer occurrence.

Proposed Course: Data collection is expected to be completed by June 1983. Data analysis is expected to be completed by November 1983. Published results are expected by the summer of 1984.

Date Current Contract Initiated: September 30, 1981

Current Annual Level: $112,000

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER ZO1 CP 04258-14 B

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)
Studies of Cancer Incidence and Mortality and Related Etiologic Factors

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Earl S. Pollack	Chief	BB	NCI
OTHERS:	Haitung King	Senior Research Scientist	BB	NCI
	Frances Locke	Statistician	BB	NCI
	Joseph Scotto	Health Services Director	BB	NCI
	Tu Ji-Tao	Visiting Fellow	BB	NCI

COOPERATING UNITS (if any) University of Bergen, Norway; Kuakini Hospital, Honolulu; University of Minnesota; Universidad del Valle, Cali, Colombia; Louisiana State University; Shanghai Cancer Registry; Memorial Sloan Kettering Cancer Center; Puerto Rico Department of Health

LAB/BRANCH
Biometry Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

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☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Research on cancer incidence and mortality continued in a number of studies on specific cancers. Migrant populations from Japan, Norway, China and Puerto Rico are being studied to identify factors associated with specific cancers. Among Hawaiian Japanese men a relationship was found between beer consumption and rectal cancer and between other alcoholic beverages and lung cancer. Among Norwegian migrants to the U.S., there was a suggestion of increased risk of colon cancer among those with high beer consumption. Differences in incidence of specific cancers have been found in the U.S. Chinese populations. For example, the rate of nasopharyngeal cancer and of liver cancer in the San Francisco Chinese is double that in the Hawaiian Chinese while that for thyroid cancer in the Hawaiian Chinese is almost triple than in the San Francisco Chinese. The mortality rate for colon cancer among the Puerto Ricans in New York City is much lower than one would expect in a migrant population. This is being explored through a study of cancer incidence.

Project Description

Objectives:

To describe and analyze cancer morbidity and mortality in human populations; to identify factors that may be associated with the occurrence of cancer; and to identify causative or promoting factors for specific sites of cancer through comparison of high-risk and low-risk populations, taking advantage of natural experiments such as comparison of migrant groups with their country of origin.

Methods Employed:

Several projects are reported on here, all of them primarily descriptive epidemiological studies. The Japan-Hawaii Cancer Study is a prospective study in which an initial physical examination was given and a detailed questionnaire administered through interview with primary emphasis on dietary information on a cohort of 8,006 Japanese men in Hawaii. The cohort is then followed through hospital records, death certificates and other sources to identify cases of specific cancers that have occurred since the initial contact. A similar methodology has been employed in the Lutheran Brotherhood Study in Minnesota on a cohort of Norwegian migrants to the United States but the initial information was obtained through interview with no physical examination being given; the same is true with the cohort of individuals being studied in Norway. The analysis of data on Chinese migrants has been carried out through comparisons of mortality data between Taiwan, Singapore, Hongkong, and more recently the Peoples Republic of China, and Chinese populations in the United States. Comparisons of both cancer incidence and mortality data are now being made through the use of the Shanghai Cancer Registry and the cancer registries in San Francisco and Hawaii. A descriptive epidemiologic study of stomach and colorectal cancer among Puerto Rican born persons in New York has begun and the comparisons with the Puerto Rican population in Puerto Rico and the New York white population may suggest the design of a specific analytic study.

Major Findings:

Japan-Hawaii Cancer Study:

A cohort of 8,006 Japanese men, born between 1900 and 1919 and living in Hawaii, has been followed since the mid-1960's to relate dietary factors, as well as a large number of demographic and physical examination variables, to the occurrence of cancers of the stomach, colon, rectum, lung and prostate. During this year an analysis was carried out in the Biometry Branch relating usual alcohol consumption to the occurrence of these cancers. The analysis suggested a trend relationship between total alcohol consumption and cancers of the rectum and lung, but not for any of the other three sites. The major contributor to the relationship with rectal cancer appeared to be high beer consumption and for lung cancer, high wine and whiskey consumption. Because of the nature of these findings and the numbers of cases involved, it was decided that we would use another year of follow-up information to determine

whether these findings still hold. Preliminary analysis of the lung cancer data indicate that they do, but they are of marginal statistical significance.

Meanwhile, work on this project continues in Honolulu in a number of areas, including analysis of dietary fiber, vitamin A and vitamin C intake; study of family history of stomach cancer; study of mutagens in relation to intestinal metaplasia of the stomach and a number of others.

Comparison of Cancer Mortality and Incidence Among Chinese in the United States and the Peoples Republic of China:

The Chinese represent a unique resource for epidemiologic investigations on cancer. Among other favorable factors, the majority of residents in a major Chinese settlement are known to share a common geographic (county) and/or dialect origin of their ancestors on the homeland; such a readily identifiable background is likely to facilitate the formulation of research hypotheses and speculation on study findings. Thus, in the United States, Chinese immigrants came mainly from eight counties, including Zhong-Shan, in Guangdong province; seven of which are parts of a single Fo-Shan prefecture. These immigrants are concentrated in New York City, Los Angeles, Honolulu and particularly, San Francisco. In addition to cancer mortality statistics, incidence data are available for all the four areas.

Studies on cancer among U.S. Chinese in the past have suffered from the unavailability of comparable statistics on homeland populations, on the basis of which the transitional effect at both geographic and generational (nativity) levels may be ascertained. Such a limitation was largely resolved by the recent release of the 1975 national mortality survey data, the accessibility of limited incidence statistics for a few Chinese localities and the presence of Dr. Tu Ji-Tao as a Visiting Fellow from Shanghai. Our current effort is directed mainly to comparing cancer experience among Chinese in this country, China, and other parts of the world in a triple analysis consisting of a) cancer mortality among Chinese in the United States, Guangdong province, and Hongkong; b) cancer incidence among Chinese in the United States, Shanghai and Zhong-Shan; c) cancer mortality among Chinese in the United States, Fujien, and Taiwan provinces, and Singapore. The underlying reasoning of the last mentioned is that the majority of the Chinese in Taiwan Province and Singapore originally came from Fujien province.

Preliminary analysis of cancer mortality among Chinese in the U.S., Guangdong, and Hongkong indicated that, for all cancers combined, a substantive elevation in risk was shown for idai (foreign-born) and Hongkong males, compared to their erdai (native-born) and Guangdong counterparts. Among females, an enormous deficit in risk of close to 50 percent was also indicated for Guangdong, whereas relatively moderate rates were displayed for U.S. and Hongkong Chinese. Examination of age-specific mortality further noted that, among U.S. and Hongkong Chinese of both sexes, there was an increasing risk in mortality with age. Among Guangdong males and females, however, such an elevated risk continued only up to around age 50, after which there was a leveling off, accompanied by a slight decline after age 70.

Overall and by specific cancer site, there was a higher idai than erdai mortality, exceptions among females notwithstanding. Of particular interest were the patterns of displacement in relation to the site-specific risk-level noted for Guangdong and Hongkong Chinese (to be referred to hereafter as GHC). Thus, for those cancers associated with high risk in the GHC populations, a downward transition was exhibited in U.S. Chinese, with erdai displaying a further depressed rate. In contrast, low-risk cancers observed for GHC followed a different pattern, with idai initially incurring a rise in mortality and erdai subsequently experiencing a lower risk. Among the cancers associated with a decreasing risk were nasopharynx, esophagus, liver, cervix, and perhaps stomach. Cancers exhibiting an upward displacement included colon, lung, breast, and leukemia.

The ordering of risk for specific cancer sites among U.S., Guangdong, and Hongkong Chinese may be speculated in reference to population composition in the three areas. Take, for example, nasopharyngeal cancer. In Hongkong, nearly 90 percent of the Chinese population were Cantonese; many of them originated in Canton and Fo-Shan. The Hongkong rates thus reflected the experience of these high-risk populations. The lower mortality noted for Guangdong than for Hongkong was likely to be attributable to the relatively depressed mortality shown for most other areas in that province. Similarly, while a large segment of U.S. Chinese originated in the Fo-Shan area, there was a noticeable admixture of migrants from other parts of China where lower rates prevailed; mortality for U.S. Chinese therefore became less excessive.

Analyses of mortality and incidence data on the areas listed under b) and c) remain to be completed pending the release of needed information from China.

Cancer Incidence and Mortality Among U.S. Chinese:

Preliminary analysis is being made of cancer incidence and mortality on U.S. Chinese and whites, based on SEER data for 1973-77, in addition to comparable data on the Chinese in San Francisco and Hawaii for the years around 1970. Comparison of mortality and incidence experience would reveal some of the shortcomings inherent in mortality data, such as survivorship differentials and, therefore, provide a truer picture of prevailing cancer patterns among U.S. Chinese.

1. Chinese vs. White - In general, the analysis confirms a picture of high rate levels among Chinese for the nasopharynx and liver, and low rate levels for the prostate, breast, and urinary system.

2. Mortality vs. Incidence

a) Mortality rates failed to reflect rising incidence rates among Chinese for such sites as prostate, lung, stomach and rectum among males, and uterus among females.

b) While thyroid does not appear prominently in mortality observations due to high survival rates, its incidence shows a higher risk for Chinese than for whites.

3. San Francisco vs. Hawaii

a) Incidence rates confirm a long-standing mortality observation that Chinese males in San Francisco have substantially higher liver cancer rates than those in Hawaii, and that in both areas, Chinese rates are higher than white rates. Lung cancer incidence is higher among Chinese males in San Francisco than in Hawaii, but in both areas, Chinese rates are lower than for whites. For both sites, the whites in the two areas have similar rates.

b) For thyroid cancer, the opposite pattern appears, with higher rates for Hawaiian than for San Francisco Chinese.

c) Prostate incidence is higher for both Chinese and whites in Hawaii than in San Francisco. Since this is not true for mortality, the difference might be a result of case finding methodology.

4. Age Specific Rates - Incidence rates for Chinese females continue to fail to rise to the level of whites after age 50. Whether or not this is a mere reflection of idai age characteristics at the moment remains to be seen.

The findings of these analyses will be compared with incidence and mortality data available from the PRC, particularly with reference to homeland area of origin for U.S. Chinese, with a view of eliciting certain etiologic factors.

Norwegian Migrant Studies:

Further analysis of the Lutheran Brotherhood data on Norwegian migrants to the U.S. reveals the following:

- There is an increased risk for colon cancer among persons with large consumption of fresh/frozen fish, smoked/salted ham or pork, vegetable soup, rutabaga, cauliflower, canned fruit and beer.

- The risk for colon cancer is enhanced for heavy users of meats/fats only when there is correspondingly low use of vegetables or grain fiber.

- There was a positive association between alcohol consumption and cancer of the pancreas, but there was no significant association for alcohol and cancers of the rectum, stomach, lung, bladder, prostate or leukemia.

- Milk, eggs, soup, bacon or side pork, chicken and apples were positively associated with stomach cancer mortality.

- Consumption of total meat, beef and fresh pork ham was not related to stomach cancer.

- Vitamins A and C may be protective against lung cancer, and perhaps stomach cancer. The Minnesota study shows a possible increased risk between dietary vitamin A and colon cancer.

- In a special recent study on the effect of coffee consumption, no significant excess risks were noted for cancers of the pancreas or bladder. These findings were consistent in both the Norwegian and Minnesota studies.

Among the cohort under study in Norway, it is possible to study both cancer incidence and mortality. High vegetable consumption appears to be associated with decreased risk of colorectal cancer. Vegetables such as cabbage, rutabaga, red beets, carrots and cauliflower are consumed more frequently in Norway while lettuce, cucumbers, tomatoes, peas, beans and maize are consumed more frequently in the U.S. Beer consumption and processed meat consumption may be associated with higher colon cancer risk.

Puerto Rican Migrants:

An earlier cancer mortality study among Puerto Ricans in New York City revealed stomach cancer rates that were intermediate between the high rates in Puerto Rico and the low rates among the New York white population. Colon cancer mortality rates among this group, on the other hand, were close to the low rates of Puerto Rico. In most other migrant studies, the colon cancer rates of the migrants begin to rise rapidly to approach the high rates of the U.S. A study has been initiated under contract with Memorial Sloan-Kettering Hospital to analyze stomach and colorectal cancer incidence among Pueto Ricans in New York City in comparison with that among Puerto Ricans in Puerto Rico and that of the New York City white and black populations. If the above finding cannot be explained by artifacts, a case-control study will be conducted to identify factors associated with this pattern.

Significance to Biomedical Research and the Program of the Institute:

These studies attempt to identify high- and low-risk population groups which can then be studied further for the identification of possible etiologic agents. These descriptive studies permit the development of more specific hypotheses for analytic studies. The study of migrant populations attempt to suggest environmental factors that may be associated with the incidence of certain forms of cancer. The assumption is that if these factors can be identified it may then be possible to initiate preventive measures thus reducing the risk from those particular forms of cancer.

Proposed Course:

The analysis of the alcohol consumption data for the Hawaiian Japanese will be completed. To ensure continued funding for this cohort study, the Principal Investigator has been encouraged to apply for a grant. This has been done and the outcome should be known shortly. Analyses of data from both the Norwegian and Minnesota studies will be accelerated on a number of topics, with collaboration among authors from NCI, Minnesota and Norway. The analyses of cancer among the Chinese populations will continue with a focus on developing hypotheses for further study. The exploratory study of cancer of the stomach, colon and rectum among Puerto Ricans in New York City and Puerto Rico will be continued.

Publications:

Correa, P., Heilbrun, L., MacLennan, R., Newell, G., and Pollack, E.S.: Cancer of the colon and rectum: Report of a workshop. Third Conference on Cancer Epidemiology and Registries in the Pacific. In Henderson, B.E., Kolonel, L.N., and Donovan, J.T. (Eds.): NCI Monograph 62. In press.

King, H., and Locke, F.B.: Health risks and life style. In Rothschild, H. (Ed.): Risk Factors for Senility. London, Oxford University Press. In press.

Stemmerman, G.N., Nomura, A., Heilbrun, L., Pollack, E.S. and Kagan, A.: Serum cholesterol and colon cancer in Hawaiian Japanese. JNCI 67: 1179-1182, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04260-22 B

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Consultation on Clinical Trials

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D.P. Byar	Chief, Clin. & Diag. Trials Section	BB NCI
OTHER:	M.H. Gail	Medical Statistical Investigator	BB NCI
	S.B. Green	Medical Researcher	BB NCI
	D.L. Levin	Senior Investigator	BB NCI
	L.R. Muenz	Mathematical Statistician	BB NCI
	D.K. Corle	Computer Systems Analyst	BB NCI
	L.V. Rubinstein	Staff Fellow	BB NCI

COOPERATING UNITS (if any)
Division of Cancer Treatment, NCI
Division of Resources, Centers, and Community Activities, NCI

LAB/BRANCH
Biometry Branch

SECTION
Clinical & Diagnostic Trials Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

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☒(a1) MINORS ☒(a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)
The purpose of this project is to provide consultative services in statistical and epidemiological methodology in the design, interpretation, and evaluation of clinical trials and other studies requiring this kind of expertise. For some trials the Section provides full statistical support including development of detailed study plans, randomization of patients, collection, processing, editing, and analysis of data, and preparation of scientific papers. At present, members of the Section are involved in studies concerned with prostate cancer, bladder cancer, testis cancer, breast cancer, lung cancer, brain tumors, mycosis fungoides, and Burkitt's lymphoma. Other important work has included an analysis of the predictive value of animal tumor models used in screening potential chemotherapeutic agents, and analysis of several studies dealing with skin test antigens and tumor markers designed to evaluate their potential both in diagnosing cancer and in following its course after treatment. In addition to these major projects, a variety of other consultative activities, including review of scientific articles, arise in response to special requests.

Project Description

Objectives:

A major objective is to provide consultative services in statistical and epidemiological methodology for the design, conduct, interpretation, and evaluation of randomized clinical trials. This objective includes providing full statistical support for some major clinical trials of cancer treatment. For example, we are currently involved in studies of lung, breast, testis, and brain cancer. A second major objective is to provide consultation and scientific collaboration with investigators conducting other types of studies requiring statistical expertise. Some current examples include psychological aspects of breast cancer, analysis of data from a registry of patients with mycosis fungoides, non-randomized comparisons of radiotherapy with endocrine treatment for patients with prostate cancer, and an analysis of the predictive value of animal models used in screening potential chemotherapeutic agents.

Methods Employed:

Standard methods of biometry, statistics, probability, epidemiology, and computing techniques with necessary modification as required by the particular problem. New techniques are developed by the personnel working in the Section to handle specific problems (see Project No. Z01 CP 04409-07 B).

Major Findings:

The most important projects of the Section will be described separately. Generally, in consultations on clinical trials, members of the Section assist the investigators in developing detailed study protocols, in determining the numbers of patients necessary for the study, in deciding what data should be recorded and at what intervals in time, and in developing forms for the recording of data. They advise on proper methods of analysis of the final data or undertake these analyses themselves. Such assistance is generally acknowledged in the publication of findings by the medical investigators.

1. Prostate cancer. Carefully collected data on over 4000 patients with prostate cancer were obtained from the randomized clinical trials conducted by the Veterans Administration Cooperative Urological Research Group (VACURG) between 1960 and 1975. In the last year, Dr. Byar and Mr. Corle completed a study of the prognostic value of the acid phosphatase and its use in cancer staging. They demonstrated that the level of the prostatic acid phosphatase, even in the normal range and in patients whose prostate had been removed, was prognostic for progression. These results suggest that cancer of the prostate, like breast cancer, metastasizes early in some patients and that some systemic therapy should be used in addition to or instead of surgery. Dr. Byar in collaboration with Dr. Hovsepian, formerly of Stanford V.A. Hospital, prepared a report concerning their work on serial examination of x-rays in patients with prostatic cancer presenting with metastases. They found that hormone treatment may lead to pseudo-progression followed by resolution of lesions. Recognition of these changes is important in determining criteria of response in studies of

treatment for prostatic cancer because they could easily be misdiagnosed as true progression.

Also in the last year, Mr. Corle and Dr. Byar have continued analysis of the third VACURG study in which both estrogens and progesterone were used alone and in combination to treat patients in stages III (local extension) and IV (distant metastasis). No significant differences in survival were detected. The combined treatment caused a more marked fall in the serum testosterone, but this effect was not reflected in the comparisons of survival or tumor progression. Further work is underway to relate changes in serum testosterone to prognosis and to understand how endocrine therapy works.

Dr. Byar and Mr. Corle have been using the VACURG data for patients treated with hormones or orchiectomy in comparisons of survival, adjusted for stage and grade, with data from three centers (Stanford, Calif.; St. Louis, Mo.; and Boston, Mass.) where similar patients were treated by x-ray therapy. No large-scale randomized clinical trial has been completed comparing x-ray therapy to any other form of treatment, so these nonrandomized comparisons will be important in assessing the role of irradiation in treating this disease. All data have now been received and the analysis is underway.

2. Bladder cancer. Dr. Byar helped the Genito-Urinary Group of the EORTC design a trial comparing placebo and pyridoxine in patients with stage I bladder cancer (recurrent papillomas). In this trial tryptophane load tests are being performed before and during treatment because experimental evidence that suggested the use of pyridoxine indicated that it might work by suppressing the levels of urinary metabolites of tryptophane which have been shown to cause bladder cancer in mice. Patient entry was terminated in December 1981 after some 300 patients were randomized to the two treatments. Dr. Byar is assisting the EORTC statisticians in monitoring this trial, and he periodically analyzes the data independently.

3. Testicular cancer. Dr. Green is responsible for study design and analysis of the Intergroup Study of Testicular Cancer, a nationwide randomized trial comparing adjuvant combination chemotherapy following surgery for resectable stage II disease versus using chemotherapy only for relapses. This study is also following stage I patients to determine factors which may predict which tumors will recur. By April 1982, 138 patients had been admitted to the randomized trial, and 145 stage I patients were being followed. Patient accrual is continuing, and the data are being systematically edited. During the past year, Dr. Green prepared two interim analyses which were distributed to study participants.

4. Data center for breast cancer studies. The current files contain data on 3600 women with breast cancer, many of whom have had estrogen receptor assays performed. Data from these patients are being used to create a natural history data based of stage II patients who received no adjuvant therapy. We are hopeful that this data base will provide a suitable set of untreated controls for other researchers conducting adjuvant studies. Analyses by Mr. Corle and Dr. Byar of data from 391 patients with advanced disease and detailed chemotherapy treatment information indicated that patients with very high levels of estrogen receptor protein are more likely to respond to chemotherapy treatment than patients with

lower ER values. Also, serum and background data have been collected from over 10,000 women for evaluation of biological markers for breast cancer in cooperation with the Markers Group of the Breast Cancer Task Force. Blood was drawn just before biopsy or mastectomy from 700 women with newly diagnosed breast cancer. Several panels of sera have been sent out for analysis during this year. An official announcement of the availability of this valuable resource is planned in the coming year. The Section is responsible for the collection, editing, and analysis of all data, and for providing an updated inventory of material in the serum bank. This project will be continued for a number of years.

5. Inflammatory breast cancer in Tunisia. Analyses of treatment in the randomized clinical trial for this aggressive form of inflammatory breast cancer have been completed by Dr. Muenz and have been published. In the last year data on estrogen and progesterone receptor levels have been compared with those in normal American controls and American women with non-inflammatory breast cancer. The results refuted a speculation that inflammatory tumors were more often associated with low receptor levels.

6. Personality factors in fibrocystic and malignant breast disease. With Dr. Mary Jansen of the American Psychological Association, Dr. Muenz has analyzed a retrospective study of 222 women divided into three groups of roughly equal size having either malignant breast disease, benign breast disease, or neither condition. After statistical adjustment for between-group differences in demographic factors, a significant (and substantial) difference remains with respect to factors often included in the "type A" personality. Women in the fibrocystic group see themselves as aggressive, dissatisfied, and ambitious, while women in the cancer group see themselves as passive, inferior, and long-suffering. These self-perceptions agree with published speculation. The women without breast disease occupy an intermediate position between the other two groups.

7. Breast cancer skin test antigens. Dr. Levin served as statistician on a study by Dr. Faye Austin of the NCI Laboratory of Viral Carcinogenesis, evaluating the effect of virus augmentation of cultured breast tumor cell lines on the sensitivity and specificity of skin tests using antigens derived from the tumor cells. The study identified two tumorline extracts with potential for use in clinical tests.

8. Trials for lung cancer. Drs. Gail and Rubinstein are statisticians for the Lung Cancer Study Group, which is comprised of six major centers with the capacity to recruit over 150 stage I lung cancer patients per year. Six prospective randomized trials are in progress. Dr. Gail is responsible for three protocols. The first, a double-blind trial of intrathoracic BCG immunotherapy versus conventional therapy in stage I patients with resected disease has nearly completed its accrual phase, with over 473 patients on study. An analysis of intrathoracic BCG toxicity and a report on the early effects of BCG treatment on recurrence and survival have been published. The usefulness of immunological parameters for prognosis and for monitoring these patients has been evaluated, and analyses of patterns of recurrence, prognostic factors, and pathological correlations are in progress. The second study compares radiotherapy and conventional therapy in stage II/III resectable patients with

squamous cell disease. The third study compares a regime of cis-platinum, adriamycin, and cytoxan (CAP) with BCG plus Levamisole in stage II/III patients with resectable adenocarcinoma or large cell carcinoma. Dr. Rubinstein is responsible for three protocols. The first compares CAP plus radiotherapy and radiotherapy alone in patients with partially resected non-small cell lung cancer. The second study compares CAP therapy with placebo in the subset of stage I patients with T1N1 or T2NO disease. The third compares limited resection with lobectomy in patients with peripheral stage I disease. In addition, Dr. Rubinstein has been surveying reasons for non-entry into present Lung Cancer Study Group trials in an effort to improve accrual and has been studying characteristics of the available patient population for accrual into future trials. Dr. Rubinstein is also working on the analysis of patterns of recurrence mentioned above, and he is maintaining a registry and natural history catalog of stage I patients with T1NO disease.

9. Brain tumor clinical trials. Drs. Green and Byar have continued to work extensively with the Brain Tumor Study Group on the design and analysis of a number of large-scale randomized clinical trials. During the past year the design of a new phase II trial to compare the chemotherapeutic agents AZQ and PCNU was completed, and patient accrual was begun. Drs. Green and Byar continued their analysis of three previous phase II trials, with particular emphasis on BTSG 78-20 which completed accrual during the past year. Analysis of BTSG 75-01 was completed; this phase III study showed that Procarbazine and BCNU were equivalently effective for the treatment of malignant glioma, but high-dose corticosteroids did not improve survival compared to the conventional doses of steroids used in treating cerebral edema. Drs. Green and Byar began their analysis of two subsequent phase III studies which have completed accrual: BTSG 77-01 comparing early versus late surgery, and BTSG 77-02 investigating four treatments: fractionated radiotherapy plus BCNU, standard radiotherapy with Misonidazole plus BCNU, and standard radiotherapy plus Streptozotocin, all of which are compared to standard radiotherapy plus BCNU. These analyses were presented to the BTSG membership along with an early interim analysis of the ongoing phase III trial, BTSG 80-01, which is still accruing patients.

10. Screening of new anti-cancer drugs to predict clinical activity. Drs. Byar and Green have collaborated with Dr. Maurice Staquet of the EORTC on an analysis of the clinical predictive value of the NCI tumor panel of eight screening systems (in mouse models) plus a P388 leukemia prescreen. The animal data on over 1900 drugs were supplied by the Drug Evaluation Branch, DCT, NCI. Reviews by Dr. Staquet of the medical literature and files of the NCI and EORTC identified 69 drugs which were considered as clinically evaluated. Drs. Byar and Green performed a detailed analysis of the individual screens and various combinations of screens with regard to yield, sensitivity, specificity, and clinical predictive value. Based on this analysis, a rationale was developed for a three-stage strategy which would identify almost all of the potentially active drugs discovered with the present strategy, but at a much reduced cost.

11. Makari Skin Test. Dr. Levin has been serving as statistician for two studies of the Makari Skin Test. The first evaluates the test as an aid to diagnosis, and the second determines the prognostic value of the test in predicting subsequent recurrence of disease after surgical resection of colorectal, lung, and breast cancer. These studies, sponsored by the Stauffer

Chemical Company, are being conducted in several collaborating medical centers in the U.S. and in England with the assistance of Dr. Ronald Herberman, Chief, Laboratory of Immunodiagnosis, NCI. As of May, 1982, 44 patients have been entered into the study from the U.S. and 185 patients from England. Preliminary analysis showed that the test has sensitivity and specificity of about 90%.

12. Tumor markers. Dr. Levin has been collaborating with Drs. Saul Rosen and Jay Morrow of NIADDK on a study of human glycoprotein hormone subunits as indicators of cancer. The study is looking for endocrinologic ways to distinguish between multiple myeloma and MGUS (monoclonal gammopathy of undetermined significance).

13. Intra-ocular melanoma. Dr. Levin has been collaborating with Dr. Peggy Tucker of the NCI Environmental Epidemiology Branch on a study to identify prognostic factors in intra-ocular malignant melanoma. The major activity to date has related to cleaning up the data file and preparing for data analysis.

14. Burkitt's lymphoma project. Dr. R. Bigger, of the NCI Environmental Epidemiology Branch, and Dr. Gail have been analyzing records from 388 patients with Burkitt's lymphoma who were treated in Ghana since 1966. The risk of disease recurrence following treatment was highest in the 6-9 year old group, which also exhibited the highest incidence rates. Males were at no higher risk of recurrence than females, although the male to female incidence ratio was 2:1.

15. Mycosis fungoides. For the past three years Drs. Byar and Green have been collaborating with Dr. Stanford Lamberg of Johns Hopkins University on analyses of data from patients registered by the Mycosis Fungoides Cooperative Group. Dr. Byar has continued analyzing the prognostic importance of histological features seen in biopsy specimens, adjusting for the effects of other variables.

16. Human chorionic gonadotropin. Dr. Muenz has analyzed the data from a study organized by the Food and Drug Administration to standardize criteria for the certification of human HCG home pregnancy-test kits. He presented his results to an international conference on clinical laboratory standards. His analysis permits a decision regarding the future international reference preparation for HCG assays; the current standard is in short supply and a new one must be chosen.

17. Other consultative activities. Dr. Byar has been serving on the NCI Chemoprevention Working Group. This group advises the Board of Scientific Counselors of DRCCA, NCI, on scientific matters relating to chemoprevention.

Dr. Gail and Dr. McIntire, of the NCI Diagnosis Branch, have been organizing a program of multiple marker studies to diagnose lung cancer and monitor its course after treatment. Statisticians from participating centers will present independent analyses of the same data. The aim of this joint effort is to improve quantitative methods for the design and analysis of clinical protocols for evaluating immunodiagnostic tests as aids to diagnosis, prognosis, and monitoring of disease. Emphasis will be on finding ways to combine several immunologic tests to produce optimal discrimination for diagnosis. It is hoped that useful marker combinations for lung cancer will be identified and tested.

Drs. Byar and Green have been asked to consult on the analysis of two clinical trials carried out by others, a trial of adjuvant therapy for operable pancreatic cancer conducted by the GI Tumor Study Group, and a trial of chemotherapy with and without Tamoxifen in patients with stage II breast cancer conducted by the National Surgical Adjuvant Breast Project.

Dr. Gail collaborated with Saul Rosen of the NIADDK Clinical Endocrinology Branch to evaluate pregnancy-specific β_1-glycoprotein as a marker for carcinoma of the breast.

Dr. Rubinstein has been assisting Dr. Jeffrey Arbeit of NCI in analyzing a study of alanine to glucose conversion in tumor-bearing and non-tumor-bearing rats.

Dr. Levin has continued to serve on the faculty of Georgetown University, helping to teach the freshman course on biostatistics in the Medical School, and has begun collaboration with the Uniformed Services University of the Health Sciences.

Significance to Biomedical Research and the Program of the Institute:

The variability of the course of cancer in individual patients means that the assessment of treatment differences, determination of the usefulness of diagnostic tests, or the proper interpretation of data from observational studies are often statistical problems. Members of the Section are frequently consulted for advice or collaboration on such problems. Besides the projects listed above, there are numerous other short-term consultations dealing with specific studies, proposal reviews, site visits, and review of manuscripts submitted for publication. Some of the consultations involve extensive trials which represent considerable efforts of the National Cancer Program. The Section represents an important resource for expert assistance in study design, implementation, and statistical and computer analysis of studies being carried out by many other groups.

In any healthy research environment, individuals skilled in many disciplines are necessary. The Section provides expertise in statistical matters relating to the study of cancer in humans. The ability to provide meaningful consultation is greatly enhanced by having four M.D.'s in the Section who are also well-equipped statisticians. Members of the section are also skilled in computer applications to analysis of medical data.

A further advantage to the Institute is that actual day-to-day experience as a statistical support center for several large-scale clinical trials and active involvement in consultation on other projects provides an ideal environment for identifying important methodological questions of general applicability to the design, conduct, and analysis of clinical trials.

Proposed Course:

In the coming year, we plan to continue our involvement in many of the studies described above. Major areas of emphasis will include the lung, testis, and brain cancer trials being conducted by the DCT.

Publications:

Austin, F.C., Boone, C.W., Levin, D.L., Cavins, J.A., Case, R., Pharm, M. and Klein, E.: Breast cancer skin test antigens of increased sensitivity prepared from vesicular stomatitis virus-infected tumor cells. Cancer 49: 2034-2042, 1982.

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Wright, P., Feld, R., Mountain, C., Cooper, J., Eagan, R., Gail, M., Ginsberg, R., Hill, L., Holmes, E., Lukeman, J., McGuire, W., Oldham, R., Pearson, G., and participating investigators: A prospective double-blind clinical trial of intrapleural Bacillus Calmette Guerin (BCG) in patients with stage I non-small cell lung cancer. In Salmon, S.E., and Jones, S.E. (Eds.): Adjuvant Therapy of Cancer III. New York, Grune and Stratton, 1981, pp. 265-276.

CONTRACT IN SUPPORT OF THIS PROJECT:

INFORMATION MANAGEMENT SERVICES, INC. (NO1-CP-01025)

Title: Biomedical Computing Software Services

Contractor's Project Director: Janis Beach

Project Officer (NCI): Donald K. Corle

Objectives: The contractor provides system design and computer programming support services for the research projects conducted by the Section (See project numbers Z01 CP 04409-07 B and Z01 CP 04260-22 B).

Methods Employed: The contractor's staff is skilled in the areas of biomedical data abstracting and editing. The staff writes computer programs primarily in the FORTRAN and COBOL programming languages and is proficient in the uses of packaged programs (e.g., SAS, SPSS, BMDP). All programming is performed on the IBM/370 or DEC-10 computer systems at the Division of Computer Research and Technology.

Major Contributions: The contractor's major contributions include development of editing, file maintenance, and statistical programs, processing of raw data, preparation of special data sets for analysis, and performance of some analyses under the direction of Section members. The contractor periodically edits and updates data bases for studies of prostate, lung, brain, testis and breast cancer. The contractor has written most of the computer programs for managing these studies and has maintained the documentation of systems and procedures. The contractor has worked closely with Drs. Gail and Rubinstein in preparing

detailed reports of the six clinical trials of the DCT Lung Cancer Study Group and has attended the biannual meetings of this group to provide technical advice concerning forms design and data processing. The contractor has worked with Drs. Green and Byar in developing computer programs for interactive statistical analysis; many improvements were made to the recode, Boolean selection, and screening program modules.

Significance to Biomedical Research and the Program of the Institute:

The justification for this project is related to the significance of the two projects designated above. This contract assures accuracy of the data, timeliness in carrying out tasks, and permits Section involvement in a broader variety of research projects.

Proposed Course:

The contractor has worked with the Section for all of Fiscal Year 1982. This contract will be continued until its termination in July, 1983, at which time a competitive renewal will be sought.

Date Contract Initiated: July 11, 1980

Current Annual Level: $335,000

Man Years: 9

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04265-17 B

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Consulting in Statistics and Applied Mathematics

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. J. Gart	Chief, Mathematical Statistics & Applied Mathematics Section	BB NCI
OTHERS:	H. M. Pettigrew	Mathematician	BB NCI
	R. E. Tarone	Mathematical Statistician	BB NCI
	D. G. Thomas	Mathematical Statistician	BB NCI
	J. Nam	Mathematical Statistician	BB NCI
	A. M. Smith	Statistician (Health)	BB NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Biometry Branch

SECTION

Mathematical Statistics and Applied Mathematics Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.5	3.0	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

It is the purpose of this study to collaborate with NCI researchers on mathematical problems related to many areas of cancer research. Consulting assistance in statistical methodology and applied mathematics is provided for NCI investigators and to some extent for NCI contractors. In general, the study is devoted to accelerating the use of quantitative methodology in various aspects of the NCI intramural and extramural programs.

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 investigators, 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 is applied to biomedical problems. Often variations of existing techniques are developed to suit the special requirements of a particular problem.

Major Findings:

During this year, the staff advised and collaborated with many investigators in the major divisions of research in the National Cancer Institute as well as some outside contractors and other Government agencies. The various projects are grouped below in terms of the divisions and areas of the projects.

Division of Cancer Cause and Prevention - Office of the Director

Dr. Tarone continued to collaborate with Dr. Thomas Cameron on a paper detailing the results of a study comparing survival, prostate tumor rates and breeding efficiencies of two strains of rats.

Division of Cancer Cause and Prevention - Field Studies and Statistics

Dr. Gart is collaborating on two large prospective studies on the relationship of diet and cancer. One study done under contract at the University of Minnesota and a second in the University at Bergen in Norway. Much of the analyses of this study was formulated by Dr. Gart and implemented on the computer by Mr. Thomas and Mrs. Smith. With Professor L. Schuman and Professor E. Bjelke and others, Dr. Gart is co-author of two papers being prepared for publication. They consider the following:

1) Lung cancer mortality in the Minnesota study was negatively associated with dietary indexes of vitamin A and vitamin C, and

2) Lung cancer incidence in Norway was negatively associated with vitamin A intake.

Other results of the studies indicate that pancreatic cancer is positively linked to alcohol, chewing tobacco, and snuff, but not to coffee as has been claimed. Dr. Gart collaborates on these projects with Mr. Scotto who is project officer of both studies.

Dr. Tarone advised Dr. Robert Depue of the Office of the Associate Director, FS&S, regarding analytical methods for a case control study of testicular cancer.

Mr. Nam continues to collaborate with Mr. Joseph Scotto of the Biometry Branch on the nonmelanoma data from the Skin Cancer Survey and SEER (the Surveillance, Epidemiology, and End Results) Program. They are examining the possible association of incidence and seasonality which is related to solar UV (ultraviolet) intensities.

Mrs. Smith aided Mr. Joseph Scotto and Dr. Thomas Fears of the Biometry Branch with the implementation of one of her computer programs in their study of the changes in nonmelanoma skin cancer morbidity in the six years since the Third National Cancer Survey in 1971-72.

Dr. Tarone advised Ms. Margot Hanson and Mr. William McKay of the Clinical Epidemiology Branch concerning various methodological problems involved in the statistical analysis of directly standardized cancer mortality rates.

Mr. Thomas maintained and modified the section's library of computer routines and advised various staff members on their use as well as other technical aspects of computer programming. Numerous computer installations throughout the world have requested and received copies of the programs developed and used in this section. Our program "Trend and Homogeneity Analyses of Proportions and Life Table Data" is now part of the BCTIC computer code collection distributed by the Biomedical Computing Technology Information Center, Nashville, TN for the Department of Energy.

Mrs. Smith did much of the data processing and support work for many of the consulting projects detailed herein.

Dr. Gart continued to serve on the FS&S Review Group.

Division of Cancer Cause and Prevention - Carcinogenesis Intramural Program

Dr. Tarone continues to work on the design and statistical analysis of experiments performed by Dr. Katherine Sanford, Dr. Raymond Gantt and Mr. Gary Jones of the Laboratory of Cellular and Molecular Biology and Dr. Ram Parshad of the Howard University College of Medicine. These experiments are performed to investigate factors which influence fluorescent light-induced and X-ray-induced chromosome damage in human cells and to attempt to explain increased susceptibility to such damage in malignant cell lines.

Mr. Nam continues to collaborate with Dr. Paul Levine of the Carcinogenesis Intramural Program in a study of survivorship of Chinese NPC (nasopharyngeal carcinoma) cases in Singapore as it relates to the HLA (human leukocyte antigen) system and Epstein-Barr virus antibody levels.

Dr. Tarone continued to provide statistical assistance to Dr. Kenneth Kraemer of the Laboratory of Molecular Carcinogenesis in a study of aryl hydrocarbon hydroxylase induction levels in psoriasis patients. Dr. Tarone also assisted Dr. Kraemer in the analysis of survival data obtained from a literature search of patients with xeroderma pigmentosum.

Mr. Nam advised Dr. Richard Yamamoto of the Laboratory of Carcinogen Metabolism on the statistical analyses of bacterial mutagenesis assays of several derivatives of 2,4-Diaminoanisole sulfate (a component of many hair dyes), and continues to advise Dr. Yamamoto on the analyses of other experimental data.

National Toxicology Program

Dr. Tarone provided statistical assistance to Dr. Jerrold Ward of the Tumor Pathology Branch on a variety of problems related to the analysis of tumor incidence data in animal carcinogenesis experiments.

Dr. Tarone continued to assist Dr. Kenneth Chu of the Technical Resources Branch and Dr. Virginia Dunkel of the Food and Drug Administration in the analysis of a study of the reproducibility of microbial mutagenicity assays. Dr. Tarone continued to advise Dr. Chu on various aspects of the analysis of epidemiologic data.

Division of Cancer Biology and Diagnosis

Dr. Tarone continues his collaboration with Dr. Jay H. Robbins and Ms. Susanna Barrett of the Dermatology Branch and Dr. Dominic Scudiero of the Chemical Carcinogenesis Program of the Frederick Cancer Research Facility in their experiments to study the in vitro survival of lymphoblast and fibroblast cell lines from patients with the cancer prone disease, xeroderma pigmentosum, and with a variety of hereditary primary neuronal degenerations after exposure to the DNA-damaging agents such as ultraviolet light, X-rays, and MNNG.

Dr. Pettigrew has continued to advise Dr. Pietro Gullino of the Laboratory of Pathophysiology on a variety of topics.

Dr. Tarone advised Dr. Edward Leonard of the Laboratory of Immunobiology regarding the design and statistical analysis of experiments performed to compare the monocyte chemotactic response of cancer patients to that of normal controls.

Dr. Tarone provided statistical assistance to Dr. Saraswati Sukumar of the Laboratory of Immunobiology in experiments performed to evaluate the treatment of MNU-induced rat mammary tumors with intralesionally administered cell walls of Mycobacterium bovis strain Bacillus Calmette-Guerin.

Various Other Activities

Dr. Pettigrew is working with Dr. Sanford Rosenthal of the Laboratory of Biochemical Pharmacology, NIAMD, on the analysis and publications of data on the effects of different dietary levels of sodium on the longevity of mice.

Dr. Pettigrew advised Dr. Annette Maluish of the USUHS on the analysis of data from the NK assay on blood samples from cancer patients to determine the effects of BCG on immune response.

Dr. Tarone and Dr. Gart continued to collaborate on the writing of two chapters for an international Agency for Research on Cancer monograph on the statistical analysis of long-term animal carcinogenesis experiments.

In connection with the latest Surgeon General's Report, Dr. Gart advised Dr. Joanne Luoto of the Office of Smoking and Health on statistical aspects of studies of the possible relation between passive smoking and lung cancer.

Dr. Tarone served as an expert consultant to the Board of Scientific Counselors of the National Toxicology Program.

Dr. Pettigrew continues to serve as a member of the Interagency Regulatory Liaison Group (IRLG) to review the NCTR/FDA EDO1 Workshop held at Deer Creek State Park, Mt. Sterling, Ohio, September 14-16, 1981.

Significance to Biomedical Research and the Program of the Institute:

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

Proposed Course:

Several of the projects mentioned in the Major Findings section will continue. In particular, the collaboration with the various projects in epidemiology within Field Studies and Statistics, as well as in the Carcinogenesis Research Area, the Division of Cancer Biology and Diagnosis, and other areas will be progressing.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CP 04267-17 B

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)
Research in Mathematical Statistics and Applied Mathematics

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. J. Gart	Chief, Mathematical Statistics & Applied Mathematics Section	BB NCI
OTHERS:	H. M. Pettigrew	Mathematician	BB NCI
	R. E. Tarone	Mathematical Statistician	BB NCI
	D. G. Thomas	Mathematical Statistician	BB NCI
	J. Nam	Mathematical Statistician	BB NCI
	A. M. Smith	Statistician (Health)	BB NCI

COOPERATING UNITS (if any)
None

LAB/BRANCH
Biometry Branch

SECTION
Mathematical Statistics and Applied Mathematics Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.5	3.0	0.5

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

It is the purpose of this project to conduct research in mathematical statistics, probability and applied mathematics, and especially to develop new statistical methodology which is particularly applicable to the biomedical sciences. Particular subjects of interest are the methodology of analyzing survival curves and proportions, statistical methods in cancer epidemiology and statistical genetics, such as the analysis of the relative risk.

Project Description

Objectives:

To conduct research in mathematical statistics, probability, and applied mathematics; to develop new statistical methodology which is especially appropriate to biomedical 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 to 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.

Robert E. Tarone continued his research on Empirical Bayes methods for analyzing frequency data. A paper presenting a method of using historical control data in testing for a trend in Poisson means has been accepted for publication. His joint research with John J. Gart on the application of score tests also continued, and a paper giving some results of this research has been accepted for publication. Dr. Tarone is investigating optimal methods for analyzing survival curves produced by the in vitro irradiation of cell cultures with X-rays or with ultraviolet light. He continues his investigations of distribution-free tests for censored distributions. Dr. Tarone and Dr. Gart are conducting research on asymptotically efficient estimators of a common relative risk or of a common odds ratio.

John J. Gart has continued his research on several topics in statistical methodology. Among these are (1) investigation of the properties of the relative risk for case-control studies with multiple matched controls. The bias and relative efficiency of various proposed estimators are being calculated, and (2) investigation, with Hugh M. Pettigrew, of the higher order corrections to the mean and higher order moments of various transformations of binomial proportions. Particularly, these include the logit and logarithmic transformations which are routinely applied in so-called loglinear methods. In this work, Donald G. Thomas has done considerable computer programming of the exact calculation of the various moments and of the transformations and the exact bias of various estimators.

Donald G.Thomas and John J. Gart are also implementing, via a computer program, an analysis of the odds ratios in prospective or cohort studies. The analysis is a stratified version of a previously published paper by

these authors on trend and homogeneity analyses of proportions and life table data. In addition, univariate and bivariate logistic regression is incorporated in the methodology. This methodology has proven particularly useful in analyzing a prospective study of diet and cancer. A paper describing the methodology has been submitted for publication.

Hugh M. Pettigrew is investigating models for tumor growth (such as the Gompertz function) and models for mammary tumor systems in rodents. He is investigating the sources of variation inherent in the NK Assay with the goal of improving the design of clinical studies in order to increase the ability of the assay to detect real changes in immune response. He is continuing his research in the mathematical theory of epidemics and considering methods to compare linear trends in proportions in samples in which the observations are correlated.

Jun-mo Nam derived an approximate formula for sample size determination for detecting a linear trend in proportions. It enables one to answer some important questions in carcinogenesis bioassay. A paper based on this research has been submitted for publication. Jun-mo Nam, with John J. Gart, is investigating the asymptotic bias of the unconditional maximum likelihood estimator of the common slope of several logistic regressions in the analysis of binary data. They are also investigating the bias of Bernstein's method for estimating gene frequencies of ABO-like systems, a correction to reduce such bias, and application of these results to testing the goodness of fit of the Hardy-Weinberg law. Jun-mo Nam continues his study on an efficient method for the identification of cyclic trends in incidence.

Alroy M. Smith provides computer programming support on many of the research projects in the section.

Significance to Biomedical Research and 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, particularly in DCCP, or other workers in cancer research. Others are mathematical models 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:

Many of the projects described in the major findings will be continued, e.g., analyses of transformations and loglinear methods, analyses of relative risk in case-control studies, research on censored survival tests, and statistical methods in genetics. In addition, new research initiatives will include the development of new statistical methods and mathematical models in various biomedical problems that come to our attention during the year.

Publications:

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04269-11 B

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Biomedical Computer Systems - Consultation, Service

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J. Michael Stump	Acting Chief, Computer Science Section	BB	NCI
OTHER:	Mary Cusano	Computer Systems Analyst	BB	NCI
	Calvin Hollingsworth	Computer Specialist	BB	NCI
	Vivian Pelham	Computer Systems Analyst	BB	NCI
	Valerie Van Holten	Computer Specialist	BB	NCI
	Ruth Wolfson	Computer Programmer	BB	NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Biometry Branch

SECTION
Computer Science Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
9.5	6	3.5

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objective of this project is to facilitate the research activities and other operations of the National Cancer Program through the application of computer science, information technology, management science and operations research and by minimizing the organizational, operational and logistical constraints on scientific investigations and related activities which involve all aspects of data processing and computing, including collection, entry, purification, storage, computation, display and retrieval, analysis and dissemination.

Project Description

Objectives:

The Computer Science Section (CSS) provides consultation and assistance to the scientific and administrative staff of the National Cancer Institute, and as necessary, to other governmental agencies, private institutions, and individual investigators who collaborate with the National Cancer Institute in its mission. Consultation and assistance are given on individual studies, as well as on multi-center studies, involving epidemiologic, laboratory and clinical investigations, cancer surveillance programs, cancer control programs, and information and reporting systems for cancer centers.

The Section participates in research and development work with NCI investigators and computer scientists and specialists at universities and other institutions in order to develop new and improved methodologies in the application of computers to biomedical research.

Methods Employed:

Members of the Section apply systems analysis techniques to the planning, design, organization, administration, operation and evaluation of research projects having data management and statistical computing requirements. This technical assistance is provided through individual consultation and by directing the activities of computer systems analysts and programmers under contract to the National Cancer Institute.

The scope of the Computer Science Section's consulting activities is focused primarily on the Biometry Branch's scientific programs and studies, particularly the Surveillance Epidemiology and End Results (SEER) Program. Members of the Section have also increased consultation to Biometry Branch investigators on special studies. Resulting methodology and technology, where applicable, are also applied to other Field Studies and Statistics Program projects.

In the light of recent funding cuts experienced by the SEER Program, a major initiative of the Section was to develop and distribute uniform and standardized software routines to requesting registries in the SEER Program. In addition, the Section is assisting the registries in developing a software exchange program. The objective of this effort is to utilize to the fullest extent possible all software available within the Program that has a demonstrated applicability in any of the SEER registries.

Major Findings:

1. The Surveillance, Epidemiology and End Results Program.

This year, with the assistance of our computer support contractor, the Section completed the development and testing of a registry data management system for the Connecticut Tumor Registry. Documentation and actual computer routines were also disseminated to several state health departments to assist them in developing population-based cancer reporting systems. This project was under the overall direction of the Acting Section Chief, and Valerie Van Holten served as the lead analyst.

The Computer Science Section provided technical consultation to the Puerto Rico Department of Health Cancer Control Program. This year, Michael Stump and Mary Cusano provided specific consultation on the selection and procurement of an in-house computer and the recruitment of a systems analyst.

The Section has relinquished its operational responsibilities for processing the annual SEER submission tape to our data support contractor. Mary Cusano provided documentation and consultative assistance during the transition. Ruth Wolfson maintained the responsibility for processing of the SEER compatible tape from the Israel Tumor Registry.

Vivian Pelham, a recently recruited systems analyst, has assumed responsibility for automating the statistical algorithms required to generate match weights for cancer patient linkage purposes. Once completed, this software module will have applicability in all the SEER registries regardless of the matching scheme being used. Dr. Charles Brown, a statistician in the Office of the Chief, Biometry Branch, is providing statistical consultation.

The Computer Science Section organized and conducted a two-day workshop for the systems analysts and computer programmers of all SEER Program participating registries. The workshop was also attended by representatives of other population-based registries in the United States. The workshop focused on the sharing of computer-related technical information and software.

2. Other Biometry Branch Consultation.

Valerie Van Holten provided consultation to staff of the Biometric Research and Analytic Studies Section on the Family Health Study. Ms. Van Holten modified software developed for the Connecticut Tumor Registry system in order to provide comprehensive editing, updating and reporting on the Family Health Study data base. Mary Cusano and Calvin Hollingsworth provided technical consultation to Mr. Joseph Scotto of the Biometry Branch on studies of melanomas, foreign-born cancer patients and financial costs of cancer.

3. Division of Resources, Centers, and Community Activities.

Due to the fact that the new Division of Resources, Centers, and Community Activities does not yet have technical support, Michael Stump consulted with Mr. Thomas Dundon, project officer for the Statistical Analysis and Quality Control (SAQC) Coordinating Center, in the development of an administrative reporting system for the Comprehensive Cancer Centers.

4. Contract Selection Committee Activities.

Senior members of the Computer Science Section served as members on a number of contract selection committees. Michael Stump was invited by Ms. Barbara Bynum, Director, Division of Extramural Activities, to serve on the NCI Intramural Support Contract Review Committee. This committee reviews all contracts originating in the office of the Director, NCI and the Divisions, involving information systems and other managerial support systems. Mary Cusano and Valerie Van Holten served as members of selection committees for DCCP activities.

5. Frederick Cancer Research Facility (FCRF).

Michael Stump has been a member of the FCRF EDP Working Group for two years. This group is responsible for monitoring the data processing portion of Litton Bionetics FCRF contract. In addition, Mr. Stump also served on the contract selection committee responsible for recommending a new contractor to provide these services to FCRF for the next five years.

6. Other Consultation.

Mr. Stump served as a member of the contract selection committee for data processing support and analytical services for the National Institute of Drug Abuse on the Drug Abuse Warning Network (DAWN) project.

Significance to Biomedical Research and the Program of the Institute:

The systematic capture, organization, and display of complex and diverse data is of considerable importance in the planning, conduct, and management of the resource 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:

Consultation and technical support will continue to be provided to various NCI research activities.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04409-07 B

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Statistical Methodology Research

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D. P. Byar	Chief, Clin. & Diag. Trials Section	BB NCI
OTHER:	M. H. Gail	Medical Statistical Investigator	BB NCI
	S. B. Green	Medical Researcher	BB NCI
	D. L. Levin	Senior Investigator	BB NCI
	L. R. Muenz	Mathematical Statistician	BB NCI
	D. K. Corle	Computer Systems Analyst	BB NCI
	L. V. Rubinstein	Staff Fellow	BB NCI
	L. J. Wei	Cancer Expert	BB NCI
	S. Piantadosi	Medical Staff Fellow	BB NCI

COOPERATING UNITS (if any)

LAB/BRANCH
Biometry Branch

SECTION
Clinical & Diagnostic Trials Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.0	4.0	1.0

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☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to conduct research in statistical methods and computer techniques with particular emphasis on those appropriate to the analysis of data from clinical and diagnostic trials and epidemiological studies of cancer. Many of the problems studied under this project arise from the consultative activities of the Section. In the past year this research has included new work on the theory of survival analysis, sequential monitoring of clinical trials, precision of adjusted survival curves, study of methods for putting confidence limits on estimates of the median survival time, work on fitting survival models incorporating covariate information, inquiry into design considerations for secondary treatments in randomized clinical trials, the use of matching on time-dependent covariates in evaluating the importance of certain events occurring during follow-up studies, adaptations of logistic regression for sequences of binary responses, and a study of multiple comparisons in two-way tables which may be helpful in interpreting subset analyses in epidemiologic studies. Further refinements have been added to the interactive data analysis programs developed in this Section which permit rapid and sophisticated analyses required for effective consulting.

Project Description

Objectives:

Many statistical problems arise in the consultative activities of the Section (see Project No. Z01 CP 04260-22 B). The investigation of these problems, the development of new statistical methods, and adaption of existing methods for special problems constitute an important aspect of the work of the Clinical and Diagnostic Trials Section. The basic objective of this work is to enrich the repertoire of statistical methods appropriate for the analysis of clinical trial data; therefore, emphasis is placed on statistical applications. However, statistical theory and application are so closely related that some of the work has a distinctly theoretical flavor.

Methods Employed:

A variety of methods of biometry, statistics, probability, epidemiology, and computing techniques, with necessary modification as required by the particular problem.

Major Findings:

The research conducted under this project covers a wide spectrum of topics. Some of the principal subjects which have been studied during the past year are summarized below.

1. Theory of survival analysis. Dr. Rubinstein, with Dr. Eric Slud of the University of Maryland, has studied methods of analyzing survival data in the presence of censoring mechanisms which may not be independent of survival time for a given patient. Under various models of dependence for death and censoring times, they have explored the behavior of the standard Kaplan-Meier survival estimator and proposed new estimators.

2. Sequential monitoring of clinical trials. Dr. Gail, in collaboration with Dr. David DeMets of NHLBI and Dr. Eric Slud of the University of Maryland, has examined the properties of group sequential tests applied to survival data to see whether proposed boundaries indeed yield the desired size and power, to determine how often they afford early trial termination, and to determine how their properties are affected by anomalies such as that which arises when sicker patients accrue first. This work, which includes studies on the correlation structure of logrank increments, was presented in October, 1981, at the Institute for Mathematical Statistics Conference on Survival Data. Dr. Gail has completed a review of methods used to monitor clinical trials. This work was presented in a conference on statistics in oncology in 1981 and will be published.

3. Adjusted survival curves. Drs. Gail, Byar, and Green have continued their investigation of methods of "direct adjustment" for survival curves, so that comparisons between different groups of patients take account of differences in patient covariates. A detailed study has been made of an approach based on actuarial direct adjustment; this has been compared with saturated and unsaturated Weibull and Cox models, using both real clinical trial data and

simulated examples. Theoretical calculations of variances of the adjusted survival estimates and their differences have been performed.

4. Confidence limits for estimates of median survival time. Dr. Eric Slud of the University of Maryland has been continuing work with Dr. Byar on comparing by simulation the small sample performance of several methods of constructing confidence limits for estimates of median survival time obtained from censored and uncensored samples of sizes 20-75. In particular they have compared the performance of several methods recently reported in the literature with methods they have devised themselves. Results indicate that all methods behaved reasonably well but that their new method may have some advantages in terms of simplicity of computation and accuracy.

5. Estimating coefficients in multivariate survival models. Drs. Byar and Green have investigated maximum likelihood estimates of coefficients for survival models incorporating covariates, studying convergence problems using the Newton-Raphson algorithm. They studied situations in which there is no solution for additive Weibull or Cox models, and they investigated the use of the multiplicative Weibull model to obtain starting estimates for the other models.

6. Secondary treatments in randomized trials. In randomized clinical trials of progressive diseases, patients often require a change from the originally assigned therapies during the course of the study because of toxicity or treatment failure. If the secondary treatment affects the endpoint of interest, then comparison of the initial treatments may be distorted. Drs. Byar and Green have been examining various options in the design of such studies and are investigating the implications for analysis. For example, should all patients be assigned the same secondary treatment, should they be randomized to alternative secondary treatments, should cross-over designs be used, or should the choice of secondary therapy be left to the physician's discretion? They are particularly interested in determining whether there are situations in which it is possible to evaluate separately the consequences of the primary and secondary treatments.

7. Prospective matched analyses. In many cancer studies comparing randomized treatments, events occur during the course of the trial which give rise to important medical questions. For example, does reoperation for tumor recurrences improve survival? Even if such questions were not considered in designing the studies, it is nevertheless desirable to try to analyze the existing data to answer them. Drs. Byar and Green have been approaching this problem by analyzing the data as if they had arisen in a prospective matched study. A very flexible computer program has been written to achieve matching both on initial covariates and those measured at the time the event occurs. Statistical comparisons of the matched groups of patients with and without the event will be achieved by means of permutation tests.

8. Logistic regression for serial data. Dr. Muenz, together with Dr. Rubinstein, has derived a generalization of the logistic regression method appropriate for the situation in which each subject contributes a sequence of binary responses, rather than just one. Examples of this situation occur in the analysis of data from chronic diseases with states of relapse and remission.

The method focuses on the transitions between disease and non-disease states and relates the probability of such transitions to covariates such as initial disease stage and treatment. The model may also be used to compare, for planning purposes, the relative efficiency of longitudinal and cross-sectional designs for follow-up studies.

9. Chi-square tests for equality in proportions. Dr. Rubinstein analyzed the standard chi-square statistics used to test the equality of two binomial proportions. By discovering the close functional relationship between the tests with pooled and unpooled variance estimators, he showed that the power advantage of the latter (discussed in the recent literature) is due almost entirely to its greater size. Exact computations of test size and power for various examples of small and moderate sample size demonstrated the superiority of using the pooled variance estimator.

10. Subset analysis in epidemiologic studies. Dr. Eric Slud of the University of Maryland and Dr. Byar have been studying by simulation the probability of turning up wholly spurious "significant" effects in case-control studies when analyzing subtables resulting from cross-classification of two binary variables (e.g., race and sex). This work is continuing in an effort to formulate general guidelines concerning subset analysis.

11. A two-sample test for positive random variables. Dr. Gail has been working with Professor Joseph L. Gastwirth of George Washington University to develop a two-sample test based on the comparison of two Lorenz curves, which may be applied to economic income data and to uncensored survival data. This work corrects inappropriate tests in the literature.

12. Statistical approaches for studying sequences of bases in DNA molecules. Together with Dr. David Lipman of the NIADDK, Dr. Muenz has been investigating the manner in which the four DNA bases are linked to form molecules of great length. The molecules are well known to be made of linked triplets of bases (codons), but there is little literature concerning the view of each such molecule as a single realization of a stochastic process with four states. Viewed as a Markov process, the molecule has a best choice of order (number of previous bases needed to specify the "current" one) and may or may not be stationary. There may be overall stationarity, but non-stationary regions. Dr. Muenz has derived new graphic and analytic methods of investigating these questions. These descriptive techniques may be useful in characterizing DNA molecules in order to measure genetic distance.

13. Interactive data analysis programs. Dr. Green has continued to design, implement, and supervise improvements for a series of interactive computer programs for analyzing clinical trial data, and new features are continually incorporated into this package. This increases our capacity to respond efficiently to requests for sophisticated data analysis. Although designed primarily for data from clinical trials, these programs have proven useful for a variety of applications requiring data processing operations such as recoding, tabulation, and selection of records, as well as statistical analyses including computation of descriptive statistics, screening of variables for prognostic significance, contingency table analysis, logistic regression, and both unadjusted and adjusted survival analysis. These programs have been used

increasingly for the specialized data analyses performed by members of the Section, by other NIH scientists, and by some workers at other centers.

14. Other activities. Dr. Green was an invited participant in the Kroc Foundation Conference on Recent History of Randomized Clinical Trials. He dealt specifically with patient heterogeneity and the need for randomized clinical trials.

Dr. Green was an invited participant in the Macy Conference on Teaching the New Biology. This conference worked on important issues in medical school education, with emphasis on the implications of recent advances in the biomedical sciences and the impact of computer technology on medicine.

Dr. Byar has written two chapters on statistical methodology for publication in forthcoming books. The first concerns the identification and analysis of prognostic factors, and the second concerns survival data from heterogeneous populations using Cox and Weibull models.

Significance to Biomedical Research and the Program of the Institute:

Much of the work described above is directly applicable to the analysis of data collected in clinical trials of the treatment of cancer, analysis of data related to diagnosis and screening of cancer, and to epidemiologic studies of cancer. Beyond that, the opportunity to engage in research in statistical methods is a necessary and important aspect of the work of a consulting statistician. The prestige of the National Cancer Institute as a leader in biomedical research is enhanced by having on its staff statisticians of a high caliber who have made original contributions to their own discipline. Looking at it from another point of view, the opportunity for professional recognition by means of original research publications is essential in attracting to the Institute unusually talented statisticians and physicians interested in careers in biostatistics. Statistical theory and applied methodology is a rapidly expanding field. If the most appropriate methods are to be used in analyzing data related to cancer, then such individuals are essential to the program of the Institute.

Proposed Course:

Some of the separate projects described in this report have been completed, but some will be continued into the next year. In general the statistical research projects are suggested by problems which arise in our consulting work, so we anticipate that many new problems will be studied as they come to our attention in the next year. The Section will continue a program of balanced activities divided between research and consultation, theory and application.

Publications:

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Byar, D.P.: Analysis of survival data: Cox and Weibull models with covariates. In Miké, V. and Stanley, K. (Eds.): Statistics in Medical Research: Methods and Issues with Applications in Clinical Oncology. New York, John Wiley & Sons. In press.

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Gail, M.H., Lubin, J.H., and Rubinstein, L.V.: Likelihood calculations for matched case-control studies and survival studies with tied death times. Biometrika 68: 703-707, 1981.

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Slud, E.V.: Consistency and efficiency of inferences with the partial likelihood. Biometrika. In press.

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Slud, E.V., and Kedem, B.: On goodness of fit of time series models: an application of higher order crossings. Biometrika 68: 551-556, 1981.

Slud, E.V., and Wei, L.J.: Two-sample repeated significance tests based on the modified Wilcoxon statistic. J. Am. Stat. Assoc. In press.

Wei, L.J.: Asymptotic conservativeness and efficiency of Kruskal-Wallis test for K dependent samples. J. Am. Stat. Assoc. 76: 1006-1009, 1981.

Wei, L.J.: Asymptotically distribution-free simultaneous confidence region of treatment differences in a randomized complete block design. J. R. Stat. Soc. [B]. In press.

Wei, L.J.: Estimation of location difference for fragmentary samples. Biometrika 68: 471-476, 1981.

Wei, L.J.: Interval estimation of location difference with incomplete data. Biometrika 69: 249-251, 1982.

Wei, L.J.: The Friedman's Urn. In Kotz, S., and Johnson, N.L. (Eds.): The Encyclopedia of Statistical Sciences, Vol. III, New York, John Wiley & Sons. In press.

Wei, L.J.: The locally most powerful test for independence with missing data. Australian J. Statist. In press.

Wei, L.J., and Gehan, E.: The Gilbert's Test. In Kotz, S., and Johnson, N.L. (Eds.): The Encyclopedia of Statistical Sciences, Vol. II, New York, John Wiley & Sons. In press.

CONTRACT IN SUPPORT OF THIS PROJECT

INFORMATION MANAGEMENT SERVICES, INC. (NO1-CP-01025)

Title: Biomedical Computing Software Services

Contractor's Project Director: Janis Beach

Project Officer (NCI): Donald K. Corle

See Project No. Z01 CP 04409-97 B for description of contract.

Man Years: 9

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER ZO1 CP 04414-07 B

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Relation of Psychosocial Factors to Cancer Incidence and Prognosis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Bernard H. Fox Manager, Social Science BB NCI

COOPERATING UNITS (if any)

School of Public Health, University of California, Berkeley, California

LAB/BRANCH
Biometry Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.60	.50	.10

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The association of psychosocial factors--stress, personality or social factors--and cancer incidence, prognosis or mortality has been proposed and argued about. The possibility of such an association continues to be examined. A prospective study on the Western Collaborative Study Group (WCGS) relating Type A and Type B behavior patterns to later cancer mortality is being pursued. Preliminary results of a 17-year follow-up show nonsignificant age-adjusted relative odds of 1.32 in favor of Type A persons being at greater risk than Type B, thus maintaining a similar positive but nonsignificant finding for the 10-year follow-up carried out earlier. Epidemiologic data from other sources are being examined and a theoretical position is being developed in regard to stress, personality and their relationship to etiology and prognosis of cancer in humans.

Project Description

Objectives:

To determine if psychosocial factors--stress, personality or social factors--are associated with initiation or rate of progress of cancer.

Methods Employed:

In the Western Collaborative Study Group (WCGS) about 3000 middle-aged men were interviewed in 1960-61 and several risk factors for coronary heart disease were measured. Among them was Type A or Type B behavior pattern. Two opposing lines of theoretical reasoning were adduced--the first suggesting that Type A persons were at higher risk of later cancer than Type B; and the second that Type B persons were at higher risk than Type A. Data from 1960-69 showed 41 cancer deaths, the proportion among persons with Type A (unadjusted for age) being 1.36 times that of persons with Type B. The present data describe follow-up through 1977, with age adjustment. In addition, epidemiologic and immunologic literature is being analyzed to provide the basis for a theoretical position in regard to the issue of psychosocial effects on cancer.

Major Findings:

Through 1977, 97 cancer deaths occurred in the cohort. An age-adjusted estimate of relative odds showed that Type A persons were 1.32 times as likely as Type B to die of cancer, although the result was not significant. It is suggested, however, that the second line of reasoning had little evidence to support it in this study, even though the first line of reasoning could not be confirmed by these results. Further work will increase ascertainment and extend the data three more years. Age-adjusted relative odds estimates will be more stable under these conditions. Analysis of the literature suggests the position that in certain cases psychosocial factors may affect cancer incidence or progress. However, even if this is so, the total contribution is likely to be only a small portion of the total etiologic fraction; effects are likely to be site-specific; and under particular conditions psychosocial factors may protect against cancer while increasing the risk under other conditions.

Significance to Biomedical Research and the Program of the Institute:

Repeatedly NCI has received questions from professionals and the public about the relation among psychosocial factors and cancer. These questions were based on a long history of belief, speculation and purported evidence that certain psychosocial factors increase cancer risk. Evidence on humans, though voluminous, is not strongly persuasive, and in some cases, contradictory. Evidence in animals is stronger, and shows both increased and reduced susceptibility to cancer. Most of the human studies have not taken into account certain factors having an important impact on outcomes. Studies with improved design are, therefore, much needed. The present project fulfills that need, in part. A positive answer would give impetus to studies on specific psychosocial factors or stress and the humoral environment associated with increased cancer risk. A negative answer would tend to shift the balance of research effort away from the psychic components of carcinogenesis.

Proposed Course:

Follow-up of cancer mortality in the WCGS will be carried out through 1980, with increased ascertainment through mail inquiries. A 50 percent increase in the size of the group dying of cancer is expected, based on a 15-20 percent increase in ascertainment and the additional deaths during the further three years of follow-up. By the end of the year the incidence data should be ready for preliminary analysis, with about 200 cases of cancer expected. These data, collected under an NHLBI grant, will probably yield sufficiently stable numbers to allow a confident position as to whether Type A or B behavior patterns predict later incidence of cancer. Further theoretical issues in regard to the relationship of psychosocial factors to the etiology and prognosis of cancer will be investigated.

Publications:

Fox, B.H.: A psychological measure as a predictor in cancer. In Cohen, J., Cullen, J.W. and Martin, L.R. (Eds.): Psychosocial Aspects of Cancer. New York, Raven Press, 1982. pp. 275-295.

Fox, B.H.: Endogenous psychosocial factors in cross-national cancer incidence. In Eiser, J.R. (Ed.): Social Psychology and Behavioral Medicine. London, Wiley, 1982. pp. 101-141.

Fox, B.H., Stanek, E.J. III, Boyd, S.C. and Flannery, J.F.: Suicide rates among cancer patients in Connecticut. J. Chronic Dis. 35: 89-100, 1982.

Fox, B.H.: Psychogenic etiology and prognosis of cancer--present status of theory. In Christ, A. and Flomenhaft, K. (Eds.): Childhood Cancer: Impact on the Family. In press.

Fox, B.H.: Lifestyle factors making for high risk of cancer. Cancer Detection Prev. In press.

Ragland, D.A., Brand, R.J., Rosenman, R.H., Fox, B.H. and Moss, A.R.: Type A behavior and cancer mortality: A preliminary report. Perspectives on Behavioral Med. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04475-06 B

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

National Nonmelanoma Skin Cancer Incidence and Epidemiology Studies

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Joseph Scotto	Director, Health Services Director	BB NCI
OTHER:	Thomas R. Fears	Mathematical Statistician	BB NCI
	Jennifer Donaldson	Epidemiology Research Assistant	BB NCI

COOPERATING UNITS (if any)
Interfederal Committee on Stratospheric Ozone Protection (ICSOP)
Environmental Protection Agency
National Oceanic and Atmospheric Administration

LAB/BRANCH
Biometry Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3	2	1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project, a collaborative effort by the NCI, NAS, EPA and other Federal agencies, was initiated to provide more data relative to skin cancer and harmful solar ultraviolet. An urgent need for these data has existed since recent scientific reports have warned about the decomposition of stratospheric ozone by nitrogen oxides and chlorofluoromethanes (CFM's). Federal regulatory agencies have already recommended bans on the use of aerosol spray cans which use CFM's as propellants. Recent National Academy of Science (NAS) reports suggest a five to 16% ozone depletion during the next century. Critical reviews will be made of all pertinent information dealing with estimates of increased human skin cancer due to excess amounts of UV-B reaching the earth's surface. As mandated by Public Law 95-95 (amendment to the Clear Air Act of 1977), the NCI is continuing its investigations in this area. As more surveys are included more reliable estimates of the degree of morbidity from skin cancer will be derived. In addition, new estimates of skin cancer risks adjusted for certain host and environmental factors (including those other than UV exposure) will be ascertained (e.g., ethnicity, skin complexion, exposure to ionizing radiation, coal tar, skin conditions other than cancer, occupation).

Project Description

Objectives:

The major objectives of this study are to provide epidemiologic data relative to the etiology of nonmelanoma skin cancer, and to evaluate the potential human health effects of harmful solar ultraviolet (UV-B, i.e., wavelengths between 290 nm and 320 nm). In particular, (1) to provide information necessary to ascertain the human health effects of UV radiation resulting from the anticipated ozone depletion in our biosphere; (2) to provide supportive basic data to reduce the degree of uncertainty in dose-response estimators; (3) to provide specific data on populations suspected to be at high or low risk of skin cancer; (4) to provide an estimate of the proportion of skin cancer in the community relative to other cancers; (5) to identify local factors in the community that may contribute to the risk of skin cancer; (6) to provide basic data in support of anticipated needed preventive care programs in this community; (7) to provide basic epidemiologic data to elucidate the multifactorial etiology of skin cancer; and (8) to estimate trends in skin cancer morbidity; and (9) to develop dose-response models which may explain initiator/promoter factors associated with UV-B radiation exposure.

Methods Employed:

Ten population-based registries were developed in various geographic locations of the contiguous United States where latitudes ranged from 47° N to 30° N. Incidence information on newly diagnosed nonmelanoma skin cancer (basal cell and squamous cell carcinoma) was collected in accordance with protocols developed by NCI. Surveys were conducted in a uniform way so as to provide data bases comparable with earlier surveys conducted by NCI in 1971-72. Relevant environmental epidemiologic data and other information on host factors were also collected using case/control methods on a sampling basis. Interviews were conducted via telephone communication. In addition UV-B measuring devices, i.e., Robertson-Berger meters, were installed and maintained at these locations in collaboration with the National Oceanic and Atmospheric Administration (NOAA). This federal agency will provide data from these locations to the NCI as they come available. Ten registries are now completed: Seattle, Minneapolis, New Hampshire/Vermont, Detroit, Utah, San Francisco, New Mexico, Atlanta, San Diego and New Orleans. Population estimates for intercensal years were developed in conjunction with demographers at the Bureau of the Census. Where possible adjustments were made for Hispanic populations who are at low risk to skin cancer. Detailed analyses provided will be specific for age, sex, cell type, anatomical site and geographic area.

Major Findings:

A final report of the basic incidence data for eight locations surveyed in 1977-78 has been published. The new data suggest that among Caucasians in the United States the annual incidence of nonmelanoma skin cancer may be well over 400,000. This annual amount is at least half that observed for all other cancers combined. While no significant increases in UV-B or

decreases in stratospheric ozone depletion may be detected in the short time since these measurements were being made by NOAA, there appears to be an increase in the incidence of basal cell carcinomas of the skin among Caucasians in Minneapolis-St. Paul and San Francisco-Oakland. The increase, 15 to 20 percent over a six year period, was not noted for squamous cell carcinomas for all anatomical sites combined. The greatest increases were seen for the male trunk and upper extremities. Lip cancers also appear to have increased among women; and, eyelid cancers have decreased for both men and women in these two geographic areas. Overall, 80 percent of the basal cell tumors arise in the face, head or neck; but the predilection for these exposed sites is somewhat less for squamous cell carcinomas. The risk for males is about two-fold greater than that for females, with greater excesses for males among squamous cell carcinoma. The north-south incidence gradient appears to be steeper for squamous cell carcinoma than for basal cell carcinoma. The amount of skin cancer among the non-Caucasian races was quite negligible. Among whites, those who do not burn and could tan easily were less likely to develop skin cancers. Those with histories of treatment for skin diseases, such as ionizing radiation exposure for acne or moles, appear to be at greater risk. Fair skinned individuals of Irish or Scottish ancestry were, as expected, more susceptible to skin cancer.

A special study of psoriasis and skin cancer was conducted in collaboration with Dr. Robert Stern (director of the National PUVA study). Preliminary findings indicate that psoriatic patients have an increased risk of skin cancer, which may be due to the combination of tar and UVB exposures over long time periods. Another special study was done in collaboration with Dr. Ken Kraemer of the Dermatology Branch, NCI. Multiple skin cancers, melanomas and other cancers, were noted to occur among Xeroderma Pigmentosum (XP) patients which may not be attributable to sunlight exposure. Age-specific incidence patterns of skin cancer among XP patients, when compared with those of the general population, reveal the accelerated pace in which this genetically disposed group develops malignant tumors.

Using eight new locations and two old locations in our analyses of the dose-response relationship of UV-B and skin cancer, we have substantially improved the degree of reliability in our estimates. Mathematical models applied to the new data indicate that a one percent increase in UV-B may result in a somewhat less than two percent increase in skin cancer. This implies that stratospheric ozone reductions of one percent may eventually result in a four percent increase in skin cancer. With large decreases in stratospheric ozone (over 10%) the subsequent increases in skin cancer may be even greater than four-fold. The increases for squamous cell types appear to double those for basal cell types of skin cancer. These estimates are currently being used by the NAS in their reports and recommendations to Congress. Further analyses of skin cancer incidence patterns by age, sex, cell type and geographic area utilizing power functions suggest that UVB has promoter as well as initiator characteristics, and that these data supply a good model for interpreting multistage theory phenomenon. New estimates will be developed utilizing information recently obtained for New Hampshire/Vermont and San Diego, California.

Significance to Biomedical Research and the Program of the Institute:

These data, when combined with those from earlier surveys, provide a basis for evaluating the potentially harmful health effects of ozone depletion in our biosphere. They also provide new leads on the relative importance of host factors and environmental factors other than UV-B which may contribute to increased risk for this disease. Results from these data help regulatory agencies establish guidelines for use of man-made products, such as chlorofluoromethane propellants in aerosol spray cans and refrigerants in air conditioners, which may effect human health. The new data provide current estimates of the degree of morbidity from skin cancer in various parts of the United States, and elucidate the need for cancer prevention programs. The dose-response models provide epidemiologic examples of non-ionizing radiation induction and skin cancer morbidity which may parallel those for ionizing radiation and cancer of non-skin sites.

Proposed Course:

Results from the case/control interview study will be published in a new monograph; thus, elucidating the environmental and host factors associated with skin cancer incidence. Intensified analyses of the newly developed data bases will continue. Incidence and dose-response analyses will include new estimates for the ten locations surveyed during the three year period, 1977-1980. In addition, new leads from the current interview surveys (e.g., excessive radiation exposure, high risk occupation/industry groups, skin conditions such as psoriasis, XP, residence mobility such as movements from northern to southern "sun spots", etc.) will be pursued. Because of the apparent difference in incidence patterns for squamous cell carcinoma, more detailed analyses and studies will be pursued which will isolate cell type and etiology. Seasonal trends and cyclic patterns will also be investigated for skin cancers as well as other malignancies (e.g., skin melanoma, eye melanoma, thyroid cancer, etc.).

We propose doing field studies utilizing newly developed personal UV-B dosimeters. At present we know how much UV-B may reach the surface of the earth, but we are severely lacking in information on the relative amounts which may reach exposed surfaces of the human skin. Our plans also include conducting new skin melanoma case/control studies into the major framework of this project. The etiology of skin melanoma also reflects an association of increased risk at locations of high insolation, but the relationship is complicated by the apparent contradiction of anatomical site distributions of malignant melanoma lesions and other associated factors.

Publications:

Fears, T.R., and Scotto, J.: Changes in skin cancer morbidity between 1971-72 and 1977-78. JNCI. In press.

Fears, T.R., and Scotto, J.: Estimating increases in skin cancer morbidity due to increases in ultraviolet radiation exposure. Cancer Investigation. In press.

Kraemer, K.H., Lee, M.M., and Scotto, J.: Early onset of skin and oral cavity neoplasms in Xeroderma Pigmentosum. Lancet, 1982, pp. 56-57.

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Nam, J., and Scotto, J.: Interpretation of Edwards' Method in Seasonality Studies, The Author's Reply. Am. J. Epidemiol. In press.

Scotto, J., Fears, T.R., and Fraumeni, J.F.: Incidence of Nonmelanoma Skin Cancer in the United States. U.S. Department of Health and Human Services, Public Health Service, Publication No. 82-2433. Washington, D.C., U.S. Government Printing Office, 1981, 113 pp.

Scotto, J., Fears, T.R., and Fraumeni, J.F. Jr.: Solar Radiation. In Schottenfeld, D. and Fraumeni, J.F. Jr. (Eds.): Cancer Epidemiology and Prevention. Philadelphia, W.B. Saunders and Company, 1982, 254-276.

Scotto, J., and Fraumeni, J.F. Jr.: Skin (Other than Melanoma). In Schottenfeld, D. and Fraumeni, J.F. Jr. (Eds.): Cancer Epidemiology and Prevention. Philadelphia, W.B. Saunders and Company, 1982, 996-1011.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04482-06 B

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Psychological and Other Predictors of Remission Time After Surgery in Clinical Stage II Melanoma

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Bernard H. Fox	Manager, Social Science	BB NCI
OTHER:	Daniel Van Kammen	Unit Chief, Section on Neuropsychopharmacology	BP NIMH

COOPERATING UNITS (if any)
Immunology Branch, NCI

LAB/BRANCH
Biometry Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.11	.1	.01

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In this study, an attempt has been made to assess the ability of certain psychological measures to discriminate those stage II melanoma patients who relapsed within one or two years after axillary node surgery, and to test their ability to predict such relapse in an independent sample. From an initial sample of 31 cases, the successful discriminators, number of nodes and a psychological variable--subjective effect of having melanoma--held up as predictors in an independent sample of 33 cases with about equal success. Analyses of the successful one-year predictors of relapse suggested no such capability for either two-year overall relapse or two-year overall mortality, but separate analyses of the two samples showed a significant difference between them, results being in opposite directions. Mortality for all 64 cases in the sample was ascertained for two years following testing, and an attempt is being made to obtain complete three-year relapse and mortality data.

Project Description

Objectives:

To test hypotheses that certain psychological measures will predict relapse status and mortality, after controlling for known physical discriminators.

Methods Employed:

Thirty-eight psychological measures (MMPI scales, SCL-90 psychiatric symptoms scale, and several other measures) were given to 31 Stage II melanoma patients a few weeks after surgical removal of axillary lymph nodes. A number of physical measures were also taken on these patients. Ability of each of the psychological and physical measures to discriminate those who did and did not relapse before one and two years was determined, both by means of a clinical algorithm and by discriminant analysis. After identifying discriminators, their ability to predict was tested in an independent sample of 33 patients. Further analysis of relapse at two years and mortality at two years has been completed, and relapse and mortality at three years is being investigated.

Major Findings:

In the initial sample, aside from the MMPI, only the number of nodes and the psychological variable, subjective effect of the melanoma hospitalization on life-style, discriminated successfully. When tested separately on a new sample of 33 patients, number of nodes and subjective effect of melanoma on life-style also discriminated relapsers successfully. Some MMPI scales discriminated on the first sample and some did on the second, but none did on both. No physical variables other than number of nodes discriminated relapsers successfully. Analysis of the one-year predictors of relapse showed no such capability for either two-year relapse overall or two-year mortality overall, but separate analyses of the two samples showed results in opposite directions, suggesting sample differences or strong variability. The preliminary indications of no difference between the groups' relapse and mortality experiences at two years have been confirmed.

Significance to Biomedical Research and the Program of the Institute:

If psychological variables are found to have independent weight in predicting length of remission, their relationship with some physical attributes should be sought to establish why they are predictors. The most important outcome would entail a cause-effect relationship of psychic state and such physical variables, thus permitting change and possible extension of remission. Even without such a relationship, a predictive capability of psychological measures would be valuable to the clinician, and under some conditions, perhaps to the family and to the patient. These results offer only interesting hypotheses about personality in the acute surgical case and shortly thereafter, but fail to support psychological relationships with relapse or death in the second year.

Proposed Course:

Relapse and mortality experience in both samples will be ascertained completely for the third year and analysis will be carried out for that year. It is planned to publish the combined results of the second and third years.

Publications:

None.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 05158-02 B

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)
Morbidity Among Long-Term Survivors of Childhood Cancer and Their Offspring

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Max H. Myers, Ph.D., Chief, Biometric Research and Analytic Studies Section		BB NCI
OTHER:	John J. Mulvihill	Chief, Clinical Genetics Section	CEB NCI
	Sandra C. Abbott	Statistician (Health)	BB NCI
	Roger R. Connelly	Statistician (Health)	BB NCI
	M. Darlene Naughton	Computer Systems Analyst	BB NCI
	Margot R. Hanson	Statistician (Health)	CEB NCI

COOPERATING UNITS (if any) University of Iowa, University of Kansas, University of Texas System Cancer Center, Yale University School of Medicine, California State Department of Health, ORI, Inc.

LAB/BRANCH
Biometry Branch

SECTION
Biometric Research and Analytic Studies Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	2	1

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Approximately 50 percent of required interviews have been completed for the study. Some data collection centers will be completing all work including related medical record abstracting by fall 1982 while others will require several more months to conclude their work. A computer system for data entry, editing and updating is nearing completion.

Project Description

Objectives:

To determine the long-term effects of cancer and its treatment on patients who reached adulthood after having cancer during childhood years.

To assess any adverse effects that might have carried over to offspring of childhood cancer survivors.

To test genetic theories of tumor etiology.

Methods Employed:

A total of 2,854 survivors of childhood cancer were identified at the five cooperating centers. Up to two sibling controls are being sought for each case. Each subject (case or control) is being asked to provide information gathered by means of an in-person interview regarding the following:

- . fertility problems
- . pregnancy wastage
- . congenital anomalies in cases and offspring
- . second primary neoplasms in cases
- . cancers in offspring
- . psychosocial morbidity

Analytical techniques will include methods for risk estimation for matched case-control triads and methods based upon stratification.

Major Findings:

This project is in the data collection phase so there are no findings to report.

Significance to Biomedical Research and the Program of the Institute:

Prior to this study there has been very little information concerning possible residual effects of cancer and its treatment on surviving childhood patients and their offspring. This study is large enough to identify important sequel events for children treated for cancer during a period prior to the most recent advances in combination chemotherapy and multi-modal therapies.

Proposed Course:

Data collection is expected to be completed by February 1983. Preliminary examination of the data will be done as sufficient numbers of cases and controls are entered into the computer file. Published results are expected by fall of 1983.

Publications:

None

CONTRACTS IN SUPPORT OF THIS PROJECT:

CALIFORNIA STATE DEPARTMENT OF HEALTH (N01-CP-01000)
UNIVERSITY OF IOWA (N01-CP-01001)
MEDICAL CENTER OF THE UNIVERSITY OF KANSAS (N01-CP-01036)
UNIVERSITY OF TEXAS SYSTEM CANCER CENTER (N01-CP-01035)
YALE UNIVERSITY SCHOOL OF MEDICINE (N01-CP-01002)

Title: Morbidity Among Long-Term Survivors of Childhood Cancer and Their Offspring

Contractor's Project Directors: Dr. Donald F. Austin, Dr. Howard B. Latourette, Dr. Frederick F. Holmes, Dr. Louise C. Strong Dr. J. Wister Meigs

Project Officer (NCI): Dr. Max H. Myers

Objectives: To determine the long-term effects of cancer and its treatment on patients who reached adulthood after having cancer during childhood years.

To assess any adverse effects that might have carried over to offspring of childhood cancer survivors.

To test genetic theories of tumor etiology.

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- . congenital anomalies in cases and offspring
- . second primary neoplasms in cases
- . cancers in offspring
- . psychosocial morbidity

Analytical techniques will include methods for risk estimation for matched case-control triads and methods based upon stratification.

Major Findings: This project is in the data collection phase so there are no findings to report.

Significance to Biomedical Research and the Program of the Institute: Prior to this study there has been very little information concerning possible residual effects of cancer and its treatment on surviving childhood patients and their offspring. This study is large enough to identify important sequel events for children treated for cancer during a period prior to the most recent advances in combination chemotherapy and multi-modal therapies.

Proposed Course: Data collection is expected to be completed by February 1983. Preliminary examination of the data will be done as sufficient numbers of cases and controls are entered into the computer file. Published results are expected by fall of 1983.

Date Current Contract Initiated: August 29, 1980

Current Annual Level: $1,014,749

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 05176-02 B

PERIOD COVERED
October 1, 1981 through September 30, 1982

TITLE OF PROJECT (80 characters or less)

Statistical Methodology - Consultation and Research

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Charles C. Brown, Ph.D. Mathematical Statistician BB NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Biometry Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.3	1.0	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to collaborate with NCI and other Federal researchers on statistical problems related to many areas of cancer research, and to conduct research on the development of statistical methodology which is particularly applicable to the analysis of data from experimental and epidemiological studies of cancer. Particular subjects of interest are methods of quantitative risk assessment, methodology for analyzing survival curves and proportions, and the analysis of epidemiologic studies, based on the multistage theory of carcinogenesis.

Project Description

Objectives:

The principal objectives are (1) to consult and collaborate with NCI and other Federal agency researchers on statistical problems related to cancer research; and (2) to conduct research on the development of statistical methodology which is applicable to the design, analysis and interpretation of experimental and epidemiologic studies of cancer.

Methods Employed:

The methods employed are the modern theories of mathematical statistics, probability, applied mathematics, and epidemiology. The development of computer programs is often used in the application of these methods.

Major Findings:

The research and consultation conducted under this project covers a wide spectrum of topics which are summarized below.

New methodologies for the analysis and interpretation of epidemiologic studies of occupational carcinogenesis based on the multistage theory of carcinogenesis are continuing to be developed in collaboration with Dr. Kenneth C. Chu of the NIEHS. Methods for the analysis of cohort studies have been developed and applied to a cohort of men occupationally exposed to arsenic. Our conclusions are that level and duration of exposure and age started exposure are important risk factors and imply that arsenic exerts its carcinogenic influence primarily at a late stage in the carcinogenic process. Similar methodologies are currently being developed for the analysis of case-control epidemiologic studies. These methodologies will be applied to studies of lung cancer associated with arsenic exposure, and mesothelioma associated with asbestos. The latter work is being conducted in collaboration with Dr. William Blot and Linda Brown of the Environmental Epidemiology Branch.

A new methodology for computing sample sizes required for comparing two Poisson outcomes has been developed in collaboration with Dr. Sylvan B. Green of the Clinical and Diagnostic Trials Section of the Biometry Branch. The work is applicable to epidemiologic and experimental studies of rare disease occurrence and has been accepted for publication.

A methodology, submitted for publication, was developed for the comparison of two or more relative survival curves adjusted for other prognostic variables. This methodology will provide the SEER Survival System with statistical comparisons of relative survival experience rather than observed survival.

In collaboration with Dr. Jurgen Wahrendorf of the IARC, new approaches are being developed for assessing interactive effects of two or more carcinogens in the analysis of epidemiologic case-control studies. This work will extend our previous work on interaction in experimental animal studies.

An analysis of urethane-induced lung tumor growth rates is being conducted in collaboration with Dr. Michael Dourson of the EPA and Dr. Ellen O'Flaherty of the Institute of Environmental Health, University of Cincinnati. The primary focus of this research is to compare the growth rates of lung adenomas induced by acute or chronic exposure to urethane.

Collaboration with John W. Horm of the Biometry Branch is continuing on the development of a system for monitoring the cancer incidence data being collected by the SEER Program. In addition, we are involved in the development of a computerized cancer incidence and mortality information retrieval system for researchers to easily access the SEER data base.

In collaboration with Herman Heise and Lynn Ries of the Biometry Branch, development of a series of U.S. life tables for ethnic groups other than White or Black is progressing. These life tables are planned to be used with the SEER Survival System to compute relative survival rates for these other ethnic groups.

Consultation has continued in the development of a record linkage component for the management of SEER data systems. This consultation is planned to continue through the implementation of the system for various SEER registries.

In the area of the assessment of human carcinogenic risk based upon epidemiologic and experimental animal studies, consultation and collaboration with researchers from the Federal regulatory agencies is continuing. The primary work in this area over this time period has been (1) preparation of a chapter in the forthcoming book, "Principles for the Evaluation of Toxic Hazards to Human Health," edited by Dr. Robert G. Tardiff and Dr. Joseph V. Rodricks; (2) collaboration with Dr. Marvin Meistrich of the University of Texas System Cancer Center on the assessment of risk to the human reproductive system; and (3) general consulting with regulatory agency researchers in the area of statistical methodology and data interpretation.

Significance to Biomedical Research and the Program of the Institute:

The relationship of statistical theory to experimental research and data analysis is an important aspect of carcinogenic research. The research objectives of the Institute and other workers in cancer research are promoted by the continued work on the development of new statistical methodologies such as those presented above. The opportunity for conducting fundamental research on mathematical statistics is essential to achieve professional peer recognition. More importantly, the possibility of doing such research is necessary to carry out consulting activities at the highest professional level.

Proposed Course:

Much of the work described in the major findings will be continued. Other research and consulting projects, not described above, are also likely to be

initiated. The current balance between research and consultation, theory and application, is anticipated to continue unchanged.

Publications:

Brown, C.C.: Comment on the regulation of carcinogens by Crouch and Wilson. Risk Analysis 1: 105-106, 1981.

Brown, C.C.: On a goodness of fit test for the logistic model based on score statistics. Commun. Stat. - Theor. Meth. II: 1087-1105, 1982.

Brown, C.C.: Approaches to Intra-species Dose Extrapolation. In Tardiff, R.G., and Rodericks, J.V. (Eds.): Principles for the Evaluation of Toxic Hazards to Human Health. In press.

Brown, C.C., and Chu, K.C.: A new method for the analysis of cohort studies: Implications of the multistage theory of carcinogenesis applied to occupational arsenic exposure. Environ. Health Perspect. In press.

Brown, C.C., and Fears, T.R.: Exact significance levels for multiple binomial testing with application to carcinogenicity screens. Biometrics 37: 763-774, 1981.

Brown, C.C., and Green, S.B.: Additional power computations for designing comparative Poisson trials. Am. J. Epidemiol. In press.

Krewski, D., and Brown, C.: Carcinogenic risk assessment. A guide to the literature. Biometrics 37: 353-366, 1981.

CONTRACT INDEX

BIOMETRY BRANCH

CONTRACT NARRATIVE
OFFICE OF THE CHIEF

BERGEN, UNIVERSITY OF (NORWAY) (NO1-CP-91043)
MINNESOTA, UNIVERSITY OF (NO1-CP-91021)

Title: Analyses of Prospective Studies on Diet, Cancer and Other Diseases in Selected Migrant Populations (Norwegian)

Contractor's Project Directors: Dr. Leonard Schuman (NO1-CP-91021)
Dr. Erik Bjelke (NO1-CP-91043)

Project Officers (NCI): Mr. Joseph Scotto
Dr. John Gart

Objectives: To determine the reasons for difference in cancer incidence and mortality in sedente populations and migrant populations of the same ethnic and genetic composition. To pursue leads relative to diet variations among the two population groups.

Methods Employed: Prospective follow-up studies of two basic cohorts (one in the USA and one in Norway) are being conducted. Dietary and demographic data obtained from responses to mail-out questionnaires distributed in 1966-67 form the original baseline data to be compared with subsequent histories of disease (via cause of death information from death certificates in the USA, and cancer registration as well as information on deaths from all causes in Norway).

Major Findings: A paper has been published and two submitted which describe the study cohort and first findings on 11-1/2 years of follow-up for the Lutheran Brotherhood study. There were 467 cancer deaths among 17,818 respondents during an 11-1/2 year follow-up of men insured by the Minnesota-based company. Cancers of the digestive system accounted for 167 deaths. Deaths from all causes totaled 2,305, with 1,025 due to heart diseases. Demographic comparison of the Lutheran Brotherhood study with the general population of United States males indicated that these respondents were (a) younger, (b) less likely to use alcoholic beverages, (c) less likely to smoke cigarettes, (d) more likely to have a higher socio-economic standard, (e) more likely to live in rural farming areas, and (f) more likely to live longer than the general population of U.S. males. A summary of findings, many of which are not conclusive at this time, include the following:

- There was a positive association (i.e., higher exposure and higher risk) reported for stomach cancer mortality and the consumption of eggs, milk, soup, bacon or side pork, chicken, and apples.
- No statistically significant negative associations (i.e., higher exposure and lower risk) were reported for stomach cancer (only 34 deaths accrued); however there were suggestions of a protective effect for high consumptions of tomatoes, cabbage, vitamin C, and green salad.

- With respect to colon cancer mortality, positive associations were noted for high consumption of the following food items: fresh or frozen fish, smoked or salted ham-pork, vegetable soup, rutabaga, cauliflower, canned fruit and beer. Excess risks were also reported for high consumption of certain food combinations, such as high animal fat and low vegetable or low grain fiber; high meat protein and low vegetable or grain fiber; and, total meat and low vegetable or low grain fiber.
- No statistically significant negative associations were reported for colon cancer and specific dietary items.
- Alcohol consumption was positively associated with pancreas cancer mortality even when controlling for age or smoking habits; but, there were no significant associations found for alcohol consumption and mortality from cancers of the rectum, stomach, lung, bladder, prostate, or leukemia.
- With respect to cancer of the prostate, positive associations were noted for high consumers of smoked or salted ham or pork, pancakes, polysaturated fat and vegetable fiber. A protective effect was apparent for high consumption of bananas and flatbread. These statistical associations, however, remain unexplained.
- High levels of vitamins A and C are reported to be negatively associated with lung cancer. Vitamin C consumption is also suggestive of providing a protective effect against stomach cancer.

With respect to the Norwegian cohort, several papers have been published, and 4 abstracts have been prepared for the XIII International Cancer Congress to be held this fall in Seattle, Washington. There were 16,713 respondents to the Norwegian dietary questionnaire which are being analyzed. Among these 1,577 new cases of cancer were diagnosed, (1,357 among males) and 900 cancer deaths (793 among males) were recorded during 11-1/2 years of study follow-up. Of the gastrointestinal cancers, there may be enough cases to conduct meaningful analyses for stomach, colon, rectum, and pancreas with 135, 77, 52 and 50 cases, respectively, among males. Among other cancers of high frequency among males, those of the lung (170 cases) are also being studied concurrently. Several analyses look at specific dietary exposures, such as alcohol consumption and smoking habits, and include all cancer sites in the analyses. The inclusion of incidence cases in the Norwegian data base make this study a more powerful one compared to the Minnesota study, where only death statistics may be evaluated. The Norwegian study also includes 259 deaths among males from gastrointestinal cancers: stomach (118), colon (43), rectum (36), pancreas (48), and other digestive organs (16). As expected, there are many more stomach cancer deaths among Norwegians than among U.S. migrants from Norway. Deaths from all causes among the Norwegian cohort amounted to 4,107 of which 2,245 were from heart disease and other circulatory system diseases, including hypertension and cerebrovascular diseases.

High vegetable consumption appears to be associated with decreased colorectal cancer risk. Vegetables such as cabbage, rutabaga, red beets, carrots and cauliflower are consumed more frequently in Norway; lettuce, cucumber, tomatoes, peas, beans and maize are consumed more frequently in Minnesota. Beer consumption and processed meat consumption may be associated with higher colon cancer risks.

The index for vitamin C intake includes fruits, potatoes and vegetables, weighted by their vitamin C content. The Norwegian cohort with 11-1/2 years follow-up experience shows that vitamin C may be associated with low risk to stomach cancer. The Norwegian data also tend to corroborate the Minnesota findings for vitamins A and C with respect to lung cancer.

Special studies on the effects of coffee consumption were conducted in both Norway and Minnesota. Results were obtained during the project officers' site visit to Norway and presented to DCCP's Board of Scientific Counselors this past February. In both cohorts no significant associations were found for coffee consumption and either pancreatic or bladder cancer. These results were in contrast to case/control studies reported by Brian MacMahon of Harvard, using data from the northeastern United States.

Proposed Course: During the past year computer files were generated in Norway to accommodate the analyses of the effects of diet on cancer incidence and mortality. Specific statistical methodologies developed at NCI and programmed for computer analyses in Norway were principally used for the current analyses of the Norwegian data base. Collaborative papers including authors from the NCI, Norway, and Minnesota will be written on several topics, e.g., dietary factors and lung cancer, and alcohol consumption and cancer mortality. Because of the many cases of cancers from nondigestive sites, we project that future analyses will include more detailed studies on cancers of the prostate (263 cases), urinary bladder (95), skin (208), leukemia and lymphoma (105). The funding for this project will end by September 1982. We expect to accelerate NCI analyses of these prospective studies when the analytical computer tapes are submitted. We anticipate developing a software computer system, which will facilitate investigator interactions with computerized data bases and newly developed statistical methodology. We plan to continue updating the Lutheran data base during the next several years; and, as the number of new cancer deaths increases, we will re-evaluate the findings for specific dietary effects.

Future plans also include the possibility of re-interviewing, via mail questionnaire, the respondents of the Lutheran Brotherhood study who are still alive. This will provide information on significant changes in dietary habits during the past 10 to 15 years.

Significance to Biomedical Research and the Program of the Institute: This project is one of a few prospective, long-term epidemiologic studies designed to discern the influence of diet as well as other environmental factors on the incidence and mortality of cancer. Isolation of these environmental elements will be useful in providing new leads on cancer etiology and in providing guidelines for preventive measures.

Date Contract Initiated: June 1966 (NO1-CP-91021)
September 1979 (NO1-CP-91043)

Current Annual Level: $129,132 (NO1-CP-91021)
$ 82,186 (NO1-CP-91043)

KUAKINI MEDICAL CENTER (NO1-CP-61060)

Title: Study of Cancer Among Japanese Migrants

Contractor's Project Director: Dr. Abraham Nomura

Project Officers (NCI): Dr. Earl S. Pollack and Dr. David Levin

Objectives: The purpose of this contract 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. 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 have been employed, including case-control and cohort studies. The matched case-control technique is particularly useful in this kind of study. Pathology protocols have been completed for cases in the study series for several sites (stomach, colon, rectum) and considerable emphasis has been placed on the correlation of the epidemiological and pathology findings. A cohort of Hawaiian Japanese males assembled by the National Heart, Lung and Blood Institute for studies of cardiovascular disease has been examined for information relevant to gastrointestinal cancers and their subsequent cancer morbidity experience is being monitored.

Major Findings: During the year an analysis was carried out in the Biometry Branch relating usual alcohol consumption to the occurrence of these cancers. The analysis suggested a trend relationship between total alcohol consumption and cancers of the rectum and lung, but not for any of the other three sites. The major contributor to the relationship with rectal cancer appeared to be high beer consumption and for lung cancer, high wine and whiskey consumption. Because of the nature of these findings and the numbers of cases involved, it was decided that we would use another year of follow-up information to determine whether these findings still hold. Preliminary analysis of the lung cancer data indicate that they do, but they are of marginal statistical significance. Meanwhile, work on this project continues in Honolulu in a number of areas, including analysis of dietary fiber, Vitamin A and Vitamin C intake; study of family history of stomach cancer; study of mutagens in relation to intestinal metaplasia of the stomach and a number of others.

Significance to Biomedical Research and the Program of the Institute: 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: Since the involvement of NCI staff in the research is not extensive, it was thought that this project might be more appropriate for grant support. Accordingly, the Principal Investigator was encouraged to apply for a grant. This was done and the results of the review of the grant proposal should be available shortly. If this is successful, the contract

will be terminated when the grant funding begins. Meanwhile, the staff of the Biometry Branch will continue to carry out some of the analyses of the longitudinal data.

Date Contract Initiated: June 1971

Current Annual Level: $542,193

SOUTHERN CALIFORNIA, UNIVERSITY OF (NO1-CP-91027)

Title: Study of Cancer Incidence in the South Pacific

Contractor's Project Director: Dr. Brian E. Henderson

Project Officer (NCI): Dr. Earl S. Pollack

Objectives: To develop a comprehensive cancer incidence reporting system for the South Pacific, including Melanesia, Polynesia and Micronesia, so that incidence rates for the various ethnic groups in those islands can be compared with those of their counterparts in Hawaii and with incidence rates in general elsewhere in order to generate additional hypotheses regarding cancer etiology.

Methods Employed: Working through the South Pacific Commission as well as directly with personnel on the island, develop and improve the reporting of all cancers occurring among residents of the South Pacific islands. Data are collected on a standard form and sent to the University of Southern California (USC) for processing. Tabulations and analyses will be carried out by USC staff and data tapes will be sent to NCI for further analyses.

Major Findings: Data collection is now being carried out with an added impetus resulting from a meeting of a consultant to the project with the Program Directors in all of the countries that are members of the South Pacific Commission. Cancer incidence data have been tabulated by the project staff for the various geographic areas of Papua, New Guinea and comparisons have been made through proportionate incidence rates. It is not possible at this time to determine the degree of completeness and accuracy of these data. Similar tabulations have been obtained for American Samoa with the same kind of limitations.

Significance to Biomedical Research and the Program of the Institute: The project is highly relevant to work now going on in the Biometry Branch for at least two reasons: 1) it will provide cancer incidence data on a set of unique population groups in a way that will permit comparison with those for populations now being studied through the SEER Program; 2) these particular population groups represent countries of origin for many of the population groups in Hawaii and therefore provide an opportunity for further formulation of specific hypotheses, although the identification of these groups as migrants to Hawaii is indeed difficult.

Proposed Course: It is anticipated that further tabulations will be produced this year and that at the termination of the contract in September 1982 a set

of data tapes on available registry data in the South Pacific areas will be provided to NCI for further analysis:

Date Current Contract Initiated: September 14, 1979

Current Contract Level: $50,382

ORI, Inc. (NO1-CP-11025)

Title: Biomedical Computing, Design and Implementation

Contractor's Project Director: Mr. John Mendenhall

Project Officer (NCI): Mr. J. Michael Stump

Objectives: This contract provides computer systems analysis and computer programming support to the Biometry Branch of the Field Studies and Statistics Program. The contractor is developing a library of statistical and data management computer programs for use in the collection and analysis of cancer incidence and end results data.

Major Findings: The contractor is providing continuing data processing support to Biometry Branch investigators. Computer systems and programs and related products are developed by the contractor in response to requests from these investigators.

Significance to Biomedical Research and the Program of the Institute: This project is critical for the ongoing operation of the research activities of the total Biometry Branch Program because it provides the necessary computer programming and systems analysis resources.

Proposed Course: The need for these services is expected to continue indefinitely, although the level of support provided to each organizational area, as well as the total level of effort, are expected to fluctuate over time with changing program requirements.

Date Contract Initiated: July 28, 1981

Current Contract Level: $950,000

Man Years: 26

ANNUAL REPORT
CLINICAL EPIDEMIOLOGY BRANCH
OCTOBER 1, 1981 THROUGH SEPTEMBER 30, 1982

Clinical epidemiology is a form of observational research in which one must make the most of natural occurrences to determine the causes and mechanisms of disease. Our approach is not traditional, but has proved to be continuously productive. Specifically, the Clinical Epidemiology Branch (CEB) seeks peculiarities in the occurrence of cancers in persons, families, communities or industries that may lead, in conjunction with recently developed laboratory research, to new knowledge of biology. In this way, study of human disorders may illuminate areas for which no animal models are yet known. Such observations may lead to new concepts of early detection and prevention.

Staff:

Elisabeth A. McKeen, M.D., a medical oncologist, resigned to go into practice after five years in our Branch. Margot R. Hanson, M.P.H., a non-medical epidemiologist resigned to enter the Uniformed Services University of the Health Sciences as a medical student. David J. Marchetto, a research assistant in our Clinical Studies Section in Boston, resigned to become a sales representative for the Hospital Corporation of America. Priscilla A. Gilman, M.D., a pediatric oncologist, expects to leave for an academic position by September, the end of her third year in the Branch as an Expert.

Thomas E. Goffman, M.D., a medical resident at Georgetown University Hospital, was a Guest Scientist in our Branch until June 30, when he became an Epidemiology Fellow in Training. He has been taking and will continue to take courses in Epidemiology at Georgetown University toward a Master of Science degree. We need to replace Ms. Hanson to supervise the three million dollar five-center study of the health of the offspring of persons who survived childhood cancer.

Since February 11, 1982, we have had two epidemiologists from the People's Republic of China as Guest Scientists: Song-lin Yu, M.D., and Xu-dong Dai, M.D. After intensive instruction in English, they have been learning computer language and usage. In the year they will be with us, they will rotate through the Analytical Studies Section of the Environmental Epidemiology Branch and the Office of the Chief, CEB. The purpose is to give them a broad perspective of epidemiology as a basis for future cooperative research between FS&S and their home institutions (Wuhan Medical College and Harbin Medical College Cancer Institute). Gilbert W. Beebe, Ph.D., a statistician-epidemiologist, is being converted from a position as an Expert to Civil Service status. As a small unit, much of our original research has depended on willing collaborators and even volunteers. The latter included not only Dr. Goffman, but also Nancy U. Potter whose contribution merited authorship and Caroline Bagley, Gary Eddey, Deborah Santos, Peter Silvain, and S. Asger Sorensen. Dr. Sorensen brought his own data to be analyzed in collaboration with us.

Books Published:

A highpoint of the year was publication of a volume, Cancer Mortality in the United States, 1950-1977, by F.W. McKay, M.R. Hanson, and R.W. Miller. It was based on data from death certificates for almost eight million persons who died of

cancer during the 28-year interval. The volume contains two- and three-dimensional graphs over time by age, sex and race, tables from which the graphs were derived, references to comprehensive epidemiologic reviews for each main cancer site, as well as three appendices: the annual tumor mortality according to the International Classification of Diseases, the codes for the sixth, seventh and eighth revisions of the ICD (the first two of which are out of print), and a republication of the important paper by C. Percy et al. of the Biometry Branch on the accuracy of death certificates. The volume, NCI Monograph 59, was intended as a resource for other workers in cancer epidemiology. The Surgeon General's Report on Smoking and Health, for example, contained 74 graphs, more than half of which came from the book.

Another important book published by the Branch during the year was Neurofibromatosis (von Recklinghausen Disease): Genetics, Cell Biology and Biochemistry, by V.M. Riccardi and J.J. Mulvihill. It is a comprehensive presentation of the state of knowledge about this overlooked disease, so full of opportunities for new understanding about the fundamental biology of cancer.

Another volume, published by the National Academy of Sciences-National Research Council, was the proceedings of a US-Italy meeting on areawide chemical contamination. The book contains case histories of such pollution, descriptions of organ-specific effects and a panel discussion concerning response strategies to such episodes. Dr. Miller served as co-organizer and co-chairman and edited the individual panel discussions.

A meeting on Chemical and Radiation Hazards to Children (84th Ross Conference on Pediatric Research) was organized and co-chaired by Dr. Miller. The proceedings, scheduled to be published in October 1982, will go to 70,000 physicians. Highlights of the meeting will be published in the Journal of Pediatrics.

Radiation:

More than half of Dr. Beebe's time was devoted to the effects on man of ionizing radiation, especially from low-dose exposures. He served on the Radiation Research Coordinating Group (RRCG), which is mainly concerned with the organization of research activities at NCI, and on the Interagency Radiation Research Committee (IRRC). The RRCG has been responsible for the contract to study cancer occurrence in Utah in relation to fallout from the Nevada weapons tests in the 1950s. The contract has now been signed at a cost of $3 million provided by NCI and $3.4 million by DOD and DOE. The IRRC is concerned, among other matters, with responses to accidental release of radiation from nuclear facilities, the revised dosimetry for the Japanese atomic-bomb survivors, and Senator Hatch's legislation to compensate people who develop cancer and claim it was due to radioactive fallout. Dr. Beebe helped prepare Dr. Brandt's testimony concerning this bill. He has also worked with Department of Justice lawyers on the question of compensation to Utah residents who have developed cancer which they claim is due to fallout from the Nevada weapons tests.

During the year, Dr. Beebe published two monumental papers on radiation effects as determined by the Radiation Effects Research Foundation (RERF); prepared a chapter on radiogenic leukemia; served on the advisory committee to the Johns Hopkins study of shipyard workers exposed to radiation, and on the NCRP Committee on Chemical and Radiation Carcinogenesis. He reported on the studies by RERF at a DOE Epidemiology Contractors' Workshop, participated in a symposium on risk assessment sponsored by the Society for Industrial and Applied Mathematics, appeared as an expert witness before Representative Gore's Committee hearing on

human research at the Oak Ridge National Laboratory, and was in close contact throughout the year with the Radiation Studies Section of the Environmental Epidemiology Branch of NCI.

Dr. Miller served on Advisory Committees concerned with cancer among atomic energy workers at the Los Alamos Scientific Laboratory and at the Hanford Environmental Health Foundation. He also served as co-chairman of the Science Council for the RERF at its annual meeting in February 1982 and as a member of the NAS-NRC Board on Research on Effects of Radiation.

This was the last year of a three-year contract with Dr. Malcolm C. Paterson and his group at Atomic Energy of Canada, Ltd, at Chalk River, Ontario. Under the contract about 30 specimens of human skin fibroblasts a year were transmitted by CEB and EEB for study by Dr. Paterson's group with regard to repair of DNA. Two papers during the year were of special interest. One described in vitro radiosensitivity of fibroblasts from members of a family in which four siblings and three other maternal relatives developed acute myelogenous leukemia. The other described radioresistance of fibroblasts from a family in which a marked aggregation of a variety of tumors occurred. Of 17 original articles published by the contractor during the three years, five were in collaboration with members of CEB or EEB.

A contract is being initiated with Dr. Richard Setlow of Brookhaven National Laboratory for studies of DNA repair with regard to heavily exposed Japanese atomic-bomb survivors who develop cancers of the types thought to be radiogenic. The results will be compared with those for a) persons whose exposures were similar but did not develop cancer or b) persons whose exposures were trivial and did not develop cancer. Multiple myeloma would be an example of the cases to be so studied.

Viruses:

Hepatitis. The relationship of hepatitis to liver cancer is being studied by Dr. Beebe in collaboration with Dr. Hoofnagle of NIAMDD, Dr. Seeff et al. of the VA Medical Center in Washington, D.C., and Dr. Norman of the Medical Follow-up Agency (MFUA) of NAS-NRC. Hepatitis occurred among about 50,000 soldiers immunized against yellow fever in 1942. A roster of 10,000 men who received the vaccine but did not develop clinical hepatitis will be created by the MFUA. Comparison of mortality in these two groups and an unvaccinated "control" group will be made. Also blood serum will be obtained from 200 men in each group to determine the percent positive for HBsAg, anti-HBs, anti-HBc and anti-HBV. The results of the serology will indicate if the hepatitis infection was with type A, B or neither. It is thought that subclinical infection with hepatitis B is likely to increase the risk of liver cancer. Subclinical disease represents chronic infection, which has been associated with cirrhosis and cancer of the liver. In addition, the study will reveal a) how long hepatitis antigen and antibodies persist, b) the percentage of chronic HBsAg carriers among persons who had acute icterus vs subclinical hepatitis, and c) the relation of serologic findings to clinically apparent chronic liver disease.

Lymphoma. Paul H. Levine, M.D., an internist primarily interested in cancers related to EBV infection, will be detailed to CEB from the Laboratory of Viral Carcinogenesis.

Genetics:

The mapping of cancer genes has been brought up to date by Dr. Mulvihill. At present, 11 chromosomes are associated with human leukemia and seven with solid tumors. Two "cancer genes" can be assigned with confidence: retinoblastoma to 13q and Wilms' tumor to 11p.

The genetic epidemiology of carotid body tumors was described based on review of 222 cases at 12 cancer centers. Familial cases occurred bilaterally and earlier in life than sporadic ones. Patterns of multiple primary tumors, often with other neural crest tissues, suggested additional neurocristopathy syndromes.

A paper on two families with marked aggregation of smoking-related respiratory cancer has been published. In both families, young members had radiogenic cancers. These findings suggest a genetic susceptibility to more than one environmental agent. Additional collection of blood and tissue samples for laboratory study is planned. Other families with aggregation of lung cancer have come to our attention.

A paper detailing the findings in 11 children with "trilateral" retinoblastoma has been published. In these children retinoblastoma affects both eyes and the pineal (the "third eye"). Two of the children also had undifferentiated tumors of the cheek, and four others has parapineal tumors. We suspect these six neoplasms to be ectopic retinoblastoma; i.e., tumor formation in retinocytes wherever they are. Thus these tumors are multifocal and are not due to pleiotropism. We now know of 23 children with pineal or parapineal involvement.

Another report published during the year in conjunction with EEB concerned three families with the Li-Fraumeni syndrome. Since the first report of these families ten years earlier, among 54 persons at risk, 10 have developed 16 tumors--far in excess of normal expectation. The study in effect was a prospective study of families that had in the past had a marked predisposition to various cancers. Thus, the presence of high risk was confirmed through further study of the same families. No progress has yet been made in understanding why these families are so prone to cancer. Another such family has been ascertained at the University of New Mexico.

With regard to neurofibromatosis (NF), the main development was publication of the proceedings of a meeting two years ago, as noted above. Genetic linkage studies of several heavily affected families suggested localization of a gene for NF on chromosome four in some of the families. Other studies made through May 1981 were to be brought to publication during the year, but the physician responsible became a trainee in radiotherapy at NCI, and has not been able to finish the work.

Polymastia was seen in 6 (19%) of 32 renal cell carcinoma patients and in 7 (11%) of 63 men with testicular cancer compared with 0% of 32 head-and-neck cancer patients, 8 (3%) of 299 male medical students, and 0.5% in published population surveys.

The largest study by the Branch concerns collection of data on the reproductive outcome among about 2500 children whose treatment for cancer allowed them to survive to the child-bearing age. Comparison will be made between the findings in this group and sibling-controls. The study is being conducted by CEB in collaboration with the Biometry Branch of NCI.

Interinstitute Medical Genetics Clinic:

The Genetics Clinic is a collaborative undertaking by six Institutes and the Clinical Center; Dr. Parry of our Branch played a leading role in its establishment. During this, its third year, 304 persons representing 60 diagnostic categories were seen--24 percent of them by members of our Branch. The Clinic provides a multidisciplinary setting for study of patients with markedly increased risk of cancer, where specimens can be obtained for laboratory investigations into the biologic mechanisms of carcinogenesis. Specimens obtained during the year included a) skin fibroblasts from the family with acute myelogenous leukemia described under Radiation above (cells from members with cancer demonstrated increased radiosensitivity in culture after exposure to gamma-radiation); b) skin fibroblasts from persons with the dysplastic nevus syndrome which exhibited enhanced in vitro sensitivity after exposure to ultraviolet light; and c) blood from multigenerational families with neurofibromatosis for genetic linkage studies. In addition, two families with marked aggregation of respiratory cancer, described above, were the focus of extensive clinical and laboratory studies, and two case-control studies were initiated to investigate the possible excess occurrence of accessory nipples in persons with a) renal cell carcinoma and b) testicular carcinoma, an association first suspected as a result of clinical observations made by Dr. McKeen.

Contract on Genetic Factors and High Risk of Cancer:

A major undertaking by Drs. Parry and Mulvihill during the year involved a multisource contract for laboratory studies of genetic factors in patients at high risk of cancer. The work to be done under the contracts involves routine chromosome analysis (three proposals received), sister-chromatid exchange analysis (eight proposals), prophase chromosome analysis (five proposals), solid tumor chromosome analysis (three proposals), and genetic polymorphisms for linkage analysis (three proposals). With so many proposals, the contract review procedure was intricate and time-consuming. The awards are about to be made. As soon as they are, specimens obtained from the clinic and elsewhere will be sent for study as appropriate under one or more of these contracts.

Studies in Boston:

In Boston, Dr. Li is identifying persons at very high risk of cancer, and through laboratory studies is seeking the reasons for their susceptibility. The patients are found by referrals by the patients themselves, their physicians, or at conferences, rounds and clinics in the Harvard complex. Advice and consultation are provided concerning management of cancer-prone patients.

During the year, in addition to the prospective study of families with the Li-Fraumeni cancer syndrome (see section on Genetics, above), extension of a study of 400 survivors of childhood cancer confirmed the previous estimate (through 1973) of a 20-fold excess in the frequency of additional primary cancers. A study in collaboration with the Biometry Branch showed a 34% decline in mortality from cancer of the testis, due mainly to new curative treatment for cancers other than seminoma.

A new syndrome of familial polyposis of the colon and acute leukemia was described in two brothers whose parents were first cousins. In another family, a patient with familial polyposis developed acute leukemia after cranial radiotherapy.

Study of her fibroblasts in culture suggested increased susceptibility to the effects of ionizing radiation. A child ascertained at Georgetown University Hospital had somewhat similar findings: acute myelogenous leukemia and glioblastoma multiform after radiotherapy and chemotherapy for an ependymoma three years before. Preparation of the report of this patient was led by Dr. Goffman, a medical resident who has spent his spare time with us as a Guest Scientist.

The study of long-term survivors of childhood cancer has revealed that those who had brain tumors generally do well as measured by their occupational and educational attainment. Another new finding is that after exposure of the thyroid to radiotherapy, a high frequency of thyroid dysfunction occurs, as indicated by elevation of TSH. Among 100 patients in the study, about 20% developed thyroid nodules and three developed carcinoma. Unexpectedly, women who had been treated with irradiation for Wilms' tumor in childhood had children who were, more often than usual, small for date at birth. Radiotherapy given during the first few months of life, regardless of the tumor type, carried a high risk of kyphoscoliosis.

Bedside Etiology:

A main effort of the Branch has been to develop clues to etiology by study of peculiarities in cancer occurrence, as noted in the summary of our activities in Boston. For the past few years our staff members have made rounds at hospitals in Washington and Baltimore, and Grand Rounds occasionally elsewhere in the country. The ascertainment of interesting cases has come through this approach, far more so if our staff member is on service at the hospital. Dr. Li reports that he finds many more cases of interest during the month that he is an attending physician on the wards at the Sidney Farber Cancer Foundation, than are referred to him from that source when he is off service. Drs. Goffman and McKeen have had success in finding such cases from their direct contact with patients at Georgetown. We have had only one good case in five years of once-a-week rounds at the National Children's Medical Center of Washington ("trilateral retinoblastoma," see above). Dr. Gilman has had limited success at Johns Hopkins.

Dr. Gilman's work in progress includes reports on genetic disorders associated with leukemia, but not known to have chromosomal abnormalities; e.g., Shwachman's syndrome, Diamond-Blackfan syndrome and infantile genetic agranulocytosis. Also in progress are reports on Ivermark syndrome with situs inversus and Wilms' tumor in siblings; congenital malignant tumors in siblings; and osteosarcoma in siblings, one with chromosomal abnormality. Reports that have been published or are in press include highlights of a meeting on the differences in lymphocytic diseases in the U.S. and Japan; hepatolenticular degeneration (Wilson's disease), penicillamine and acute leukemia; and the epidemiology of human teratomas. Dr. Gilman is planning to relocate as a pediatric hematologist/oncologist at a university, and hopes to spend her required military duty (three days every two months) in the Branch as a Guest Scientist, involving in part, studies of patients of interest ascertained through her new position.

Epidemiology Resource Development:

To reduce the cost and effort of epidemiologic studies, several new data resources are being developed. Dr. Beebe is involved in all of them. He serves on a Working Group that is testing the capacity to match names and other identifying information for follow-up through the National Death Index, which has been very active during the past year. This resource will make follow-up studies easier.

At the May 1982 meeting of the DCCP Board of Scientific Counselors a concept was approved to fund the Social Security Administration (SSA) for three years beginning on October 1, 1982 to determine the value of its information on occupation in relation to the causes of death as given on about 100,000 death certificates, 1973-1977.

Another project seeks to supplement occupational data from the SSA Continuous Work History Sample with information on occupation from the Internal Revenue Service (Form 1040). Funding began in mid-January 1982 and will continue for one year.

An agreement has been made under which SSA will provide longitudinal work histories of 100 persons who died of mesothelioma (asbestos-related) and 100 controls. The purpose is to test SSA's work histories as compared with parallel information available from next-of-kin through the New York State Department of Health, the University of Southern California, and the Veterans Administration.

Our use of the IRS address file was closed down by the 1976 Tax Reform Act. NIOSH has opened its files for our studies and MFUA has added studies of the military. A still broader access for medical research purposes is pending evaluation by OMB. Dr. Beebe has been working on this with Mr. John Fanning, a lawyer in Dr. Brandt's office.

Representative Gradison's office is interested in restoring SSA's routine mortality reporting based on claims for burial and other death benefits. Mr. Fanning is also assisting in re-establishing this reliable nationwide system.

International Activities:

Japan. Tumors derived from the neural crest were the subject of this year's workshop of the Interdisciplinary Group of the U.S.-Japan Cooperative Cancer Research Program. A highlight of the meeting, held at the East-West Center in Honolulu on March 1-2, 1982, was the participation of Prince Masahito, the Emperor's younger son. The Prince spoke for the first time ever at a scientific meeting. His subject was tumors of red, black or iridescent pigment cells in giant goldfish. He also described melanomas in 30-50 percent of croakers caught off the Kii Peninsula, an observation not previously given much attention outside Japan. The meeting concerned neurofibromatosis (NF), multiple endocrine neoplasia (MEN) syndromes types II and III, as well as familial meningioma, medullary carcinoma of the thyroid (MTC), chemodectoma, neuroblastoma, neurolemmoma, pheochromocytoma and melanoma. In the last 90 minutes of the workshop, the information presented was synthesized so a new understanding of the timing of gene actions may have been derived. To illustrate: NF involves the widest array of tissues derived from the neural crest, so the gene for NF must affect the "trunk-line" in the development of the neural crest. MEN type III has two of the same components plus MTC, so the gene must act later; and occurring still later is the action of the gene for MEN type II (same organs affected as MEN type III except for blubbery lips due to mucosal neuromas). The latest acting genes would account for familial tumors of a single type; e.g., pheochromocytoma, melanoma or meningioma.

People's Republic of China Cancer Program. The Branch has had a continuous series of activities with epidemiologists in the PRC since September 1977. In February 1982 Drs. Yu and Dai, epidemiologists, came to NCI for a year to learn various epidemiologic techniques, as described above, under the section on Staff.

Denmark. Dr. S. Asger Sorensen, from the Institute for Medical Genetics at the University of Copenhagen, spent a month in the Branch as a Guest Scientist. At Dr. Mulvihill's suggestion, Dr. Sorensen is making a follow-up study of all persons with neurofibromatosis diagnosed in Denmark over a 20-year interval and alive at the time of the study (1944-1949; N=112). The case material was analyzed and published in 1951 by Dr. Allan Borberg. Dr. Sorensen brought with him the disease histories among these patients covering the intervening 30 years. He and Dr. Mulvihill will prepare a report for publication on this prospective study of the health of patients with neurofibromatosis.

Italy. In addition to publication of the proceedings of the U.S.-Italy workshop on area-wide chemical contamination sponsored by NAS-NRC (see above), Dr. Miller participated in the annual meeting of the International Steering Committee on Seveso, which evaluates studies on people of Seveso who were exposed to dioxin in 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04325-19 CEB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Planning and Development in Cancer Epidemiology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: :	R.W. Miller, M.D.	Chief	CEB	NCI
OTHER:	F.P. Li, M.D.	Chief, Clinical Studies Section	CEB	NCI
	J.J. Mulvihill, M.D.	Chief, Clinical Genetics Section	CEB	NCI
	G.W. Beebe, Ph.D.	Expert, Biostatistics	CEB	NCI

COOPERATING UNITS (if any)
None

LAB/BRANCH
Clinical Epidemiology Branch

SECTION
All units

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.4	0.5	0.9

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objectives of this undertaking are to originate new epidemiologic approaches to the study of cancer causation in man, to develop sources of data related to specific epidemiologic problems, and to stimulate epidemiologic research in other health agencies.

Opportunities for research in cancer epidemiology are based on questions arising from clinical or laboratory observations, on unusual groupings of cancer cases in the population, and on study of the characteristics of groups of persons prior to the onset of a specific type of cancer as compared with similar persons who have not developed the disease.

Project Description

Objectives:

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

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

3. To stimulate epidemiologic research on cancer and other diseases.

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 (e.g., 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.

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 them. Conversely, the Branch seeks by its surveys to raise questions which can be answered by laboratory or clinical studies.

Major Findings:

Until a few years ago when the Privacy Act and human ethics committees were established, epidemiologists could abstract vital records and hospital charts at will. In our experience over fifteen years, this approach produced a steady flow of important new information about the etiology and natural history of cancer. Never did we have a problem with privacy or ethics, but the new restrictions stopped our research along these lines. Instead, we have turned to clinical observations of peculiarities in cancer occurrence and explored their biologic nature through laboratory research performed under contract or collaboratively.

Significance to Biomedical 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. As a 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

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:

We plan to continue and to extend etiological studies of patients with cancer and of cancer families at NIH or elswhere in the city or nation, and to encourage similar approaches internationally. Specimens from cases of interest will be obtained for laboratory studies using new procedures in an attempt to develop further understanding of factors that increase host susceptibility to environmental carcinogens. New ways of portraying cancer mortality data will be undertaken to seek peculiarities in distribution which have etiological implications. Tests of hypotheses will be made as they arise from experimental or clinical observations.

Publications:

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04377-11 CEB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Familial, Congenital, and Genetic Factors in Malignancy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.J. Mulvihill, M.D.	Chief, Clinical Genetics Section	CEB	NCI
OTHER:	E.A. McKeen, M.D.	Clinical Investigator	CEB	NCI
	D.M. Parry, Ph.D.	Geneticist	CEB	NCI
	T.E. Goffman, M.D.	Medical Staff Fellow (Epidemiology Trainee)	CEB	NCI
	S.A. Sorensen, M.D.	Guest Researcher	CEB	NCI
	P. Silvain, B.S.	Guest Researcher	CEB	NCI
	C. Bagley, R.N.	Guest Researcher	EEB	NCI

COOPERATING UNITS (if any)
Environmental Epidemiology Branch and various other clinical and laboratory groups within NCI (especially Pediatric Oncology Branch), Atomic Energy of Canada, Georgetown University.

LAB/BRANCH
Clinical Epidemiology Branch

SECTION
Clinical Genetics Section

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NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.2	2.2	1.0

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☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Study of genetic diseases with neoplastic manifestations and detailed investigation of families at high risk of cancer may help detect environmental and genetic influences in carcinogenesis, especially when appropriate laboratory assays are used. By chart review, the genetic epidemiology of carotid body tumor and Ewing sarcoma was described; of 222 carotid body tumor patients, 10% had bilateral disease, 17% had other primary tumors, and 7% had at least one similarly affected relative. By clinical examination, polymastia was seen in 19% of renal cell carcinoma patients and 11% of testicular cancer patients (compared to 0-3% in control groups). In two families, apparent excesses of lung cancer were attributed in part to smoking, in part to genetic susceptibility (because three tumors followed radiation or chlorambucil use and one family had a new syndrome of birth defects involving bone and teeth). Unusual in vitro radioresponsivity was found i one family with leukemia, one with diverse malignancies and one with the Gardner syndrome. Guest lectures, literature reviews, and committee activities were done to stimulate similar research worldwide.

Project Description

Objectives:

To identify genetic factors and disorders associated with cancer, and to promote similar studies worldwide. To document the occurrence of patterns of familial aggregation of neoplasms; to study selected disorders and families by genetic and laboratory investigations in an effort to elucidate carcinogenic mechanisms and the degree to which heredity and the common familial environment contribute to the etiology of neoplasms. To distribute biologic specimens from selected subjects to laboratory investigators for etiologic studies by biochemical, cytogenetic, immunologic, viral, and tissue culture methods. To study similarly, patients with birth defects and other heritable disorders that may predispose to malignancy.

Methods Employed:

Interviews of patients with cancer or other diseases with respect to familial occurrences especially of cancer and birth defects, as well as 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 congenital and genetic diseases or neoplasms. Establishment and maintenance of laboratory collaboration by contract and other means. Invited lectures, reviews, and committee memberships provide ways for stimulating research in cancer genetics.

Major Findings:

Reports published or in press in the last 12 months by the two permanent participants of this project comprise nine reports of original research, [concerning laboratory findings in cancer families (2), case reports (3), clinical studies (3), and statistical methodology (1)], twelve reviews, and seven abstracts for national meetings. Research reports involved 27 co-authors from the Environmental Epidemiology, Medicine, Pediatric Oncology, and NCI/VA Medical Oncology Branches of NCI, Atomic Energy of Canada, the Universities of Cincinnati and Washington, Harvard, Georgetown, Baylor, and Duke Universities, the Mayo and Cleveland Clinics, Mount Sinai, Memorial, and M.D. Anderson Hospitals, and the Fox Chase Cancer Center.

Genetic Epidemiology (for mutation epidemiology, see Z01 CP 05146-03 CEB). In the first research report from a network of patients' records at ten large cancer centers, the genetic epidemiology of 222 cases of carotid body tumor was described. Familial carotid body tumor was seen in 16 individuals from 13 kindreds including nine newly ascertained ones. The familial cases were more often bilateral but only slightly younger at diagnosis than nonfamilial cases, suggesting that this rare tumor type is not entirely consistent with Knudson's model of two-step carcinogenesis. Patterns of multiple primary tumors, often with other neural crest tissues, resembled some known neurocristopathy syndromes and suggested additional new ones. Preliminary report of a similiar chart review of Ewing sarcoma, a tumor with few etiologic leads, revealed an excess of urinary tract anomalies.

Stimulated by a consultation through the Interinstitute Genetics Program, a generalized design strategy was developed and published for maximizing efficiency of familial studies by eliminating marginally informative families when many families

are available. Members of the Cardiology Branch, NHLBI applied this design to patients with hypertrophic cardiomyopathy; a preliminary report found likely genetic heterogeneity in this disorder, previously widely considered a clearly autosomal dominant trait.

A review of the cytogenetic abnormalities associated with human cancer revealed 11 chromosomes now associated with leukemia and seven with solid tumors. Two human cancer genes can be assigned with some confidence: retinoblastoma to 13q and Wilms tumor to 11p. A book chapter, rapporteur's report, and meeting summary were published following a symposium on host factors in human carcinogenesis, cosponsored by the International Agency for Research on Cancer which previously had emphasized environmental causes of cancer.

Clinical Observations. Bedside observations of etiology and diseases associated with peculiar occurrences of cancer continue to yield insights into the origins of neoplasia and useful leads for follow-up studies. Astute clinical recognition of accessory nipples in two patients with renal cell carcinoma was confirmed in a clinical survey that found polymastia in 19% of 32 renal cell carcinoma patients in contrast to 0% of 32 head and neck cancer patients and 0.5% in published population surveys. The relative risk was 5.0. Because the index case with multiple nipples and renal cell carcinoma also had testicular cancer, physical examination and radiologic reviews were undertaken in a search for birth defects in a consecutive series of 63 white males with testicular cancer. In the preliminary analysis, seven patients (11%) had polymastia compared to eight of 299 (3%) male medical students, a relative risk of 4.6.

Three case reports were published:

1. A seven-year-old with minor dysmorphisms and abdominal Burkitt lymphoma had an unaffected identical twin; no environmental factors seemed different between the two boys. Fortuitous storage of sera drawn two years before the lymphoma was diagnosed provided a precious resource that could be used when appropriate tests are agreed upon.

2. A 67-year-old white farmer from Maine presented with large cell carcinoma of the lung and died within two months of a fulminant hypereosinophilia. His eosinophilia was considered a leukemia, often a difficult diagnosis to make, because the bone marrow cells had a previously undescribed deletion of the long arm of chromosome 15, near the site involved in the 15;17 translocation of acute promyelocytic leukemia. At autopsy, a cerebral meningioma, prostatic carcinoma, and thyroid adenoma were discovered. His relatives had a striking array of lymphomas, and included two men with prostatic cancer, a common tumor rarely reported to occur in familial aggregations.

3. The case of a 48-year-old electrician with metastatic renal cell carcinoma was instructive, not for etiology, but for clinical management. His rapid debility following nephrectomy was attributed to cancer cachexia, until (treatable) mineralocorticoid insufficiency from adrenal gland metastases was diagnosed.

Several years of study culminated in a publication of two families with fourteen individuals with lung or laryngeal cancer. All were smokers and, in one family, the occurrence of a new malformation syndrome of limb and dental defects led to the

postulation that there was an inherited predisposition to environmentally induced cancers. In addition, one relative had acute myelogenous leukemia following chlorambucil use, and likely radiogenic cancers (a facial basal cell carcinoma and a thyroid carcinoma) occurred in individuals who received radiotherapy for nonmalignant childhood conditions. The results of general laboratory assays were negative or normal, but specimens were stored for new assays, for example, using monoclonal antibodies to look for DNA-carcinogen molecules.

To clarify the Li-Fraumeni cancer family syndrome of sarcomas and breast, brain and bone cancers, a preliminary report was made of 26 families with one member having a sarcoma and a first-degree relative with any cancer. Of 1,242 members, 283 primary cancers occurred, including 27 individuals with multiple primary tumors. Breast and lung cancers occurred at an earlier age than expected in the general population.

Clinical Laboratory Collaboration. Many laboratories supported clinical investigations on peculiar occurrences of cancer, but the most productive this year was the contract-supported Health Sciences Division, Atomic Energy of Canada (Dr. Malcolm Paterson).

1. Fibroblasts from six melanoma patients from five families with the dysplastic nevus syndrome showed ultraviolet (but not ionizing) radiosensitivity, and four were also sensitive to 4-nitroquinolone-1-oxide, as measured by colony survival.

2. A woman lost four of six children to acute myelogenous leukemia, and she had rectal carcinoma 14 years after (and probably as a result of) radiotherapy for uterine cervical carcinoma. In correlation with prior results of abnormal transformation following exposure to simian virus 40, cells from the woman and her two available leukemic daughters were moderately radiosensitive, in the range of heterozygotes with the ataxia-telangiectasia gene. Her husband, two remaining normal sons, and a sister with breast cancer had normal colony survival after radiation.

3. Two members of a family with diverse malignancies gave a history of exposure to radiation. A teen-aged boy had a vertebral osteosarcoma in the field of radiotherapy given for bilateral malignant schwannomas 12 years before; his paternal great-uncle with polycythemia vera had some occupational exposure during the manufacture of heavy water. Of eight family members tested, unusual radioresistance was observed in fibroblasts from four blood relatives with cancer and one without, but not in a blood relative with synovial sarcoma or in two normal spouses. This novel finding (more cell survival after radiation than seen in normal cells) could relate to a cancer predisposition: perhaps the abnormal cells survive a radiation exposure well enough to manifest a mutation that causes cancer, whereas normal cells, given the same dose, would die.

4. Preliminary results showed unusual sensitivity to methyl-nitro-nitrosoguanidine in lines from a family with the Gardner syndrome of polyposis of the colon, osteomas, and subcutaneous cysts.

5. In a laboratory-clinical collaboration with geneticists at the University of California, Los Angeles, linkage analysis of 27 genetic markers was done on 108 persons in 11 families with neurofibromatosis. Preliminary analysis excluded

linkage with 15 loci, including HLA. Nine families gave a lod score of +2.7 (close to the generally accepted level of significance of +3.0) for Gc, a marker on chromosome 4. Since another family had a negative lod score, the overall estimate was +1.0, suggesting genetic heterogeneity of neurofibromatosis.

Reviews, Consultations, Committees, and Lectures:

A monograph, co-edited by Dr. Mulvihill, Neurofibromatosis (von Recklinghausen Disease): Genetics, Cell Biology, and Biochemistry, was written for the purpose of stimulating clinical and laboratory research on a frequent mendelian trait with many neglected biomedical facets, including a high risk for cancer. Two chapters had Branch authors. Two section members contributed chapters on epidemiology and eco-genetics in the newly released volume, Cancer in the Young, mostly an effort of the Pediatric Oncology Branch, National Cancer Institute. Shorter reviews were prepared on the clinical genetics of human cancer, the potential for carcinogenicity by the intrauterine device, and the fetal alcohol syndrome.

In an effort to recruit junior staff and to promote clinical and laboratory collaboration, teaching responsibilities were carried out at the NIH Genetics Training Program; the Johns Hopkins University School of Medicine, Department of Pediatrics; Georgetown University School of Medicine, Oncology Division; and the Uniformed Services University of Health Sciences. Dr. Mulvihill became a charter fellow of the American Board of Medical Genetics by passing its first national examination.

In the Interinstitute Genetics Clinic and Consultation Service, Branch members counseled women at high risk for breast cancer (11), diagnosed individuals referred with possible neurofibromatosis (24) or malformations syndromes (7) and evaluated and obtained biological specimens from patients with Cowden (multiple hamartoma) syndrome (5), urogenital cancer (16), familial lung cancer or leukemia (10) and multiple primary malignancies (2).

Consultation, in the form of committee membership, was given to the Committee on Epidemiology of the International Commission for Protection Against Environmental Mutagens and Carcinogens, the editorial boards of the Journal of the National Cancer Institute and the Yearbook of Cancer, the NCI/NICHD Reproductive Endocrinology Working Group, the US-Japan Joint Panel on Environmental Mutagenesis and Carcinogenesis of the US-Japan Cooperative Medical Science Program, and the Ad Hoc Review Group for the PAHO/IARC/EPA/NCI study of possible of teratogenic and carcinogenic effects of pesticides among floraculture workers in Colombia.

Significance to Biomedical Research and the Program of the Institute:

Epidemiologic surveys and detailed clinical and laboratory studies of families and individuals at high risk of cancer may help distinguish environmental and genetic influences in carcinogenesis. In addition, identification of high risk individuals has therapeutic implications, enabling surveillance and early diagnosis of neoplasms and genetic counseling for offspring.

Proposed Course:

The same approach will be continued. New laboratory methods and epidemiologic clues from other sources will be incorporated into the project as available. Especially

close watch will be made for advances in viral oncology and monoclonal antibody techniques that deserve exploration with human material. Manuscripts are in final preparation on counseling women at high risk of breast cancer, pregnancy outcomes in cancer patients, Epstein-Barr virus-related cancers and birth defects in a family, in vitro radiosensitivity in the nevoid basal cell carcinoma syndrome, genetic linkage of neurofibromatosis, the frequency of hereditary large bowel cancer, the formal genetics of hypertrophic cardiomyopathy, a family with a new limb and cranial nerve defects syndrome with melanoma and premature centromere separation, a Wilms tumor cell line derived from an adult with that embryonal tumor who also had endometrial and laryngeal cancer, and a life table analysis of a Danish cohort of neurofibromatosis patients. Contracts are nearly ready for award for genetic laboratory services and research in five areas: simple-banded cytogenetics, prophase karyotyping, solid tumor karyotyping, sister chromatid exchanges, genetic markers and linkage analysis, and in vitro ultraviolet- and gamma-radioresponsivity. A contract for support services for the enlarging program of the Section is in development.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04400-18 CEB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Clinical Epidemiology of Human Cancers

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	F.P. Li, M.D.	Chief, Clinical Studies Section	CEB	NCI
OTHER:	D.J. Marchetto	Research Assistant	CEB	NCI

COOPERATING UNITS (if any)

Sidney Farber Cancer Institute, Boston, MA

LAB/BRANCH
Clinical Epidemiology Branch

SECTION
Clinical Studies Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.8	1.0	0.8

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Studies are made to identify persons at very high risk of cancer and to investigate causes of their susceptibility. The individuals are found through special referrals by clinicians, self-referral by patients, and clinical observations at patient conferences, rounds and clinics. With informed consent, epidemiologic studies are made to identify the predisposing role of cancer genes and environmental carcinogens. Laboratory studies are performed to clarify biologic mechanisms of susceptibility to cancer. Findings to date show that cancer risk approaches 100% in persons who are carriers of certain cancer genes. Early cancer detection has been achieved in some patients, and appropriate counseling during pre-clinical stages has been provided to other patients. High risk groups include patients who had one cancer in childhood and are susceptible to develop multiple primary neoplasm. Cancer risk factors in these patients are under prospective study through a computerized Registry of Survivors of Childhood Cancer in Boston.

Project Description

Objectives:

1. To employ clinical observation at the bedside to identify causes of human cancers.

2. To investigate susceptibility factors in the development of cancers in high risk subgroups in the population.

3. To apply latest laboratory techniques to investigate biologic mechansims of predisposition to cancer.

4. To provide counseling and consultation regarding appropriate management of cancer-prone persons.

Methods Employed:

Patients admitted for cancer therapy at the Sidney Farber Cancer Institute are examined for clues to etiology of the neoplasm. When exceptional clinical observations are made, appropriate follow-up epidemiologic and laboratory investigations are conducted.

A registry has been established of 820 patients who have survived childhood cancer for at least 5 years. These patients are being studied to determine the probability of development of a new cancer and the somatic and genetic effects of the neoplasm in childhood.

Striking family aggregates of specific cancers have been identified. Family members are under study to identify reasons for the susceptibility and to detect early cancers.

Prospective studies are made to confirm predictions of high risk of cancers in individuals, families, and other groups.

Major findings:

1. Prospective study of four families with breast cancer-sarcoma syndrome showed development of 16 cancers during a 12 year period, as compared with 0.5 cases expected. The new cancers were breast cancer or sarcoma in nine instances.

2. Retrospective analysis in 1973 of 400 survivors of childhood cancer had shown an approximately 20-fold increased risk of development of a new primary cancer. Prospective observation of the cohort for an additional eight years confirms risk estimates derived from the retrospective analysis.

3. Testis cancer mortality in the U.S. declined by 34% between 1973 and 1978, despite a rise in disease incidence. The trend corresponds with development of new curative treatment for non-seminoma.

4. A new syndrome of familial polyposis coli and acute leukemia was described in two brothers whose parents were first counsins, suggesting recessive inheritance. In addition, a patient with familial polyposis coli and brain tumor developed acute leukemia after cranial radiotherapy. Study of her fibroblasts suggests increased susceptibility to the effects of ionizing radiation.

5. Studies of long-term survivors of childhood cancer revealed the following new findings: 1) high level of performance of many survivors of brain tumors in childhood as measured by occupation, educational attainment and reported physical disabilities; 2) high frequency of thyroid dysfunction after radiotherapy in high doses. Elevated TSH was the major finding. Approximately 20% of 100 study patients had nodules, including carcinoma in three patients; 3) high frequency of late effects, particularly kyphoscoliosis among patients treated in the first months of life; 4) small birth-weight infants born to women radiated for Wilms' tumor in childhood; 5) susceptibility to radiation-induced breast cancer after chest radiotherapy.

6. In collaboration with visiting scientists from the People's Republic of China, high rate areas for penis cancer in China were also shown to have increased cervix cancer rates, suggesting common etiology of the two neoplasms. In a second study comparing childhood cancer incidence in China and the U.S., differences were found for rates of myeloid leukemia and cancers of the liver, bone, testis and other sites.

Significance to Biomedical Research and the Program of the Institute:

The Clinical Studies Section identifies persons susceptible to cancer for laboratory studies of mechanisms of carcinogenesis. In addition, specialized laboratory techniques are investigated as markers to identify persons for surveillance for cancer at early stages. Follow-up studies of survivors detect late effects of disease and therapy that may lead to modifications of therapy to reduce morbidity. The collaboration with Chinese scientists provides additional knowledge of the patterns of cancer worldwide and clues to causes of certain prevalent neoplasms in the U.S.

Proposed Course:

The Clinical Studies Section intends to continue studies of childhood cancers. These projects will examine the etiologic role of genetic factors and prenatal exposures, and the late effects of these diseases. In addition, the methods that have proved useful in childhood cancer studies will be applied to study appropriate cancers in adults. These studies will examine family aggregates of cancer, and epidemiologic features of rare and seldom studied forms of adult malignancies. High risk persons will continue to be surveyed to detect cancer at treatable stages and and to receive counseling and supportive care.

Pilot studies are in progress to examine new approaches to mapping cancer mortality in the U.S. Time trends in mortality of curable and preventable cancers (e.g., cervix) are examined by geographic area to identify localities which might lag in cancer education, detection, and treatment services. In addition, a pilot study is in progress to have lung cancer patients provide, with informed consent, help in smoking cessation activities directed to relatives who are smokers.

Publications:

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Li, F.P., Hecht, F., Kaiser-McCaw, B., Baranko, P.V., and Potter, N.U.: Ataxia-pancytopenia: Syndrome of cerebellar ataxia, hypoplastic anemia, monosomy 7 and acute myelogenous leukemia. Cancer Genet. Cytogenet. 4: 189-196, 1981.

Li, F.P., Little, J.B., Bech-Hansen, N.T., Paterson, M.C., Arlett, C., Garnick, M.B., and Mayer, R.J.: Acute leukemia after radiotherapy in a Turcot's syndrome patient: Impaired cultured skin fibroblast colony formation after X-radiation. Am. J. Med. In press.

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Parry, D.M., Li, F.P., Strong, L.C., Carney, J.A., Schottenfeld, D., Reimer, R.R., and Grufferman, S.: Carotid body tumors in man: Genetics and epidemiology. JNCI 68: 573-578, 1982.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 05137-03 CEB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Bedside Etiologic Consultative Program

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R.W. Miller, M.D.	Chief	CEB NCI
OTHER:	P.A. Gilman, M.D.	Expert	CEB NCI
	E.A. McKeen, M.D.	Clinical Investigator	CEB NCI
	T.E. Goffman, M.D.	Guest Scientist	CEB NCI

COOPERATING UNITS (if any)
Johns Hopkins Medical Institutions, Baltimore, MD.; University of Maryland Medical School, Baltimore, MD.; Georgetown University Medical Center, Washington, D.C..

LAB/BRANCH
Clinical Epidemiology Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

By participation of the staff at are institutions, at the bedside and in clinical conferences, rounds and oncology clinics, patients or families with unusual features are ascertained and etiologic consultations given. Comprehensive family interviews and appropriate laboratory and epidemiologic investigations are suggested or performed yielding environmental and genetic information as a means of elucidating biologic mechanisms of carcinogenesis.

Project Description

Objectives:

1. To generate hypotheses as to etiology of human cancer from clinical observations made at the bedside.

2. To study these hypotheses through epidemiologic and laboratory investigations.

3. To identify pregestational and prenatal factors predisposing to malignancy, including genetic and environmental interactions.

4. To increase the sensitivity of primary and specialty care clinicians to clues of etiologic significance in their patients and guide them in appropriate evaluation.

Methods Employed:

At medical centers in Washington and Baltimore, Branch members participate regularly in case-review conferences, rounds, and clinics leading to identification of patients with unusual features and etiologic consultations are performed. Follow-up epidemiologic and laboratory studies are conducted at the parent institution or through the contract laboratories of CEB. Complete family interviews are included in the consultation. Two staff persons have academic appointments (Dr. Gilman, Lecturer in Pediatrics and Pediatric Oncology at Johns Hopkins, Dr. McKeen, Clinical Instructor in Medicine at Georgetown) which allow access to tumor registries and record-room research material for more complete epidemiologic data gathering.

Etiologic consultations are provided on request on the wards of NCI and in the Interinstitute Medical Genetics Clinic. Other requests for consultation come by mail or telephone, and during visits to lecture at hospitals or universities in other parts of the country. The Childhood Cancer Etiology Bulletin, issued monthly since December 1973 by the Branch to 900 recipients throughout the world, assists in this endeavor, as do the examples set by the Branch in its actions and publications.

Major Findings:

1. A single case seen on rounds at the Children's Hospital National Medical Center in D.C., noted by our staff member, led to the realization that pineal tumors occur excessively with bilateral retinoblastoma. We have since learned of 23 such cases, which suggests that the gene for retinoblastoma affects retinal anlage wherever they occur naturally or ectopically.

2. Two patients at Georgetown University with renal cell carcinoma (RCC) were noted by our staff member to have accessory nipples. A case-control study of 32 RCC patients in this area revealed that six had accessory nipples as compared with 0.5 expected. Embryologic development of the breast and kidney may have a previously unrecognized link with the biology of renal cancer. Further study may suggest a new concept in renal cell carcinogenesis.

3. Another patient with familial cancer, seen by our staff, has a chromosomal deletion which may indicate the locus for a gene that influences the development of the cancer.

4. Predisposition to malignancy in congenital bone marrow dysfunction disorders has been confirmed (three acute leukemia and one histiocytic lymphoma in less than 75 cases of infantile genetic agranulocytosis and four acute leukemia and two liver cell carcinomas in less than 200 cases of congenital hypoplastic anemia). Epidemiologic and laboratory studies are underway to elicit the mechanism(s).

Significance to Biomedical Research and the Program of the Institute:

The regular participation of CEB staff in oncology activities at the area institutions is increasing the oncologists' awareness of and ability to identify unusual associations of etiologic significance. Referral to or more detailed investigations in these patients by epidemiologists and other laboratory investigators will allow identification of genetic, familial and/or environmental factors predisposing to malignancy. By subsequently identifying high-risk features or persons, prevention or early diagnosis of malignancy can be facilitated.

Proposed Course:

It would seem valuable to explore possibilities of a more formal collaboration between pediatric oncology divisions at the area institutions and the CEB to allow improved pediatric oncology registry data, continuity of studies, case ascertainment for further investigation, and teaching. Combination of material from the different locations by an epidemiologist could yield etiologic relationships more rapidly. Dissemination of information on genetic and prenatal factors would be easier, leading to earlier diagnosis or prevention.

Publications:

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Gilman, P.A. and Holtzman, N.A.: Acute leukemia in a patient receiving Pencillamine for Wilson's disease. JAMA. In press.

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Greenberg, M.S., Anderson, K.C., Marchetto, D.J., and Li, F.P.: Acute myelocytic leukemia in two brothers with polyposis coli and ccarcinoma of the colon. Ann. Intern Med. 95: 702-703, 1981.

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SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 05139-03 CEB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

NIH Interinstitute Medical Genetics Training Program: The Genetics Clinic

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	D.M. Parry, Ph.D.	Geneticist	CEB NCI
OTHER :	J.J. Mulvihill, M.D.	Chief, Clinical Genetics Section	CEB NCI
	P.A. Gilman, M.D.	Expert Scientist	CEB NCI
	E.A. McKeen, M.D.	Clinical Investigator	CEB NCI
	S.M. Robinette, B.S.	Research Assistant	CEB NCI

COOPERATING UNITS (if any)

NIADDK, NICHD, NIDR, NEI, NINCDS, CC

LAB/BRANCH
Clinical Epidemiology Branch

SECTION
Clinical Genetics Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.75	0.65	0.10

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The Genetics Clinic is a collaborative undertaking by researchers from six different Institutes and the Clinical Center. Consequently, Clinic patients constitute a broad spectrum of genetic disease. The patient load during the Clinic's second year comprised 304 individuals representing 60 different diagnostic categories. Of these, 74 patients (24%) were seen by members of CEB. For our Branch the Clinic provides a multidisciplinary setting in which to study unusual patients who either have cancer or increased risk of developing malignancy. Patients are ascertained through special referrals from outside physicians and inhouse requests for etiologic consultations. With informed consent, the approach to the patient includes detailed physical examination, and, where applicable, epidemiologic studies of the environmental and genetic background and laboratory studies to clarify biologic mechanisms of carcinogenesis. Categories include patients with genetic diseases predisposing to malignancy, patients with birth defects and cancer, families with childhood sarcomas and breast cancer in blood relatives, and any other families with an excessive occurrence of cancer of any type.

Project Description

Objectives:

1. To identify genetic and environmental factors in the development of human cancer.

2. To utilize the insights provided by the multidisciplinary approach of the Clinic to pursue new avenues of clinical and laboratory investigation into cancer risk.

3. To provide counseling for persons at high risk of malignancy and recommend appropriate medical surveillance for the early detection of tumors.

4. To provide training to graduate physicians and medical students in the diagnosis, counseling and treatment of individuals with or at risk of genetic disease, and in the research approach to genetic disease.

Methods Employed:

Referred patients are examined to determine the extent of any pre-existing condition or birth defects and for clues to the etiology of cancer in themselves or family members. When exceptional clinical observations are made, appropriate follow-up epidemiologic and laboratory investigations are conducted. Certain categories of patients, i.e., those with testicular carcinoma, are examined and tested according to an established protocol to ensure uniform data collection. Physicians and medical students in training undertake patient interviews, physical examinations, treatment and counseling under the direct supervision of an attending physician.

Major Findings:

1. Radiosensitivity and cancer susceptibility. The gamma-ray sensitivity of skin fibroblasts from six members of a previously described cancer family was investigated using a colony forming assay. Examined fibroblasts included those from two sisters with AML, the mother with rectal cancer following radiotherapy for uterine cancer, and three unaffected individuals including the father and two sons. Fibroblasts from the three individuals with cancer showed a significant increase in radiosensitivity, while those from the unaffected individuals gave a normal response. A variety of assays for DNA repair replication, single strand break rejoining and removal of enzyme sensitive sites in gamma-irradiated DNA gave similar results in all six cell lines. Although no direct evidence for defective DNA repair has as yet been demonstrated in this family, the initial results suggest a correlation between cancer proneness in vivo and enhanced radiosensitivity in vitro.

2. Radiosensitivity and cancer susceptibility. The dysplastic nevus syndrome (DNS) is a preneoplastic melanocyte abnormality which occurs in families affected by hereditary cutaneous malignant melanoma (HCMM). Although environmental exposures, especially solar UV-irradiation, have been implicated as risk factors in sporadic melanoma, the role of such exposures in the pathogenesis of HCMM is unknown. In

vitro radiation responses were studied in six non-tumor skin fibroblast strains from HCMM/DNS patients representing five families. All six HCMM/DNS strains were found to show some degree of enhanced cell killing sensitivity, compared with normal controls, following 254 nm UV-irradiation. The abnormal survival responses appeared to relate to specific characteristics of HCMM/DNS cells since the six strains had essentially normal sensitivity to gamma-radiation. The enhanced photosensitivity was not associated with abnormal patterns in either DNA repair synthesis or UV-induced inhibition and recovery of de novo DNA synthesis. The survival results are consistent with the hypothesis that the genetically determined predisposition to malignant melanoma may directly or indirectly be the consequence of increased susceptibility to UV-induced cellular damage.

3. Case-control clinical study of patients with renal cancer. A case-control study of renal adenocarcinoma suggested an association between that cancer, accessory nipples and genitourinary anomalies. Individuals with renal cancer and such anomalies were further evaluated in the clinic. Two brothers with renal cancer and anomalies of the nipples and genitourinary tract, and their sibs, were examined for other anomalies which might be embryologically related. Also examined were two other individuals with renal cancer and a first-degree relative with brain cancer. This protocol included peripheral blood cell karyotypes, dermatoglyphics, X-rays and an extensive physical examination.

4. Clinical study of patients with testicular cancer. The discovery of an association between renal cancer, genitourinary anomalies, and accessory nipples (polymastia) suggested that patients with testicular cancer might also manifest an excess of developmental defects. All living testicular cancer patients ages 18-40 years seen at NCI during the last five years underwent a medical interview, physical examination and review of family history. Available X-rays were also examined. Definite polymastia was seen in 7/62 examined white patients (11%); this frequency was significantly higher than that in white male medical students (8/299=2.7%). Genitourinary anomalies and bony malformations also appeared to occur excessively among the testicular cancer patients. Family histories revealed nine relatives with testicular cancer, two fathers with renal cancer, two mothers with breast cancer and a sister with gestational choriocarcinoma. These data, which suggest that a common prenatal abnormality may predispose to testicular cancer, polymastia and the other developmental and neoplastic defects, are being prepared for publication.

5. Clinical and laboratory studies of two families with respiratory tract cancers. Members of two families with lung and other respiratory tract cancers were investigated. Consenting individuals underwent comprehensive evaluation, including physical examination, routine laboratory sutdies, pulmonary function testing, sputum cytology analysis, and lymphocyte karotyping. In both families, the environmental influence of smoking and, to a lesser extent, occupational exposures were evident risks. Both families had members with multiple primary malignant neoplasms and probably radiogenic cancers, suggestive of predisposition to environmentally induced nioplasia. Furthermore, one family had a newly recognized syndrome of limb and dental anomalies, and, independently, two members were carriers of a balance translocation between chromosomes 13 and 14. Efforts were made to prevent further respiratory cancer deaths, to search for laboratory markers of risk, and to store blood and tissue specimens for assays in development.

6. Genetic linkage studies in neurofibromatosis. For several years, members of CEB have been studying patients with neurofibromatosis, a relatively common autosomal dominant disorder predisposing to malignancy. One of our goals has been to try to determine on which chromosome the gene for this disease is located. This knowledge would facilitate pre-and post-natal diagnosis and stimulate research into its pathophysiology. Recently, we investigated the possible linkage of neurofibromatosis with 27 genetic markers in 11 multigenerational families. We examined and drew blood on 108 persons, including 54 affected individuals. Lod scores were calculated using the standard LIPID program. Close linkage was excluded for 15 loci including three each on chromosomes 1 and 6, including HLA, and was inconclusive for 11 others. Nine families gave a lod score of +2.7 for linkage with Gc (chromosome 4). The one other informative family had a negative lod score so that the combined lod score was only +1.0. These findings suggest that the neurofibromatosis phenotype may result from mutation at more than one locus, an interpretation supported by its high frequency and mutation rate as well as its clinical diversity. These results are being prepared for publication.

7. Etiologic consultation on an identical twin with Burkitt lymphoma. The case of a 7-year-old identical twin boy with Burkitt lymphoma demonstrated the concept of utilizing a consultation to gain insight into possible cancer etiology. There was no evidence on physical examination or in the child's medical or family history of a genetic disease predisposing to lymphoma. The families' environmental exposures were not suspect. Results from a study of U.S. childhood cancer death certificates indicated that the risk of lymphoma in the co-twin was low. Ophthalmalogic consultation was recommended to evaluate dry eyes in the mother and the affected twin; evidence of sicca syndrome could provide a link with lymphoma.

8. Pregnancy risks in women with porphyria. Clinical and laboratory observations on pregnancies in women with porphyria and their outcome were described.

Significance to Biomedical Research and the Program of the Institute:

The Clinic provides a unique multidisciplinary setting in which unusual occurrences of cancer can be identified and studied by geneticists, epidemiologists and laboratory investigators. Through their experiences in the Clinic, graduate physicians become aware of and instructed in methods of studying unusual occurrences of cancer that involve the integrated collaboration of clinic and laboratory personnel. The regular post clinic conferences and seminars provide major vehicles for dissemina-tion of new findings in cancer etiology to scientists representing a broad array of clinical and laboratory expertise and offer opportunities to establish future insightful collaborations.

Proposed Course:

The Genetics Clinic will continue to provide a unique setting for the study of genetic and environmental factors predisposing to increased cancer susceptibility. In our effort to learn about the biologic causes of cancer we will continue to ascertain and study patients with genetic diseases predisposing to cancer, familial aggregates of cancer, and patients with birth defects or unusual environmental exposures associated with tumor development. The Genetics Clinic will continue to provide unique clinical opportunities through which to train physicians to study genetic and environmental factors predisposing to increased cancer susceptibility.

Publications:

Bech-Hansen, N.T., Sell, B.M., Mulvihill, J.J., and Paterson, M.C.: Association of in vitro radiosensitivity and cancer in a family with acute myelogenous leukemia. Cancer Res. 41: 2046-2050, 1981.

Goedert, J.J., McKeen, E.A., and Fraumeni, J.F., Jr.: Polymastia and renal adenocarcinoma. Ann. Intern. Med. 95: 182-184, 1981.

Goffman, T.E., Hassinger, D.D., and Mulvihill, J.J.: Familial respiratory tract cancer. Opportunites for research and prevention. JAMA 247: 1020-1023, 1982.

Lamon, J.M., Leake, R.D., Levy, H.L., Schulman, J.D., and Shih, V.E.: Selected metabolic diseases. In Schulman, J.D. and Simpson, J.L. (Eds.): Genetic Diseases in Pregnancy: Maternal Effects in Fetal Outcome. New York, Academic Press, 1981, pp. 1-5

Miller, R.W., Maron, L. and Mulvihill, J.J.: Burkitt lymphoma and dry eyes in an identical twin: Etiologic consultation. Clin. Bull. In press.

Smith, P.J., Green, M.H., Devlin, D.A., McKeen, E.A., Patterson, M.C.: Abnormal sensitivity to UV-radiation in cultured skin fibroblasts from patients with hereditary cutaneous malignant melanoma and dysplastic nevus syndrome. Int. J. Cancer. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 05146-03 CEB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Morbidity in Childhood Cancer Survivors and Their Offspring

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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	S. Abbott, M.S.	Statistician	BB	NCI
	R.R. Connelly, M.S.	Statistician	BB	NCI
	E.A. McKeen, M.D.	Clinical Investigator	CEB	NCI

COOPERATING UNITS (if any)

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NCI, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.1	2.0	0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Fertility and reproductive history in cancer patients, especially in long-term survivors of childhood cancer, and in men and women who reproduced during cancer therapy are studied for possible mutagenicity and teratogenicity of cancer treatments, and to discover hereditary patterns of cancer. Current phases include (1) interviews of 2,800 adults who survived cancer in childhood and their sibs as controls; (2) registry of pregnancies in young adult women through correspondence with physicians in Cancer and Acute Leukemia Group B. Other possible cohorts for study and other methodologies are being explored.

Project Description

Objectives:

To document fertility and reproductive outcome in patients with cancer before, during, and after treatment. The goals are to test genetic theories of cancer etiology, to define potential gonadal toxicity of cancer treatment, both teratogenicity and mutagenicity, and to provide needed information for genetic counseling of long-time survivors of cancer. The hypothesis being examined is that cancer patients, especially, have excessive morbidity due to additional malignancies or other illnesses, and impaired reproductive performance, including an increased frequency of live offspring with birth defects or cancer.

Methods Employed and Major Findings:

Three separate phases are in different stages of completion:

1) A registry of young women with cancer was assembled from physicians of Cancer and Acute Leukemia Group B. Preliminary analysis of 137 pregnancies in 66 women has been presented, showing little if any teratologic effect, but some excess wastage of pregnancies conceived within 12 months of completing chemotherapy.

2) Intensive interviewing and record abstracting are underway among individuals in California, Connecticut, Iowa, Kansas, and Texas who had cancer under age 19, survived at least five years, and achieved at least age 18 years. The controls are up to two siblings per case. The cases include 2840 patients (26% lymphoma, 15% brain tumor, 11% soft tissue sarcoma, and 7% embryonal tumors) with a mean age at diagnosis of 15 years, and a mean year of diagnosis of 1962. Treatment regimens were surgery only in 37%, radiation only in 20%, both in 17%, and some chemotherapy in another 17%. Field work is nearing completion in Iowa and Kansas, and is well along in Connecticut.

3) Book and symposium chapters were prepared to emphasize the principles of mutation epidemiology and the potential usefulness of studies of reproduction by cancer patients in addressing the refractory question about human germinal mutagens.

Significance to Biomedical Research and the Program of the Institute:

The study of reproduction in cancer patients may help document the familiality of certain tumors especially of childhood, the predicted but poorly documented teratogenicity of modern cancer therapy, and the predicted but undocumented germinal mutagenicity of radiation and drugs. The data may be directly used in counseling cancer patients.

Proposed Course:

The case registry of Cancer and Acute Leukemia Group B is closed, although comprehensive analysis continues. The staff of the Clinical Oncology Program continues surveillance of their patients. The five-Center study is scheduled to end the collection of data by Spring 1983. Additional populations suitable for investigation

are being identified, as well as the feasibility of laboratory measures of mutation to increase the power of subsequent studies.

Publications:

Mulvihill, J.J.: Towards documenting human germinal mutagens: Epidemiologic aspects of ecogenetics in human mutagenesis. In Sugimura, T., Kondo, S., and Takabe, H. (Eds.): Environmental Mutagens and Carcinogens (Proceedings of Third International Conference on Environmental Mutagens). University of Tokyo Press, Tokyo/Alan R. Liss, Inc., New York, 1982, pp. 625-637.

Mulvihill, J.J. and Miller, J.R.: Mutation Epidemiology: Prospects for detecting human germinal mutations. In Kilbey, B.J. and Nichols, W.W. (Eds.): Handbook of Mutagenicity Test Procedures. Amsterdam, Elsevier Scientific Publishing Co., 1982. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 05194-02 CEB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

National Cancer Mortality Studies by Computer

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R.W. Miller, M.D.	Chief	CEB NCI
OTHER:	F.W. McKay	Computer Systems Analyst	CEB NCI
	M. Hanson, M.P.H.	Epidemiologist	CEB NCI

COOPERATING UNITS (if any)
National Center for Health Statistics
Bureau of the Census
DCRT

LAB/BRANCH
Clinical Epidemiology Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.4	1.3	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We have used information from the National Center for Health Statistics (NCHS) and Bureau of the Census to create a comprehensive data base concerning mortality and population information at the county level. Data are available, 1950-1978, for cancer mortality, and 1965-78, for deaths from other causes. The mortality data will be extended through 1979 when public use NCHS mortality tapes are received. Population data will be extended and corrected when the 1980 census data become available. Three-dimensional graphs employing these data are one example of the value of the data collection. A system for mapping counties in black-and-white is being developed, along with a system for analyzing projections of cancer mortality in the coming decades.

Project Description

Objectives:

1. To develop new ways for evaluating existing cancer mortality data for the United States by computer.

2. To project the numbers of cases expected in the next 20 years based on changes in the age distribution of the population; e.g., the baby boom of the 1950's.

3. To provide special data tabulations to others on request.

Methods Employed:

The data, which were collected by NCHS in a varying format from year to year, 1950-1977, have been reworked into a common format. The widely known Atlases of Cancer Mortality in the U.S. by County were the first results from studying these data. Programs have now been developed for creating three-dimensional graphs of cancer mortality rates by site, race, sex, calendar year and age-group. The graphs are drawn on a Calcomp X-Y plotter.

The most efficient use of computer time has been achieved through the use of programs written in COBOL, Fortran or Assembler. Over 30 fast-running programs are used to keep the data base current. Most programs are run often enough to keep their operation efficient.

Major Findings:

1. The three-dimensional graphs, which allow trends in cancer mortality to be seen at a glance, have been published as a 500-page reference volume. Mr. McKay reformatted approximately 30 of these graphs for inclusion in The Surgeon General's Report on Smoking and Health.

2. The corresponding graphs for the population indicate changes in numbers of cases to be expected as the age composition of the nation changes over time.

3. Combining the two can provide estimates of the needs for services in cancer care in the future, taking into account the pattern of the rates (i.e., rising, falling or unchanging).

4. Other uses of the data during the year included:

   a. Developing a method of forecasting through the year 2000, using trends of the age-specific rates from 1950 through 1977 applied to the projected populations supplied by the Bureau of the Census.

   b. Discovering an apparently serious discrepancy in the traditional methodology that has been used for analyzing cancer mortality data at the county level. Basically, standard errors of age-adjusted death rates standardized to different populations, i.e., the 1960 U.S. population vs 1950 or 1970,

are not comparable; thus, maps prepared from data that have been age-adjusted to two different population standards will not necessarily be the same. The implications of this type of discrepancy will be examined further.

c. Designing and programming a data processing system to routinely analyze county mortality data by birth cohort.

Significance to Biomedical Research and the Program of the Institute:

The computer-generated volumes of tables and graphs of national cancer mortality are widely used, and special requests are frequently received and information provided.

Proposed Course:

Further work will be done on county mortality graphs to pool data on adjacent areas of special interest, as, for example, from radioactive fallout or chemical contamination.

Publications:

McKay, F.W., Hanson, M.R., and Miller, R.W.: U.S. Cancer Mortality 1950-1977. Natl. Cancer Inst. Monogr. 59, 1982, 475 pp.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 05198-02 CEB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Clinical Epidemiology of Neurofibromatosis

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:
OTHER: P. Silvain, B.S. Guest Worker CEB NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Epidemiology Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Individuals with the autosomal dominant genetic disorder, neurofibromatosis (NF), are thought to be at increased risk of cancer, particularly neural tumors. Multidisciplinary studies of individuals with this condition have terminated as a separate project, being subsumed under Familial, Congenital, and Genetic Factors in Malignancy (Z01 CP 04377-11 CEB), whence it arose two years ago.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 05279-01 CEB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Development of Epidemiologic Data Resources

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: G.W. Beebe, Ph.D. Expert CEB NCI

COOPERATING UNITS (if any)
Occupational Studies Section, EEB
Radiation Studies Section, EEB

LAB/BRANCH
Clinical Epidemiology Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

To develop a national system for occupational mortality, IRS information on occupation is being studied as a possible addition to the Continuous Work History Sample (CWHS) maintained by the SSA, and cause of death will be added to the CWHS file. Through the NIH Census/NDI Working Group efforts are being made to match a sample of the general population against the National Death Index (NDI) to create mortality rates by occupation and industry, not now available in the national mortality data. A mesothelioma study has been started to test the value of SSA files for epidemiologic research. Access by epidemiologists to the IRS address file is being sought through legislation.

Project Description

Objectives:

1. To develop and facilitate access to data files likely to be useful for epidemiologic research.

2. To encourage the linkage of large administrative data files in the interests of epidemiologic research.

Methods Employed:

Experiments are designed to test the technical feasibility and scientific adequacy of proposals for making use of data files in research on cancer and for linking large data files to produce new information. Methods used in other countries with more advanced data systems are studied for their possible usefulness in the U.S. Legislative changes are sought in the interests of epidemiologic research.

Major Accomplishments:

1. Work was started with the Internal Revenue Service to determine if the occupational entries on the Form 1040 could be used effectively to update the Continuous Work History Sample (CWHS) maintained by the Social Security Administration (SSA).

2. A plan to test the usefulness of SSA information on employment histories was put into effect. The SSA will provide the histories of 200 men for comparison with parallel data obtained by interviews with the next-of-kin in a case-control study of mesothelioma.

3. A plan was worked out with the SSA for obtaining death certificates and cause of death on CWHS subjects in the interval 1973-1977. The study will test the usefulness of the CWHS as a tool for probing for differentials in mortality rates that may provide clues to carcinogenic hazards in the workplace. If successful, it should lead to a retroactive completion of data to 1968, and a forward projection after 1977. The U.S. would then have a national sample with which at least the major industries could be screened for carcinogenic hazards. High risks could be made the subject of specifically designed epidemiologic studies.

4. Through the NIH Census/NDI Working Group it was determined that the linking of a significant part (4.5 million) of the 1980 Census with the National Death Index (NDI) to create mortality tables in relation to industry, occupation, and other demographic, geographic, and economic facts entered on the Census "long form" was possible by computer, but that without the Social Security number (SS#) on the Census return the completeness of mortality ascertainment would probably never reach 90 percent. This work also showed that the current algorithm used by the NDI would probably match correctly only about 80 percent of deaths in the absence of the SS#. The basis was laid for a more effective matching algorithm in the future.

5. Various legislative proposals were reviewed and commented upon, and a legislative initiative undertaken, through lawyers at the Department level, to regain access to the IRS address file for epidemiologic research, an access that was destroyed by the Tax Reform Act of 1976. This proposal is under consideration at OMB.

Significance to Biomedical Research and the Program of the Institute:

The work on epidemiology data sources, which is organized through the NCI Working Group on Epidemiology Data Sources, holds promise for developing better research tools and opportunities for epidemiologic research, for pointing to differential risks that may deserve more intensive study, for monitoring changes in carcinogenic risk at the mortality level, and for judging the impact of preventive programs in industry.

Proposed Course:

The search for neglected data bases will be continued. With the start that has been made in the area of occupational mortality it is expected that one or another of the various approaches to the development of a national system will in time succeed. Efforts will be continued to develop all the apparent options for creating this information: systematic coding of the information on the death certificate, now largely neglected; the CWHS with or without information on occupation, or perhaps an even larger sample of Social Security account numbers; linkage of an adequately large piece of the 1980 Census to the National Death Index; and linkage of the Current Population Survey to the National Death Index. Efforts will be continued to ease the restrictions on the access of health researchers to major Government files such as the IRS address list and the Social Security records of employees by industry of employment.

Publications:

Beebe, G.W.: Discussion of Smith, M.E. and Silins, J.: Generalized iterative record linkage system. In American Statistical Association 1981 Proceedings of the Social Statistics Section. 1982, pp. 138-139.

Beebe, G.W.: Prospects for national data on occupational mortality. In Proceedings of a Conference on Epidemiologic Methods for Occupational and Environmental Health Studies. Washington, D.C., Society for Occupational and Environmental health. In press.

Beebe, G.W.: Record linkage and needed improvements in existing data resources. In Banbury Report 9. Cold Spring Harbor, New York, Cold Spring Harbor Laboratory, 1981, pp. 661-673.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 05280-01 CEB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Carcinogenic Effects of Ionizing Radiation

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: G.W. Beebe, Ph.D.	Expert	CEB	NCI

COOPERATING UNITS (if any)
Occupational Studies Section, EEB
Radiation Studies Section, EEB

LAB/BRANCH
Clinical Epidemiology Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A-bomb survivors, AEC/DOE workers, and the population exposed to fallout from atmospheric tests at the Nevada Test Site have been studied for their potential to provide low-dose risk estimates for radiogenic cancer. Only some combination of epidemiologic studies at higher doses, with experimental and theoretical work, will provide a reliable guide to such risks. Sources of variation in risk estimates for radiogenic cancer are explored for their significance to research on carcinogenic mechanisms and to give direction to epidemiologic research.

PHS-6040

Project Description:

Objectives:

1. To evaluate the carcinogenic risk of low levels of ionizing radiation.

2. To determine the limits of knowledge of the carcinogenic effects of ionizing radiation and suggest research needed to extend that knowledge.

3. To suggest how knowledge of differential risks of cancer from exposure to ionizing radiation may be used in research on carcinogenic mechanisms.

Methods Employed:

A continuing analysis is made of the literature on the carcinogenic effects of ionizing radiation. Critical reviews are prepared and needed research outlined. Membership on various research committees provides opportunities for both gaining new information and testing the soundness of interpretations.

Major Accomplishments:

1. A variety of exposures to low doses of ionizing radiation were studied for their potential contribution to the estimation of the carcinogenic effects of low doses. A critical analysis of the A-bomb survivor experience was published. The ORAU study of AEC/DOE contractors was reviewed for a congressional committee. The progress of the Hopkins study of the nuclear shipyard workers was monitored as a member of the committee advisory to the study. The Tri-State experience with fallout from the Nevada Test Site was studied in connection with the RFP issued by the NCI and the compensation bills in the Congress. From none of these experiences did it seem likely that low-dose risk estimates of any considerable scientific value would be forthcoming.

2. An earlier review of what we have learned from human studies of the effects of ionizing radiation was published, and an analysis was made of what might be expected to emerge from the studies of the A-bomb survivors in the years ahead. A paper was prepared for the NCRP on radiation carcinogenesis to parallel a contribution on chemical carcinogenesis. Methods used in calculating radiogenic risks were published, and an analytic paper was prepared on risk assessment from the standpoint of a tool for exploring differentials in risk that may have significance for a deeper understanding of the carcinogenic process, e.g., that the hierarchy of carcinogenic risks by site bears little relation to that for natural incidence.

Significance to Biomedical Research and the Program of the Institute:

The work on carcinogenic effects of ionizing radiation provides up-to-date descriptive information on empirical risks, direction for epidemiologic research

efforts, a factual basis for regulatory standards, and a stimulus to thinking about the underlying biologic meaning of observed risk differentials in the human data.

Proposed Course:

The analysis of radiogenic cancer risk estimates will be continued, with special attention to differential risks associated with host factors, other environmental risk factors, specific tissues and organs, time from exposure, and such characteristics of radiation as its quality and dose-rate.

In addition, plans are being made for a large study of the 1942 postvaccinal hepatitis epidemic that occurred in the Army following mass immunization against yellow fever. A great deal can be learned from this experience about the relation between one or more of the hepatitis viruses and subsequent hepatoma. Epidemiologic evidence points to HBV as the likely agent of that epidemic, but a serologic survey will be required to make the positive identification. A mortality follow-up of adequately large cohorts of men chosen in relation to their vaccination experience and the presence or absence of an acute illness associated with the vaccination, should then provide new information on the risk of hepatoma following exposure to the virus in adult life, and the possible dependence of hepatoma on prior cirrhosis. In addition, the serologic survey would provide a much-needed estimate of the carrier rate for chronic active infection 40 years after exposure.

Publications:

Beebe, G.W.: The atomic bomb survivors and the problem of low-dose radiation effects. Am. J. Epidemiol. 114: 761-783, 1981.

Beebe, G.W.: Case studies: The Atomic Bomb Casualty Commission. In Dardanoni, L. and Miller, R.W. (Chm.): Plans for Clinical and Epidemiologic Follow-up fter Area-wide Chemical Contamination. Proceedings of an International Workshop. Washington, D.C., National Academy Press, 1982, pp. 114-125.

Beebe, G.W.: The estimation of risk from whole-body exposure to ionizing radiation. In Proceedings of the Seventeenth Annual Meeting of the National Council on Radiation Protection and Measurements: Critical Issues in Setting Radiation Dose Limits. Washington, D.C., National Academy Press, 1982, pp. 72-87.

Beebe, G.W.: Ionizing radiation and health. Am. Scientist 70: 4, 35-44, 1982.

Beebe, G.W.: Review of the book Hiroshima and Nagasaki, by Committee for the Compilation of Materials on Damage Caused by the Atomic Bombs in Hiroshima and Nagasaki. New York, Basic Books, 1981. Am. Scientist 70: 209, 1982.

Beebe, G.W.: Assessment of Risk from Exposure to Ionizing Radiation. In Prentice, R.L. and Whittemore, A.S. (Eds.): Environmental Epidemiology: Risk Assessment. Philadelphia, SIAM. In press.

Beebe, G.W.: Determinants of the carcinogenic response to ionizing radiation. In Upton, A.C. (Eds.): Comparative Carcinogenesis by Chemicals and Ionizing Radiation. National Council on Radiation Protection and Measurements. In press.

Miller, R.W. and Beebe, G.W.: Radiation Leukemia and Lymphoma in Man. In Upton, A.C. (Ed.): Radiation Carcinogenesis. New York, Elsevier North-Holland. In press.

ANNUAL REPORT
ENVIRONMENTAL EPIDEMIOLOGY BRANCH
OCTOBER 1, 1981 THROUGH SEPTEMBER 30, 1982

This is the seventh report of the Environmental Epidemiology Branch which was created in December 1975. The objective of the Branch is to generate and test ideas concerning the environmental and host determinants of cancer by a broad range of epidemiologic studies based on knowledge and application of clinical medicine and oncology, statistical methodology, new developments in carcinogenesis, and resources best available at the national level.

Joining the Branch this year as a research associate was Dr. Douglas Blayney, an internist and medical oncologist. Dr. Edward Trapido (Sc.D. in epidemiology from Harvard University) and Dr. Abbie Ershow (Sc.D. in nutrition from Harvard University) were appointed as Staff Fellows. Also joining the Branch for varying periods were visiting scientists from several countries, including China, Japan, Italy, England, France, and West Germany.

RESEARCH PROGRAM

Geographic Studies

Demographic patterns--To provide a systematic means for identifying geographic variation and clustering, the Branch has analyzed U.S. cancer mortality on a county level and prepared computer-generated color maps for 35 cancer sites. First, an atlas of cancer mortality was published for the white population, followed by a companion atlas for the non-white population covering the years 1950-1969. Maps for non-neoplastic diseases were published during the year, emphasizing conditions that predispose to cancer or share etiologic factors. Mapping of cancer mortality for 1970-1975 was also conducted for some of the more common tumors. Perhaps most striking was the updated map for lung cancer among white males, which revealed a shifting geographic pattern compared to the earlier period. Rates were high in broad stretches of the South, and elevated mortality was no longer seen in northern metropolitan centers.

Increases in mortality over time were documented for non-Hodgkin's lymphoma, particularly the histiocytic type, for multiple myeloma, especially among blacks, and for malignant melanoma. A continuing survey of Alaskan natives revealed an increasing incidence of "western" tumors (e.g., lung, breast, colon) which were uncommon in this population several decades ago. With visiting scientists from the People's Republic of China, reports were prepared showing a concordance of mortality from cancers of the uterine cervix and penis, with highest rates for both tumors in central China, and a correlation of colorectal cancer and schistosomiasis in Chinese provinces bordering the Yangtze river where the parasitic disease is endemic.

Field studies--To seek explanations for the geographic and temporal variation in cancer across the U.S., correlations were sought with demographic and environmental data available at the county level. The clues derived from the cancer maps and correlation studies have been pursued actively by the Branch, the final step being the testing of specific hypotheses by case-control investigations in high-risk sections of the country. Branch investigations of lung cancer in high-risk coastal areas of Georgia and Florida,

and lung cancer and mesothelioma in coastal Virginia, revealed significantly increased risks associated with employment in the shipbuilding industry, particularly during World War II. A summary analysis of lung cancer combining data from more than 2,500 interviews in these surveys placed the relative risk, adjusted for smoking, for employment in the industry in the 1940's at about 1.4. This suggests that as many as 100,000 extra lung cancer deaths may eventually result among the cohort of some 4.5 million Americans who worked in ship construction and repair during World War II.

Also completed this year was a case-control study of lung cancer in eastern Pennsylvania. A significantly increased risk was found among men who worked in the steel industry, the area's major employer. The excess was primarily among long-term employees, particularly those who began work before 1935, but was not confined to a single trade within the industry. Some excess risk was also seen for zinc smelter workers employed at least 15 years, perhaps due to arsenic exposure. The findings implicate occupation as an important cause of lung cancer in the area, and suggest that exposure within the steel industry may contribute to an extent greater than previously recognized. Respiratory cancer is also the focus of case-control interview surveys in New Jersey with the State Department of Health, in coastal Texas with the University of Texas School of Public Health, and in southern Louisiana with Louisiana State University. The latter study includes pancreas and stomach cancers, which also cluster in this area.

Reported this year were results from a case-control survey of esophageal cancer among black men in Washington, D. C., where the mortality rates exceed those in all other U.S. cities. Heavy alcohol consumption was the dominant risk factor. Nutritional deficiency was also found to play an independent role, with decreased intake of fruits and vegetables, fresh meats, and dairy products. A case-control study of cancer of the mouth and throat among women in North Carolina revealed that snuff dipping accounts for the excess risk of these tumors in the rural South. There was no evidence of an association with employment in the textile industry, as previously suspected. An increased risk was associated with electronics manufacture, but the numbers of individuals involved were small. Use of mouthwash was also suggested as a risk factor, but only among abstainers from alcohol and tobacco, a finding consistent with reports from two other surveys of oral cancer.

To clarify the relationship between woodworking and nasal cancer, a case-control interview study is being conducted in Virginia and North Carolina. To help explain the clustering of renal cancer in the north central area of the U.S., the Branch carried out a case-control study with the University of Minnesota. Nearly 600 kidney cancer patients and twice as many controls were interviewed. Cigarette smoking was the major risk factor for cancer of the renal pelvis, with greater than 5-fold excesses among smokers of both sexes, and accounted for about a 50 percent increased risk of renal adenocarcinoma. Also completed was a case-control study of colorectal cancer in areas of rural Nebraska, where mortality rates were high. Elevated risks were found among persons of Czech descent, especially those with high fat diets and familial occurrence of digestive tract cancer.

Occupational Studies

Epidemiologic studies of occupational groups are valuable since workers often have heavy and prolonged exposures to carcinogens which are also found in the general environment. Cancer patterns are determined through long-term follow-up of persons employed in specific plants and industries, and comparisons made with the general population or other industrial groups when available. Proportionate mortality studies are conducted when population data are unavailable. Case-control studies of particular cancers are carried out in areas where occupations of interest are concentrated, utilizing personal interviews or available employment records.

The relationship between cancer and employment in the chemical and petroleum industry has been under intensive investigation. A retrospective cohort study of male chemists employed at a chemical company showed a slightly elevated risk of melanoma, and cancers of the colon and prostate, although the total number of cancers was lower than expected. Among a small number of chemical plant workers having contact with benzene, there was a significant excess of deaths from hematopoietic cancers (three leukemias and one multiple myeloma) consistent with previous reports of benzene-exposed workers. Proportionate mortality studies of cancer among active and retired petrochemical workers in Texas revealed an excess of deaths from leukemia, lymphoma, and myeloma, and cancers of the brain, stomach, and skin. A larger percentage of cases than controls worked in areas involved in transport of refinery products and in production of motor oil. In a study of servicemen in chemical processing companies during World War II who had been exposed to tetrachloroethane and other chlorinated compounds while impregnating clothing against mustard gas, the cancer mortality experience was elevated as a result of leukemia, lymphoma, and cancers of the genital organs.

A proportionate mortality investigation among members of the International Molders and Allied Workers Union revealed an excess of lung cancer. The risk was mainly among younger workers (<65 years at death) in iron foundries, and not among workers in steel and nonferrous metal foundries. In a cohort study of iron ore miners, a significant excess of stomach cancer was noted among those working above and below ground, while lung cancer was excessive only among the foreign-born. An updated survey of mortality among copper smelter workers revealed that a respiratory cancer excess previously observed during the period 1938-1963 continued during 1964-1977. The risk was mainly in work areas of the plant where airborne arsenic levels were high and it increased with cumulative exposures.

Surveys of farmers and workers in agricultural-product industries have suggested associations with certain cancers. A case-control study of leukemia among Wisconsin farmers showed increased risks for farmers born more recently, dying at younger ages, and residing in counties where fertilizer usage and dairy production were heavy. In a parallel study of non-Hodgkin's lymphoma in Wisconsin, farming was more common among cases than controls, with increased risks among younger farmers residing in counties high in acreage treated with insecticides, planted with wheat, or planted with small grains. Case-control interview studies in Iowa, Minnesota, and Kansas were initiated to clarify factors in the agricultural environment that may contribute to these tumors. Finally, the pattern of causes of death among workers in the tobacco industry

revealed no peculiarities, although more deaths from colon cancer occurred than were expected.

An analysis of causes of death among male professional artists revealed an excess of leukemia and cancers of the bladder, kidney, brain, colon, and prostate. The excesses for leukemia and bladder cancer were more striking among painters, while the excess for prostate cancer was limited to sculptors. Among female artists there were higher proportions of cancers of the rectum, lung, and breast. These patterns may be partly from exposures to pigments and dyes, metal fumes, and solvents. Comparison of causes of death among veterinarians with the general population revealed significant elevations for cancers of the lymphatic and hematopoietic system, colon, brain, and skin. The excess of leukemia appeared due to the lax use of radiation protective equipment. A proportionate mortality study of embalmers, who have regular contact with formaldehyde, revealed increased frequencies of death from cancers of the skin, kidney, and brain, particularly among those licensed more than 35 years or first licensed before age 30. Because of the public health importance of formaldehyde, which is carcinogenic in laboratory animals, several large-scale cohort studies of formaldehyde-exposed workers were launched this past year. A study of ceramic plumbing fixture manufacturers showed an elevated frequency of lung cancer, perhaps related to the heavy use of talc in the casting of these appliances.

Radiation Studies

Studies of populations exposed to ionizing radiation are being conducted to investigate further the relationship between cancer risk and exposure to high doses and to improve estimates of risk associated with lower doses. An immediate practical need is for risk estimates on which to base decisions about the use of nuclear and radiological technology in medicine and industry. The study of radiation-induced cancer is also a promising approach to understanding carcinogenesis, and a multidisciplinary conference was held this year to focus on insights provided from such studies.

An international radiation study of cervical cancer includes over 200,000 women treated by radiation or surgery. Recent findings suggest that (1) after a minimum latent period of about 10 years, the risk of radiogenic cancer remains throughout life and does not decrease; (2) radiation-induced cancers do not tend to occur earlier than other naturally occurring cancers; (3) very high doses to limited volumes of tissue may induce cancers; (4) large doses received by cells that have high mitotic activities, as in the bone marrow or colon, might cause more "cell-killing" than large doses to other organs with less reproductive activity; (5) the radiation regimens used to treat cervical cancer are not so effective in inducing leukemia as are other radiation exposures that have been studied, although a slight risk might be associated with radiation received by marrow outside the pelvis; (6) the young and the very old are at greatest excess risk for cancer induction; (7) the constancy of the relative risk with age at exposure suggests that radiation may interact in a multiplicative fashion with other factors that cause cancer; and (8) ovarian irradiation may lower breast cancer risk at all ages.

A new breast cancer incidence study among atomic bomb survivors in Japan confirms earlier data. The dose-response function continues to show no departure from linearity, the temporal distribution of excess risk is unrelated

to dose, and women exposed at ages 10-19 have the highest excess risk. An apparent anomaly in earlier data, in which women exposed at ages 40-49 were at significantly decreased risk, disappeared. However, women at ages 40-49 and 50+ did not experience a significant elevation of risk. For the first time, women who were exposed at ages 0-9 showed a dose-related excess risk of breast cancer.

The second follow-up of women who received multiple chest fluoroscopies during pneumothorax treatment of tuberculosis reaffirms that repeated relatively low radiation doses pose some future risk of breast cancer, that the risk may be cumulative, and that a woman's lifetime risk is likely determined in large part during early adult life. No excess of total cancer deaths, leukemia, lung cancer, or lymphoma has occurred among fluoroscopically examined men or women. These findings indicate that the carcinogenic effect of multiple low-dose x-ray exposures is unlikely to be greater than currently assumed, and may be less for lung cancer and leukemia.

In a study of women treated with radioactive iodine or surgery for hyperthyroidism, no increased cancer mortality or incidence was observed. There was a slight excess of cancer of the thyroid and other organs with high 131-I exposure, but the numbers of cases were relatively small. No increased risk of breast cancer was observed. A study of non-Hodgkin's lymphoma revealed a positive correlation between radiation dose to the bone marrow and the risk of acute nonlymphocytic leukemia, independent of chemotherapy duration. A study to evaluate the risk of head and neck malignancies among children irradiated for enlarged tonsils is continuing, including physical examinations, blood studies, and chromosome evaluations. Three case-control studies were completed: (1) an interview study of 200 thyroid cancer cases and 400 population controls; (2) a study of 225 children who developed second primary cancers following treatment for childhood cancer in 6 countries; and (3) a study of childhood cancer in twins associated with prenatal x-ray.

Ongoing projects include a collaborative investigation of 10,000 children irradiated for ringworm of the scalp in Israel; a study of 170,000 x-ray technologists; a case-control study of approximately 2000 cases of leukemia and lymphoma to evaluate the risk of lifetime diagnostic x-ray exposures; and several studies in cancer registries or clinical trials to evaluate the carcinogenic effects of radiation therapy and chemotherapeutic agents.

Nutritional Studies

This year the Branch expanded its research to clarify dietary factors in cancer etiology. Several case-control studies in high-risk areas have probed for nutritional exposures. In Washington, D. C., the U.S. metropolitan area with the highest mortality rate for esophageal cancer among black males, an interview study revealed that poor nutrition and heavy alcohol consumption were the major risk factors. The risk due to poor nutrition was independent of that associated with alcohol, and could not be linked to a particular vitamin or food group deficiency. In contrast, a case-control study of oral and pharyngeal cancer among white women in North Carolina, a population with high mortality rates for those cancers, suggested that fruit and vegetables were the food group consistently lacking in the diet of the cases. Both carotene and vitamin C intake are highly correlated with fruit and vegetable consumption. Another study focused on the unexpected excess mortality from colon cancer in a cluster

of rural counties in Nebraska. The excess risk was primarily among people of Czech ancestry and seemed associated with a high intake of meat and dairy products. The role of dietary fat was also suggested by a case-control study of breast cancer in Alberta, Canada, where total beef and pork consumption was related to increased risk. This association remained after adjusting for age at menarche and age at menopause, even though beef and pork consumption was correlated with younger age at menarche and older age at natural menopause among the Alberta control population. Correlations between diet and anthropometry have previously been noted only in international comparisons. A study of colorectal cancer in Florida retirement areas is in progress to evaluate the low rates in the South, including areas with a large percentage of migrants from the North. Also under way is a study to evaluate the role of dietary factors in increasing the risk of breast cancer among Japanese migrants to the U.S.

Several studies are being conducted to evaluate the influence of vitamin A and carotene in reducing the risk of epithelial cancers. A dietary component was added to case-control studies of lung cancer in New Jersey and Texas to ascertain whether retinol, carotene, vitamin C, fruits and vegetables in general, or some other correlate is associated with reduced risk, and to examine the interaction of diet with smoking and other risk factors. A case-control study of invasive and in situ forms of cervical cancer is under way to measure the intake of various micronutrients, both by interview about usual adult dietary patterns and by laboratory assay of blood samples.

The Branch continued to develop and utilize national data resources that might contribute to research on nutrition and cancer etiology. Several analytic studies involve HANES I, the first Health and Nutrition Examination Study of the U.S., conducted in 1971-1974 on 23,000 people. In collaboration with other Institutes and the National Center for Health Statistics, the Branch is tracing and reinterviewing the 1982-1984 adults examined in HANES I in an effort to relate dietary habits with the subsequent risk of cancer.

Medicinal Agents

Demographic, clinical, and laboratory observations are monitored for candidate drugs that can be evaluated for carcinogenic effects utilizing special resources developed by the Branch. These include clinical trials for long-term effects, follow-up of specific patient populations, intensive case-control investigations, and record linkage studies within pre-paid health plans. These studies are valuable in the clarification of drug effects and in the development of insights into mechanisms of carcinogenesis.

This year several studies were undertaken to assess the hazards of various hormonal preparations. A study of 345 women who developed breast cancer while enrolled in one pre-paid health plan in California revealed a 40% excess risk associated with conjugated estrogens used at menopause. There was a dose-response relationship with three different measures of dose, rising to a two-fold excess for the long-term users. In a study combining data from three health plans in California, there was a two- to three-fold excess risk for breast cancer associated with long-term use of estrogens among women having undergone bilateral oophorectomy. The influence of oral contraceptive use was evaluated among 963 breast cancer patients and 858 controls participating in a large mammography screening project. Overall there was no increase in risk among

long-term users. However, non-significant excess risks were associated with contraceptive use among women exposed in the perimenopausal period and among several subgroups, including women with a family history of breast cancer or a previous biopsy for benign breast disease.

This year the Branch further expanded its program to evaluate the potentially carcinogenic effects of alkylating agents, anti-metabolites, and other medications used in the treatment of cancer and some non-neoplastic conditions. The studies often utilize specific clinical trials in collaboration with the Division of Cancer Treatment. In a follow-up of ovarian cancer patients participating in randomized trials, there was a substantially elevated risk and a dose-response relationship associated with alkylating agents. An evaluation of nine clinical trials involving the use of methyl-CCNU revealed an excess risk of leukemia among patients treated with this drug. In a follow-up of 517 patients with non-Hodgkin's lymphoma at the NIH Clinical Center, there was an excess of acute non-lymphocytic leukemia related to therapy, particularly total body irradiation, and an excess of lung cancer that seemed unrelated to specific treatment. Nearing completion is a study of cancer risk in rheumatoid arthritis patients treated with alkylating agents. With the Late Effects Study Group an intensive study is being conducted on children with multiple primary cancers to clarify the role of therapy in the development of second cancers.

Recent concerns have been raised about the promoting effects of diazepam (valium) and thyroid supplements on breast cancer risks, but case-control studies have uncovered no associations. Another study is under way to evaluate the long-term effects of dapsone used by patients with Hansen's disease, since this drug is carcinogenic in laboratory animals.

Case-Control Studies

The Branch is conducting a variety of case-control studies that are not limited to high-risk areas or targeted to test one particular hypothesis (e.g., occupation, radiation), but are designed to evaluate a wide variety of etiologic leads. This year emphasis was placed on analyses of large case-control studies of bladder cancer and breast cancer. In the National Bladder Cancer Study, there was no substantial elevation in risk associated with the use of artificial sweeteners in the total study group, but subgroup analyses provided some evidence consistent with a weak carcinogenic effect. Other preliminary findings included no obvious associations with use of hair dyes, a diminished risk with never having drunk coffee (but no dose-response relationship among coffee drinkers), and a two-fold risk with multiple urinary tract infections. The breast cancer study was carried out within the context of a large screening program, and allowed an evaluation of the interaction between risk factors. Familial susceptibility to breast cancer appeared to be mediated through hormonal factors that operate early in a woman's life, and a synergistic interaction with the occurrence of surgically-confirmed benign breast disease. Evaluations of in situ and other forms of "minimal" breast cancer indicated epidemiologic features which resemble those of larger invasive breast cancers and are unlike those of benign breast disease.

Also nearing completion are studies of intraocular melanoma, testicular cancer, ovarian cancer, kidney cancer, cutaneous T-cell lymphoma, bladder cancer in children, nasal cancer, and in situ and invasive cancers of the uterine

cervix. The exposures being assessed in these studies include occupation, diet, smoking, reproductive and sexual characteristics, drugs, personal habits, and various host factors.

Family Studies

Studies of cancer-prone families provide special opportunities to clarify the role of genetic susceptibility and environmental interactions in carcinogenesis. These investigations are conducted jointly with the Clinical Epidemiology Branch and with clinical and laboratory scientists at NIH and elsewhere. The development of an integrated manual and computerized record-keeping system has provided a framework for an expanding data base that grew by over 200 new families in the past year, bringing the total to more than 2,100 families. Utilizing this capability, statistical genetic approaches were applied in the analysis of patterns of cancer occurrence in families prone to sarcomas and diverse neoplasms, segregation and linkage analyses of melanoma-prone families, and linkage analysis of HLA in families prone to Hodgkin's disease.

The interdisciplinary approach continued to provide insights into the mechanisms of host susceptibility to cancer, as illustrated by the evaluation of familial melanoma and its relation to the dysplastic nevus syndrome (DNS). Based on a detailed study of 400 members of 14 melanoma-prone families, the clinical and pathologic features of DNS have been precisely defined. The discovery of many new primary melanomas in study participants underscores the value of close surveillance to detect early surgically-curable lesions. The role of these nevi in at least one-third of the cases of non-familial melanoma was also established this year. In vitro ultraviolet (UV) radiation sensitivity in cultured skin fibroblasts of patients suggests a biologic basis for host-environmental interactions in this dominantly inherited syndrome. A primary area of activity has been establishing and coordinating a free-loan program for distributing three educational videotapes which were developed in collaboration with the University of Pennsylvania to disseminate information about this syndrome to concerned health professionals and the general public. It is estimated that 55,000 persons viewed these materials this year.

A survey of 25 kindreds prone to diverse neoplasms, including bony and soft-tissue sarcomas, breast and brain cancers, leukemia, and other tumors has been analyzed. Utilizing life-table techniques, it was possible to quantify the familial risk of mesenchymal and epithelial neoplasms. In vitro study of fibroblast cells from seven members of one kindred showed that affected and high-risk individuals, but not spouses, are resistant to cell killing by gamma radiation. Biochemical characterization of this novel phenotype may have important implications for understanding mechanisms of carcinogenesis, especially since family members appear unusually susceptible to carcinogenic exposures, including radiation.

This year immunogenetic studies in families prone to various lymphoproliferative disorders linked an HLA-MB antigen to Hodgkin's disease susceptiblity, and revealed HLA haplotype sharing in three sibs with hairy cell leukemia. In a study of "heritable" renal cancer (patients with familial occurrence, early onset, or bilaterality), a statistical approach was developed for calculating HLA phenotype frequencies from 2 and 3 locus haplotype

frequencies. One particular 3 locus phenotype (HLA - A3, B7, DR2) was excessive and may be related to ethnic susceptibility to kidney cancer. New laboratory approaches, such as fluorescence-activated cell sorter analysis and recombinant DNA gene cloning technology, are being applied to the evaluation of families prone to chronic lymphocytic leukemia, in which shared cell surface immunoglobulin determinants have been identified.

Clinical observations prompted a case-control study of renal carcinoma that identified an excess of polymastia and genitourinary abnormalities in patients and their close relatives. A parallel survey of testicular cancer patients also revealed an increase of polymastia along with genitourinary and bony malformations. In studies of familial ovarian cancer, three women developed disseminated intra-abdominal carcinomatosis of unknown primary site following prophylactic oophorectomy. Since the histology of these malignancies was indistinguishable from an ovarian primary, it appears that the "target organ" at risk includes a spectrum of mesothelial structures sharing a common embryologic origin with ovarian epithelium.

A survey of offspring and siblings of 149 children with Wilms' tumor reported to the Connecticut Tumor Registry during 1935 to 1973 revealed an excess risk of other cancers, including rhabdomyosarcoma and Hodgkin's disease. In collaboration with the Sloan-Kettering Institute, studies were carried out on families prone to colon cancer, and new analytic approaches developed to quantify the shifts in colonic crypt-cell proliferation associated with cancer risk. A patient with male breast cancer from a family prone to diverse malignancies showed in vitro sensitivity to ionizing radiation and bleomycin; thymic irradiation as a child may have contributed to the development of this patient's tumor. A repository of biologic specimens on high-risk families remains a valuable source of materials for experimentalists investigating susceptibility mechanisms in carcinogenesis.

Infectious Agents

Viruses have not been causally tied with certainty to the origins of any human cancer, although the search continues on several fronts. The Branch became involved this year in two new areas of investigation. The human T-cell leukemia/lymphoma virus (HTLV) is a newly discovered type C retrovirus consistently isolated from cases of T-cell leukemia. In conjunction with investigators from the Laboratory of Human Tumor Cell Biology, Branch members have identified clusters of T-cell lymphoid malignancy associated with HTLV infection in certain regions of Japan, the Caribbean, and the southeastern United States. In these endemic areas, a distinctive form of T-cell malignancy with shared clinical and cytologic features has been linked to infection with HTLV. A comprehensive series of parallel epidemiologic studies in different areas is being planned.

Over the last 18 months clusters of Kaposi's sarcoma (KS) and opportunistic infections have become evident in homosexual males. These potentially fatal illnesses appear to complicate an epidemic immunodeficiency syndrome of unknown cause. In an effort to clarify mechanisms, the Branch conducted a study of apparently normal homosexual males, and found a high frequency of T-lymphocyte aberrations that appeared related to the heavy recreational use of amyl nitrite. Similar findings in homosexuals with KS suggest that this drug may be

immunosuppressive in the setting of sexually transmitted viral infections, with the cytomegalovirus being a chief suspect. Studies involving Danish homosexuals suggest a transmissible agent is involved, and coordinated epidemiologic investigations in the U.S. and Denmark are under way to further clarify risk factors for KS and the underlying immunodeficiency disorder prevalent in homosexual populations.

One Branch member continued studies on African Burkitt's lymphoma (BL), which has been related to infection by the Epstein-Barr virus (EBV) and perhaps malaria. With the University of Ghana Medical School, a survey of BL revealed an overall decline in incidence and a shift in clinical pattern resembling that seen in low incidence areas. In African children, high EBV antibody is considered a marker of increased risk for developing BL. In Ghana, as elsewhere, males showed a 2-fold excess of BL, but females had higher titers to EBV at every age. Thus, whatever factor promotes the excess risk in males appears independent of EBV response. In other studies, the EBV titers did not change in BL patients who survived many months (up to one year) in remission before relapsing, so this is not a useful means of predicting relapse. The relation of malaria to EBV infection was explored in a cross-sectional survey; although it is a known immunosuppressant, malaria had no effect on EBV response. Thus, if both agents are involved in the origins of BL, they probably operate independently. In progress are HLA studies of BL in an effort to clarify immunogenetic mechanisms. The Branch is also planning follow-up and case-control studies to evaluate the role of herpes virus type 2 infection on the risk of cervix cancer.

Immunoepidemiology

Several studies are being conducted to evaluate the role of immune function in the development of cancer. The risks of cancer of different sites are quantified for various groups of patients, and the characteristics and determinants of unusual risks are sought. The populations under study include renal transplant recipients, patients with diseases which alter immune function (e.g., Hansen's disease, end-stage renal disease, rheumatoid arthritis, lupus erythematosus), patients receiving drugs which alter immune function, and persons "hyperimmunized" by repeated vaccinations. Detailed analysis of renal transplant recipients indicated a 25-fold increased risk of lymphoma which appears within a year of transplantation, tends to arise in the central nervous system, is greater among recipients of cadaver than sibling grafts, and is more prominent among recipients in the earlier years of transplantation compared to more recent periods. With the University of Minnesota, a registry of cancers occurring in patients with genetic immunodeficiency disease has revealed a pattern of cancer risk resembling that noted among transplant recipients. On-going research in immunoepidemiology also includes case-control evaluation of immunologic parameters for specific tumors such as mycosis fungoides, and multidisciplinary studies of cancer-prone families to detect subclinical evidence of immunodeficiency.

Veterinary Studies

One Branch member conducted surveys of cancer and other diseases in pet animals, particularly dogs, based mainly on data collected by 16 veterinary medical teaching hospitals and clinics in the U.S. and Canada. By epidemiologic

comparisons with human cancer, these studies are designed to clarify risk factors in human cancer and related diseases, uncover animal models that may be useful in further research, and identify sentinels that may act as early predictors of environmental hazards.

This year a survey of the proportional morbidity of various cancers in the dog was correlated to the amount of industry in the locale of the dog's home (assessed by postal zip code). Bladder cancer had the strongest positive association, followed by nasal and oral cancers. Similar geographic correlations between human bladder cancer and industrial activity suggest that the pet dog may be an early sentinel for environmental hazards to man. A study of canine nasal carcinoma could not confirm a direct relationship with the size or length of the dog's nose. Several breeds were identified with excessive risk, and mongrel dogs had the same risk as all purebred dogs combined, further suggesting the influence of environmental factors. A study of canine biliary cancer indicated an association with infestation by blood-letting intestinal parasites among cases of cholangiocarcinoma.

The frequency of canine transmissible venereal tumor (TVT) in North America showed an inverse correlation with latitude, and an association with increasing temperature and rainfall. Review of the epidemiology of canine TVT revealed similarities to human Kaposi's sarcoma. Both are sarcomas of uncertain histogenesis, more aggressive and common in immunosuppressed hosts, endemic in tropical Africa, and apparently associated with latitude and rainfall.

Methodologic Studies

Several Branch members contributed to the adaptation and development of statistical methods useful in epidemiologic studies. A widely used general text which features a library of programs for epidemiologic analysis using a programmable calculator was updated and expanded into a second edition. A new method based on recursion formula was developed which allows rapid computation of exact conditional likelihood estimates of the relative risk, extending the applicability of this useful technique. The extent to which unconditional logistic analyses overestimate odds ratios from matched data sets was examined in a simulation study. One report described the features of design and methods of execution that helped reduce bias and assure quality control in a large case-control study. Several reports expanded methodology for use in occupational and other cohort studies. Another demonstrated a bias with the serially additive expected dose (SAED) method for assessing occupational exposure risks and proposed a correcting modification. Other reports presented new tests for equality of, and trends in, standardized mortality ratios (SMRs). A detailed method of regression analysis for SMRs was presented and linked to a Cox analysis for both cohort and case-control studies, thus unifying the various analytic techniques. Methodologic issues were also explored with respect to descriptive and correlational studies, estimating dose-response relationships for radiation-induced cancers, evaluating familial aggregations of cancer, and calculating HLA phenotype frequencies. For developing and describing an "occupation and exposure linkage system for the study of occupational carcinogenesis", one staff member received an author's award for the best paper published over the past year in the Journal of Occupational Medicine.

Reviews

The Branch continued to provide comprehensive and critical reviews of etiologic factors in cancer. One staff member served as co-editor of a reference volume entitled Cancer Epidemiology and Prevention. This year reviews were prepared on several cancers, including the lung, pancreas, biliary tract, multiple myeloma, non-Hodgkin's lymphoma, bone cancer, soft-tissue sarcoma, and non-melanoma skin cancer. Also reviewed was the series of Branch studies generated by the cancer mapping project. The epidemiologic evidence for environmental carcinogenesis and genetic susceptibility was considered in detail. Particular environmental hazards were reviewed, including ultraviolet radiation, medications, occupational exposures, parasites, water pollution, and food additives. Several review papers were written concerning the health effects following exposure to ionizing radiation, including a general overview, a review of cancers following medical irradiation, the effect of radiation on the immune system, the statistical aspects of estimating cancer risks from low doses of ionizing radiation, the epidemiologic issues concerning low-dose radiation studies, the implications of studies on radiogenic breast cancer for models of human carcinogenesis, the risk of cancer following treatment with radioactive iodine, and the long-term effects of radiation upon the human fetus and upon children. The proceedings of a conference on Radiation Carcinogenesis, held in May 1982, are being edited by Branch staff.

OTHER ACTIVITIES

The Branch continued to provide a liaison for epidemiologic research in the National Cancer Program and for environmental cancer studies being conducted in various agencies in the Federal Government. A great deal of advice and support was given to clinicians, experimentalists, public health officials, and many other groups. Members served on the editorial boards of various journals and on advisory groups and committees connected with cancer centers, several Federal and State agencies, and other national and international activities. Staff members helped in preparing reports on chemical carcinogens and other activities coordinated by the International Agency for Research on Cancer and the International Union Against Cancer. Several meetings and projects this year were related to bi-national agreements with the People's Republic of China, Italy, France, and Japan. At times staff members became heavily involved in controversial public policy issues and debates (e.g., low-level radiation, occupational hazards, food additives).

The Branch continued efforts to identify and utilize epidemiologic resources best available at the national level. Initiatives were taken to stimulate and develop cooperative projects with several government agencies possessing routinely collected data resources that can be utilized for epidemiologic studies (e.g., Social Security Administration, Internal Revenue Service, Department of Labor, Bureau of Census, National Center for Health Statistics). Another important activity of the Branch has been the on-the-job training of staff at the post-doctoral level, the supervision of medical students during their elective periods at school, field research opportunities for doctoral candidates at Schools of Public Health, and the assignment of visiting scientists with variable experience in epidemiology.

Although the Branch encourages an atmosphere of academic freedom and the development of new ideas and approaches, these undergo critical review and evaluation through several mechanisms. These include frequent section and branch meetings; close contacts with support service and collaborating groups; various formal review mechanisms by internal and external committees; several working groups (e.g., data resources, radiation studies, family studies, drug studies); interagency committees; the Clinical Center Review Committee involving clinical investigations; careful scrutiny of questionnaires and protocols prior to and during clearance through governmental channels; ad hoc external review groups for major studies (e.g., national bladder cancer project, formaldehyde study); the NIH Coordinating Epidemiology Committee; and a variety of advisory bodies that oversee Institute activities, notably the Board of Scientific Counselors of the Division of Cancer Cause and Prevention.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES . PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04378-07 EEB

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

U.S. Cancer Mortality Survey

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	T.J. Mason	Head, Population Studies Section	EEB	NCI
OTHER:	J.F. Fraumeni, Jr.	Chief, Environmental Epidemiology Branch	EEB	NCI
	R.N. Hoover	Head, Environmental Studies Section	EEB	NCI
	W.J. Blot	Head, Analytical Studies Section	EEB	NCI
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	R.I. Ramsbottom	Computer Specialist	EEB	NCI
	A.E. Blair	Acting Head, Occupational Studies Section	EEB	NCI
	L.W. Pickle	Mathematical Statistician	EEB	NCI
	H.M. Hayes	Veterinarian	EEB	NCI

COOPERATING UNITS (if any)

National Center for Health Statistics, Bureau of the Census, National Oceanic and Atmospheric Administration

LAB/BRANCH

Environmental Epidemiology Branch

SECTION

Population Studies Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.0	3.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) The overall objective of this project is to examine the cancer mortality experience in the United States relative to cancer etiology. Special emphasis is placed upon the selection of areas in the U.S. for intensive study. Publications from this area of interest have facilitated the design of ongoing analytical investigations to test specific etiologic hypotheses. Analyses of the cancer mortality experience in New Jersey through 1975 were completed. The geographic units were counties and municipalities. This refinement permits identification of areas for additional study. Three municipalities in Salem County were found responsible for our previously identified bladder cancer excess. This finding is consistent with occupational exposure in this area. Of specific interest is the finding that rates for lung cancer among women were found to increase at a much faster rate than any other cancer. Analyses of lung cancer for the entire U.S. dating 1950-1975 detected a pronounced shift in the geographic patterns for white males. Highest rates during 1920-1975 were in the South, both in rural and urban areas particularly at younger ages. An Eighth Revision ICD system for 1968-78 is near completion, and will greatly expand our capabilities on this project.

Project Description

Objectives: To examine the cancer mortality experience in the United States relative to cancer etiology. Special emphasis is placed upon the selection of areas in the U.S. for intensive study.

Methods Employed: This project involves computer analysis of over 6 million death certificates by site, sex, race, state, and age. The investigation is ongoing, updated each year, and expanding. Data for all causes of death are utilized from 1968.

Major Findings: An atlas of the descriptive epidemiology of cancer mortality in New Jersey was published. This investigation expanded on our earlier studies of cancer mortality at the county level. Analyses were performed at the municipality level in an attempt to identify additional places where analytic studies would seem warranted. Also, the time period was extended to include comparison data for the United States through 1975 in 5-year calendar periods. Of specific interest was the refinement of our earlier finding of exceptionally high rates of bladder cancer among white male residents of Salem County, New Jersey. The municipality-specific analysis found this excess among only three of the municipalities in this county. This finding is consistent with our prior hypothesis of occupational exposure in this area.

Data were available for the state of New Jersey from 1949 through 1976. During this study period, mortality rates for all cancer combined increased among males, but decreased among females. To a large extent, the increasing male rates reflect both the great increase in lung cancer and also colon cancer. Rates for the two major causes of cancer mortality among females, cancer of the breast and ovary, were stable over the period under study. However, rates for lung cancer among women were found to increase at a much faster rate than any of the cancers studied.

Although mortality from lung cancer increased throughout the U.S. during 1950-1975, the rate of change varied according to sex, race, and geographic sector. A pronounced shift in the geographic pattern of lung cancer was seen in white males. The elevated rates in urban counties of the north which were seen in the 1950's were not observed in the 1970's. The highest rates during 1970-75 were in the south, both in rural and urban areas, particularly at younger ages. The sharpest rise in U.S. mortality rates was reported among nonwhite males. Cohort analysis revealed that for males born before 1885 age-specific-rates among whites exceeded those among nonwhites by nearly 50 percent, but for those born after 1915 a 50 percent excess was seen among nonwhites. Among black males the highest mortality in the 1970's occurred in urban areas of the south. Among females the rate of increase in lung cancer mortality escalated in the 1970's, but racial and geographic differences tended to be minor. Cigarette smoking undoubtedly accounts for a substantial part of the shifting patterns of lung cancer, but it is likely that other factors are involved, including industrial exposures (e.g., to asbestos in shipyards) and possibly nutritional deficiencies and other determinants yet to be identified.

Significance to Biomedical Research and the Program of the Institute: This survey provides a continually expanding data set which has generated specific etiologic hypotheses concerning cancer. The capability of subdividing the data set into specific racial and geographic subsets (e.g., county level analyses) also provides an opportunity to also test specific etiologic hypotheses.

Proposed Course: The project will continue to pursue etiologic questions, and specifically will address the dynamic changes of rates for malignancy as a function of calendar time and geography.

Publications:

Blot, W.J., and Fraumeni, J.F., Jr.: Changing patterns of lung cancer in the United States. Am. J. Epidemiol. 115: 664-73, 1982.

Blot, W.J., and Fraumeni, J.F., Jr.: Geographic Epidemiology of Cancer in the United States. In Schottenfeld, D., and Fraumeni, J.F., Jr. (Eds.): Cancer Epidemiology and Prevention. Philadelphia, W.B. Saunders Co., 1982, pp. 179-193.

Stenhagen, A., Mogielnicki, A.P., Altman, R., and Mason, T.J.: Descriptive Epidemiology of Cancer Mortality in New Jersey: 1949-1976. Trenton, New Jersey Department of Health, 1981, Volume I. 484 p., Volume II, 376 p.

CONTRACT IN SUPPORT OF THIS PROJECT:

ORI, INC. (NCI-CP-01054)

Title: Biomedical Computing: Design and Implementation.

Objective: To provide systems design analysis and programming for intramural research projects.

Methods Employed: Research projects are reviewed for priority, and appropriate managers, analysts, and programmers are assigned tasks.

Major Contributions: Almost all studies resulting in recent publications from the Branch have had tasks performed by the contractor to facilitate their completion. The team approach that has evolved meets the varied needs of the Branch in a very responsible fashion.

Proposed Course: To provide comparable support through September, 1983.

Current Annual Level: $708,645.

Man Years: 21.0

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04401-06 EEB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Immunologic Factors in Cancer Etiology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R.N. Hoover	Head, Environmental Studies Section	EEB NCI
OTHER:	J.F. Fraumeni, Jr.	Chief, Environmental Epidemiology Branch	EEB NCI
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	A.F. Kantor	Staff Fellow	EEB NCI

COOPERATING UNITS (if any)

University of Minnesota; Immunodeficiency Cancer Registry; Veterans Follow-up Agency of the National Academy of Sciences; Oxford University

LAB/BRANCH
Environmental Epidemiology Branch

SECTION
Environmental Studies Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) The purpose of this project is to identify and study populations with altered immunologic states experiencing unusual rates of malignancy. Risks of cancer of different sites are quantified for various groups of such patients, compared with each other, and characteristics and possible determinants of unusual risks are sought. The populations studied include renal transplant recipients, patients with inherited and acquired immune deficiency syndromes, and groups of immunostimulated persons. Both cohort studies of these groups of patients and case-control studies of such patients who also developed cancer are being conducted. Markedly elevated risks of non-Hodgkin's lymphomas, soft-tissue sarcomas, lower urinary tract cancers, hepatobiliary malignancies, skin cancers, and several other sites have been noted. The patterns of excesses noted show some similarities and some differences between the various groups studied.

Project Description

Objectives: (1) To identify and study populations with altered immunologic states experiencing unusual rates of malignancy. (2) To study these populations in order to identify the characteristics and possible determinants of any unusual risk.

Methods Employed:

1. Further analyses were undertaken concerning 16,000 patients who received renal transplants. The number of cancers observed among various subgroups of this population were compared to that expected based on cancer incidence rates prevailing in the general population. These analyses included a separate evaluation of the risk of nonmelanotic skin cancer in this population and a search for unusual space-time clustering of lymphomas.

2. A collaborative arrangement has been established with the End-State Renal Disease Program of Health Care Financing Administration to evaluate cancer risks among transplant recipients and patients on dialysis. The study population currently includes over 50,000 dialysis patients and more than 5,000 transplant patients. Survival analyses have been carried out, and detailed analyses of the cancer experiences of this population are currently underway.

3. The collaboration with the University of Minnesota continued via the contract mechanism (NO1-CP-43384). In the past year, a number of separate analyses have been done in an attempt to characterize the risk of cancer among patients with genetically determined immunodeficiency diseases.

4. The follow-up study of the malignancy, mortality, and reproductive experiences of 3,000 former employees of Ft. Detrick in Frederick, Maryland, has continued. A large proportion of these workers fall into a group which would be considered "hyperimmunized". Extensive attempts to track down the vital status and the current address for those living have been performed for those individuals who were not readily contacted, based on the information received from Ft. Detrick. The data are currently being analyzed.

5. A follow-up study of patients with Hansen's disease, previously conducted by the Branch, has been updated. Approximately 1,700 patients with Hansen's disease hospitalized at one institution from 1930 onward have had their clinical records abstracted and have been followed up for mortality and for cancer incidence. These data are currently under analysis.

6. Work has continued on the creation of longitudinal medical histories for all persons admitted to Veterans Administration hospitals since mid-1963. This project is in the phase of systems design and verification of the completeness of information. This resource will provide an ever-

expanding database to be used for analytical studies on persons whose exposure and/or outcome diagnoses, as well as procedures, are of specific interest.

Major Findings:

1. There is a 25-fold excess risk of lymphoma among renal transplant recipients. This excess appears within a year of transplant, and the tumors show an unusually high frequency of brain involvement. There were four strong and significant trends in the excess risk of lymphoma: there was a substantial reduction in risk over time; there was an elevation in risk with repeated transplantation; there was a progressive reduction in risk with increasing time interval from transplant; and there was an inverse association between "closeness" of the relationship of the donor to the recipient and the risk of lymphoma. Other tumors occur at approximately twice the expected frequency. This excess appears more gradually following transplantation and is due to excesses of cancers of the bladder, lung, liver and bile ducts, soft tissues, and malignant melanomas.

2. Transplant recipients also have an excess risk of nonmelanotic skin cancer. This excess is restricted to squamous cell lesions, and there is no excess of basal cell cancer. The excess increases with increasing interval from transplant and occurs in both high- and low-risk areas for skin cancer. No association with any particular underlying renal disorder was evident, nor was there any significant relationship with any characteristic of donor or recipient on which information was available.

3. Persons with genetically determined immunodeficiency syndromes have a high-risk of lymphomas, similar to that among transplant recipients. These patients also appear to be at high risk of Hodgkin's disease and lymphatic leukemia in children. In addition, they also have elevated risks of soft-tissue sarcomas, malignant melanomas, and stomach cancer (adults). Among children with non-Hodgkin's lymphoma in this population, surface marker characteristics indicate that these are primarily of B-like phenotype, in distinction to the predominantly "null" or T-phenotype of unselected children.

Significance to Biomedical Research and the Program of the Institute: Host factors clearly comprise a major determinant of the response of humans to environmental carcinogenic exposures. One of the components of these factors is the immune status of the individuals involved. Laboratory research has indicated a central role for the immune system in determining who develops a malignancy among those exposed to a carcinogenic agent. The presence of a number of identifiable groups with markedly altered immune states allows an assessment of these factors in the production of human malignancy. In addition, such human observations have already uncovered major associations which have altered some preconceived notions of the relationship between the immune system and cancer.

Proposed Course:

1. The registry of patients with end-stage renal disease will be utilized to continue and expand the studies of patients receiving immunosuppressive drugs for these conditions, including an attempt to conduct a case-control study of the differences in tissue-typing information for those kidney transplant recipients who have gone on to develop malignancy, versus those in whom a malignancy has not developed. This resource will also allow the assessment of the risk of malignancy among patients with chronic uremia, an immunosuppressive condition itself.

2. The collaboration with the immunodeficiency and cancer registry will continue, with periodic evaluation of the data collected. A case-control study will be continued in the next year to assess the severity of immunosuppression and immunostimulation in children with these genetic states and cancer, compared to those with similar genetic conditions who did not develop a malignancy.

3. Patient populations seen at the NIH with other conditions that result in altered immunity (e.g., SLE) will be identified and followed up in order to obtain mortality and cancer morbidity information.

4. The study of hyperimmunized employees from Ft. Detrick, Frederick, Maryland, will be completed.

5. The analysis of the cohort with Hansen's disease will be completed.

6. Large groups of patients with a variety of diseases involving altered immunologic states will be identified in the VA hospitalization file and followed for subsequent morbidity and mortality due to cancer.

7. Attempts will be made to identify population groups, other than those receiving renal transplants, who received substantial amounts of immunosuppressive therapy. If appropriate, attempts will be made to establish cancer morbidity and mortality studies in these groups.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04410-06 EEB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Studies of Cancer Prone Families

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
12.5	10.5	2.0

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SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to conduct and coordinate interdisciplinary studies on high-risk populations in order to clarify the role of genetic mechanisms and host-environmental interactions in carcinogenesis. Clinical studies of high-risk families have suggested etiologic relationships between the premalignant dysplastic nevus syndrome and cutaneous melanoma, both hereditary and sporadic types. Investigative laboratory collaborations have clarified the relationship between lifestyle, subclinical immunologic perturbations and the epidemic of Kaposi's sarcoma and other disorders in male homosexuals. Seroepidemiologic study of the newly discovered candidate human leukemia retrovirus, HTLV, has defined patterns of virus infection and disease relationships. Mechanisms of host suceptibility are seen in DNA repair defects in familial melanoma, and families prone to diversity of rare cancers.

Project Description

Objectives: (1) To document the occurrence of cancer in high-risk families, and to study such families by clinical, epidemiologic, and laboratory investigations, in an effort to elucidate genetic mechanisms and host-environmental interactions contributing to carcinogenesis. (2) To develop educational materials and provide counseling to high-risk families. (3) To coordinate the distribution of tissue and blood specimens from such families to interested investigators for etiologic studies by cytogenetic, immunologic, viral, endocrine, biochemical, tissue culture, and other methods. (4) To apply innovative analytic approaches to these studies, including statistical genetic approaches.

Methods Employed: Protocols for study of individual families or groups of families are developed, outlining study aims and methods, and are reviewed by section professionals to maximize efficient use of personnel and laboratory resources. Patients are interviewed with respect to prior medical, occupational, and environmental history, and familial occurrence of cancer and other disorders, and are examined for clinical features associated with heightened risk. Family medical history is systematically documented utilizing a family medical history questionnaire developed by section professionals. Clinical history is documented using vital records, and hospital and physician charts, and operative specimens are sought for systematic review by collaborating pathologists. Data are abstracted and systematically entered and verified on a computerized record-keeping system. Specialized questionnaires are developed for documenting specific etiologic information. Biologic specimens are collected from informative members of high-risk families, stored in biospecimen repositories, and transmitted to collaborating laboratories. Analysis includes application of computerized genetic, as well as traditional, approaches.

Major Findings:

1. Family Studies Data Base: The integrated computerized and manual data base developed by section professionals over the last six years has been further refined and a manuscript describing the innovative features of this system was prepared for publication. During the last year approximately 600 new families were ascertained bringing the total to over 2,100. This reflects the continued ascertainment of new families, including those resulting from more than 40 unsolicited inquiries from private citizens following section research projects being reported in the lay press, and the continued referral of new families from the NCI Office of Cancer Communications. Progress continues to be made in defining precise routines for data verification, and a coding and computer data management manual has been developed. Currently there are computerized records on 32,850 members of 825 families, and data entry is proceeding on families selected for in-depth studies. The computerized clinical data base is linked to specimen inventory and laboratory results files, simplifying recordkeeping and improving opportunities for computer-based analysis. Programs for updating precise specimen location and destination of biospecimens in various repositories have been completed.

2. Studies of Hereditary Cutaneous Melanoma (HCM): A major focus of the Family Studies Section has been the study of HCM and the dysplastic nevus syndrome (DNS), now entering its seventh year. The primary area of activity in the past year has been establishing and coordinating a free-loan program for distributing the three educational videotapes which were developed to facilitate dissemination of information about this syndrome to concerned health professionals and laypersons. Currently, 330 copies of each program are in circulation. It is estimated that 55,000 persons will have viewed these materials by December 1982. This overwhelming response has led to a major reassessment of NCI policies regarding the demand for high quality video materials. Our epidemiology research nurses have completed a manuscript for the nursing literature describing the DNS and a nursing care plan for identifying and managing patients at increased risk of melanoma, and have also completed a survey of psychosocial determinants of patient compliance with our surveillance program. A report of this latter study was the subject of a formal presentation at the Oncology Nursing Society's Annual Meeting and the Master's degree thesis of a former Family Studies epidemiology nurse. A formal evaluation of the effectiveness of the family videotape as an educational tool is scheduled to begin in the summer of 1982. Manuscripts currently in preparation include: (a) a clinical summary of 14 melanoma-prone families; (b) a report focusing on newly-diagnosed melanomas in high-risk family members; (c) a color atlas detailing the DNS; (d) segregation and linkage genetic analyses of data collected from 14 high-risk families; (e) a survey of HLA haplotypes in HCM families; (f) the ultrastructure of dysplastic nevi; (g) a case report of dysplastic scalp nevi in children from high-risk families; (h) a report of UV radiation sensitivity in HCM family members; and (i) descriptions of kindreds prone to melanoma and breast cancer, melanoma and lymphoma, and both cutaneous and intraocular melanoma. Finally, data abstraction and follow-up have been completed on a series of 400 consecutive, newly-diagnosed malignant melanoma patients. Data analysis, which is just now beginning, should permit quantification of melanoma risk in the relatives of patients with melanoma, and identification of other cancers which occur excessively in this population.

3. Studies of Human T-cell Leukemia/Lymphoma Virus (HTLV): During the last year a major new focus of section professionals has been the epidemiologic study of a recently discovered candidate human leukemia virus, HTLV. These studies have been carried out in close collaboration with the Laboratory of Human Tumor Cell Biology. Section professionals have developed and implemented a far ranging interdisciplinary protocol aimed at defining: (1) the distribution and determinants of HTLV infection; (2) the relationship of HTLV infection to disease in man, particularly, the association with lymphoid malignancy; and (3) the role of host and immunogenetic factors. Results of our studies indicate that, unlike many other human candidate tumor viruses, HTLV has a relatively low prevalence in certain populations. In particular foci of prevalent HTLV infection have been identified in certain regions of Japan and the Caribbean, and among Blacks in the Southeastern United States. In each of these areas of

endemic HTLV infection, there appear to be clusters of a distinctive form of T-cell malignancy with shared clinical and cytologic features. A series of three cases of HTLV associated T-cell malignancy with aggressive hypercalcemia is the subject of a manuscript submitted for publication. Another manuscript focuses on the clustering of HTLV infection in families. A comprehensive series of parallel epidemiologic studies in different regions of the U.S. and several areas of the world is planned. One such study in collaboration with the University of West Indies is the subject of a physical anthropology graduate student doctoral dissertation for one member of the section.

4. Investigative Studies of Kaposi's Sarcoma (KS): Over the last 18 months an emergent problem has been the epidemic of KS and opportunistic infections in homosexual men. To investigate this outbreak, a multidisciplinary clinical, epidemiologic, and laboratory evaluation was performed on two homosexuals with Kaposi's sarcoma and 15 healthy homosexuals in the high-incidence New York City area. Half of the healthy men had T-lymphocyte abnormalities, including low helper/suppressor ratios, and these abnormalities were almost exclusively found in recreational users of amyl nitrite. Similar findings in homosexuals with Kaposi's sarcoma suggested that amyl nitrite may be immunosuppressive in the setting of repeated virus infections and may be of etiologic significance in the recent outbreak of these rare diseases. A larger case-control study to evaluate the relationship of subclinical immunologic perturbation involving a combined laboratory and interview case-control study is planned.

5. Studies of Families Prone to Sarcoma and Other Neoplasms: The syndrome of diverse familial neoplasms, including bony and soft-tissue sarcomas, breast and brain cancers, leukemia, and other tumors, first described by Branch personnel in 1969, continues to be a major research focus. A survey of 25 such families has documented over 200 primary malignant neoplasms with a remarkable preponderance of rare types. The preliminary analysis of this cohort, presented at a national cancer meeting, utilized life table analysis to quantify the risk for several mesenchymal and epithelial tumor sites. Results suggested that pancreas may also be one previously unrecognized form of epithelial cancer associated with the syndrome. Age-specific comparisons documented that a younger age of onset is typical for all sites, and this excess risk persists for mesenchymal but not epithelial sites in older age. Follow-up of the original four families reported by Li and Fraumeni reveals a strikingly high incidence of rare tumors characteristic of the syndrome in close relatives of the original probands.

   Subsequent cancers in a black family with features of this syndrome, Turcot's Syndrome, and familial polyposis suggest common etiologic factors or the overlap of several well-characterized genetic syndromes. Long-term follow-up of one large kindred documented the occurrence of new neoplasms in three family members, including a second primary osteosarcoma which developed in the field of prior radiotherapy for bilateral malignant neurilemmoma. Gamma irradiation DNA repair studies performed on skin

fibroblasts from this patient and seven other close relatives over three generations showed that cells from clinically affected and high-risk individuals, but not spouses, are resistant to cell killing. Biochemical characterization of this novel phenotype has important implications for understanding mechanisms of carcinogenesis. Studies on cell strains from more distant branches of this family and from probands of five other well-characterized families should help to define the utility of this phenotype as a possible marker of cancer risk, and may correlate with the clinical impression of increased susceptibility to environmental carcinogens, including ionizing radiation, in this syndrome.

A manuscript has been submitted describing the laboratory abnormalities observed in a young man with radiation-related breast cancer and his relatives, all of whom are members of a family prone to diverse neoplasms. In vitro studies have documented that the patient, his sister, and his mother are unusually sensitive to cellular injury induced by ionizing radiation and bleomycin (a radio-mimetic chemical carcinogen). The data suggest an interaction between host and environmental risk factors in the proband's breast cancer.

6. Studies of Familial Ovarian Cancer: A review of prophylactic oophorectomy biopsy material from women in 16 families prone to ovarian cancer has failed to substantiate earlier suggestions that there might be a pre-neoplastic abnormality in the ovaries of high-risk family members. A report describing three women who developed disseminated intra-abdominal carcinomatosis of unknown primary site following prophylactic oophorectomy has been prepared. Histologically, these malignancies were indistinguishable from metastatic ovarian cancer, suggesting that the genetic cancer susceptibility in these women extends beyond tissues strictly confined to the ovary. This observation provides important support for the hypothesis that the "target organ" at risk in this setting includes a spectrum of mesothelial structures which share a common embryologic origin with ovarian epithelium. Currently in preparation is a report describing the clinical features and genetic characteristics of these 16 families. An evaluation of the association between cancer risk and the red blood cell GPT locus is planned for the fall of 1982.

7. Risk Factors in Genitourinary Neoplasia: The bedside observation that several patients with renal cancer had supernumerary nipples led to a case-control study conducted in collaboration with investigators from the Clinical Epidemiology Branch, NCI. It was found that 19 percent of kidney cancer patients (compared with no control patients and 0.3 case expected from population estimates) had supernumerary nipples. Other minor congenital anomalies which appeared in this group included duplicate renal arteries, renal cysts, and a variety of genitourinary abnormalities. Family histories confirmed an increased likelihood for similar abnormalities, as well as clusters of renal and brain cancers in relatives. A follow-up case-control study to confirm these findings, to ascertain the diagnostic accuracy of supernumerary nipples, and to better define the host factors involved in cancer of the kidney is in progress. Data from the HANES dermatologic survey have been used to define a

population-based estimate of supernumerary nipples and to calculate that this anomaly carries a relative risk of 50 for renal cancer.

In a parallel survey of testicular cancer patients, a significant excess (11 percent versus 2.7 percent in controls) of polymastia was found. Other genitourinary and bony malformations were found in an array similar to that seen for renal cancer. This study has helped refine the hypothesis of inherited mesenchymal birth defects as risk factors in selected adult malignancies. A cohort of five testicular cancer families in whom two or more close relatives have developed testicular cancers is under study. Risk for testicular cancer has been documented through both maternal and paternal lineage and close relatives appear to have a variety of genitourinary abnormalities, which are in excess of the expected occurrence. An interdisciplinary laboratory protocol has been implemented to evaluate genetic markers of familial risk.

A survey of knowledge and attitudes about testicular cancer is being implemented by the Family Studies nurse epidemiologist to aid in developing effective approaches for primary prevention and early detection through routine self-examination.

Follow-up of a bladder cancer family first reported in 1967 revealed the development of an additional case in the sibship and in a first cousin. In all cases, cigarette smoking was implicated in the development of their bladder cancers; and two now have cigarette-associated, second primary respiratory neoplasms. In one bladder cancer case, heavy saccharin usage might have acted as a tumor promoter. All three cases had a rapid N-acetyl transferase phenotype, and two had detectable urinary N-nitrosodibutylamine. These findings suggest the interaction of host susceptibility with environmental exposures.

A search was made for cancers among offspring and siblings of 149 Connecticut-born children with Wilms' tumor reported to the Connecticut Tumor Registry during 1935 to 1973. Nasopharyngeal rhabdomyosarcoma developed in the daughter of a man with unilateral Wilms' tumor whose sister also had Wilms' tumor. Hodgkin's disease developed in the daughter of a woman who had unilateral Wilms' tumor. One other patient had a sibling with Wilms' tumor and three had a sibling with other cancers (two Hodgkin's disease, one testicular seminoma). The survey suggests an excess risk of other forms of cancer among the progeny and siblings of Wilms' tumor patients.

Cytogenetic study of 35 high-risk renal cancer patients (6 with a history of renal cancer in a close relative, 5 with bilateral disease, and 23 with young age of onset) revealed no cases with a translocation involving chromosomes 3 and 8, an abnormality previously reported in one renal cancer family. Two cases had pericentric inversions of 1 chromosome and there was one case with a Turner's mosiac. These data confirm the rarity of the type of translocation previously reported.

8. Immunological Studies: The first familial aggregation of hairy cell leukemia (HCL) was discovered, evaluated, and reported. During the course of the project, a third sibling developed HCL; he shares an HLA-haplotype with the two previously affected family members. This provides further evidence for an HLA-associated susceptibility factor in familial HCL. Biologic specimens have been obtained from all 11 sibs and the offspring of the 4 brothers who share the disease-related HLA haplotype. Extensive laboratory evaluation of the MHC and peripheral blood mononuclear cell surface markers is now in progress.

Families prone to chronic lymphocytic leukemia are being systematically studied to establish whether cell surface markers are shared between familial cases. In one family, several members with normal white blood counts and lymphocytosis are being studied with the FACS-II to determine if early perturbation in B-cell clones can be detected as a precursor to chronic lymphocytic leukemia (CLL). Follow-up of a family in which a father and four of his five offspring were diagnosed with CLL confirms that all cases share the same cell surface phenotype. Surprisingly, one of the cases with classical CLL in 1974 has undergone a spontaneous clinical remission, yet continues to harbor a clone of cells with cell surface characteristics of the malignant clone. Immunogenetic study of this family reveals the presence of shared B-cell typing reactions which segregate independently from known HLA-A, -B, -C, and -Dr specificities in the cases, but not in normal family members. Cytogenetic abnormalities involving extra chromosome 12 material were documented in 2 cases and may correlate with the severity of clinical disease. Collaboration with recombinant DNA geneticists at NIH and the University of Wisconsin is under way, with plans for cloning the immunoglobulin genes from familial cases in order to study their detailed structural organization.

Among 526 patients with cutaneous T-cell lymphomas, 21 reported first-degree relatives with lymphoproliferative or hematopoietic malignancies. Intensive case finding, coupled with pathology review, confirmed 29 such cases in these 21 kindreds. Hodgkin's disease was over-represented in this group, accounting for one-third of the cases. These data form the basis for the hypothesis that genetically-determined immunoregulatory abnormalities may represent a shared pathway of oncogenesis in diverse lymphoproliferative and hematopoietic malignancies. To follow up on these results and to complement a large case-control study of cutaneous T-cell lymphoma (CTCL) now in the field, 100 consecutive CTCL patients will undergo HLA tissue-typing. These patients will be a subset of those participating in the case-control study, thus permitting detailed correlation between HLA phenotype and epidemiologic variables. Data collection for the case-control study has been completed. Finally, detailed laboratory evaluation of what is only the fifth familial aggregation of CTCL is now under way. This analysis is focusing on HLA and immunologic risk factors.

A project designed to evaluate the role of the major histocompatibility complex (MHC) in the etiology of familial Hodgkin's disease continues in an expanded form. The study now includes 7 Hodgkin's disease families, a

series of 65 sporadic Hodgkin's disease cases, and a control group of 210 normal individuals. All familial Hodgkin's disease patients directly studied, and a significantly elevated proportion of the non-familial cases, express the MHC specificity now designated MB-1. Thus, MB-1 is associated with increased risk of both familial and non-familial forms of Hodgkin's disease. A formal segregation analysis of the seven families is also under way, in an effort to determine if familial Hodgkin's disease is inherited in a classic Mendelian fashion. The next phase of this project will involve evaluation of suppressor T-cell function in high-risk family members.

Cells from members of a family prone to acute lymphoblastic leukemia were studied at the recent International Tissue Typing Workshop and by collaborators at Georgetown University. Results of this evaluation demonstrated that the affected sibs were homozygous for Dr4-related reactivities and certain MT specificities. This result validates the original hypothesis that homozygosity for major histocompatibility determinants is associated with an increased risk of leukemia, a conclusion that is emerging in the recent medical literature. However, in another ALL sibship, the cases did not share the same haplotype and HLA types did not correspond to those of the previously reported family.

A data set of HLA genetic marker data on 891 members of a cancer-prone family was created by combining HLA data from several laboratories. This data set was edited and merged with the Family Studies data base in order to analyze the association between HLA type and diverse forms of familial cancer. Frequencies of HLA types in this total family member data set are similar to those reported for North American Caucasians in the 1980 Histocompatibility Testing Workshop.

In collaboration with NIADDK, 249 Pima Indians from Arizona were typed for HLA markers and data analysis was performed by a section member. HLA phenotype frequencies in Pimas were similar to published values for other North American Indians. The MT3 phenotype, however, was not found with the expected HLA-DR associations, suggesting the presence in this population of an undefined HLA-DR allele associated with MT3. A manuscript reporting these findings has been submitted for publication.

Analysis of HLA types from high-risk patients from a cohort of renal cancer patients led to the development of an innovative statistical analytic approach for calculating HLA phenotype frequencies from 2 and 3 locus haplotype frequencies. Utilizing this approach one particular 3 locus phenotype, HLA-A3B7DR2 was excessive in the study cohort and may be linked to ethnic susceptibility to kidney cancer.

9. Late Effects of Cancer Therapy: A series of investigations by section personnel have dealt with long-term effects of chemo- and radio-therapy and have quantified the risk for second primary neoplasms in various cohorts (details in report of the Radiation Studies Section, EEB). An extension of these formal epidemiologic investigations involves an in-depth study of second primary cancers in long-term survivors of Hodgkin's

disease. Of the 200 patients treated with MOPP at the NCI between 1965 and 1975, approximately 100 are still alive. To date, 63 have been approached for data and biospecimen collection. Bone marrow for cytologic examination, culture, chromosome analysis, and storage; skin fibroblasts for long-term culture; and peripheral blood for immunologic investigation are being collected. The laboratory phase of this project will focus on a search for biological markers of increased leukemia risk. A parallel case-control study of this NCI cohort is also under way to determine the rate of second malignancies and relationship to type of therapy.

10. Miscellaneous: Studies of the relationship of autoimmunity to cancer are continuing, with the evaluation of 30 kindreds identified through a proband with dermatitis herpetiformis and 25 kindreds with gluten sensitive enteropathy. In a preliminary report of these studies, family members were found to have an increased incidence of autoimmune disorders in first- and second-degree relatives compared to a cohort of spouse control families. However, the patterns of disease differed in the two study groups, suggesting different immunogenetic determinants. There was no excess of gastrointestinal, lymphoproliferative, or other forms of malignancy when compared with the expected incidence for the U.S. population.

11. Reviews: A comprehensive review of the epidemiology of multiple myeloma and Waldenstrom's macroglobulinemia, with special emphasis on familial and genetic aspects, showed that different genetic mechanisms operate in these conditions. For macroglobulinemia, a dominantly inherited predisposition to autoimmunity and lymphoproliferation is suggested; while for myeloma, a recessive factor (possibly associated with HLA) may explain the pattern.

    A review of the epidemiology of non-Hodgkin's lymphoma (NHL) identified two distinctive patterns of familial aggregation for the tumor: (1) teenage male sibs with extra-nodal (primarily gastrointestinal) NHL and (2) adult female sibships with nodal NHL. The many conditions associated with an increased risk of NHL were reviewed, and chronic antigenic stimulation appear to underlie many of the known lymphoma precursor states. These data were presented at an NIH Combined Clinical Staff Conference on Recent Advances in NHL. Recent reviews summarized the familial and genetic aspects of cancer causation, and demonstrated the utility of interdisciplinary laboratory studies in dissecting risk mechanisms.

Significance to Biomedical Research and the Program of the Institute: Studies of cancer-prone families may help to detect genetic mechanisms and host-environment interactions in carcinogenesis, and also help to identify those individuals most likely to benefit from screening programs aimed at early diagnosis of cancer. These unique, high-risk populations offer an opportunity to evaluate patient and physician education techniques and assess the impact on health status of the patients over time. Studies of the distribution and determinants of infection by the newly discovered human retrovirus, HTLV offer promise for documenting an etiologic role for the first human type C

retrovirus. Interrupting the virus infection may result in primary prevention.

Proposed Course: The same basic methodologies will be applied, emphasizing a more systematic approach to studying groups of families with shared features, rather than individual families. The development of a computerized data base and the availability of statistical genetic techniques have added impetus to this more targeted approach. Laboratory collaboration will continue to expand, utilizing the fibroblast cell strains, sera, and other biospecimens collected over the last eleven years. Immunologic analysis, utilizing the FACS-II, the availability of recombinant DNA approaches for gene cloning, and cytogenetic analysis with prophase banding are areas of laboratory collaboration that show particular promise. A major new thrust will involve expansion of etiologic studies of the new candidate human leukemia virus, HTLV.

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CONTRACTS IN SUPPORT OF THIS CONTRACT

BIOTECH RESEARCH LABORATORIES (NCI-CP-21007)

Title: Laboratory Support for Processing and Storing Biological Specimens for Persons at High Risk of Cancer.

Objectives: To process and store biospecimens from persons at increased risk of cancer.

Methods Employed: Peripheral blood mononuclear cells, red cells, serum, plasma, urine, stool, and tumor tissue are processed and cryopreserved using standard techniques. Frozen material is entered into the biospecimen repository, from which it is disbursed to collaborating scientists at the request of the Project Officer. Appropriately written documentation is prepared for computer data entry, to record needed background information on each sample and data required for biospecimen tracking and inventory purposes.

Major Contributions: The new laboratory has been equipped and staffed, and the first series of quality-control experiments successfully completed. The lab is now operational, and provides critical support to the research activities of EEB Family Studies Group investigators.

Proposed Course: An expansion of the level of effort provided by the contract is planned to accomodate a marked increase in the volume of biospecimens being processed. This increased work-load has resulted from the initiation of two major new projects: (a) sero-epidemiologic studies of the human T-cell leukemia/lymphoma virus (HTLV) and (b) epidemiologic studies of the "gay-related immunodeficiency diseases", including Kaposi's sarcoma.

Current Annual Level: $197,000.

Man Years: 4.1

FLOW LABORATORIES, INC. (NO1-CP-21021)

Title: Biological Specimen Repository for Patients at High Risk for Cancer.

Objectives: To maintain and develop a repository of skin fibroblast and epithelioid strains on high-risk patients and members of families at high-risk for cancer.

Methods Employed: Primary skin biopsy explants are processed using standard tissue culture techniques and are screened for contamination. These cell strains are frozen in liquid nitrogen and distributed to collaborating investigators on written request.

Major Findings: Approximately 250 new specimens were submitted to the repository over the past year. Approximately 300 separate cell strains were sent to collaborating investigators at 12 different institutions. A number of publications resulted from the use of these cell strains, including a series of studies which focused on DNA repair response to ultraviolet and gamma radiation. Abnormalities were identified in a family prone to acute myelogenous leukemia, a patient with radiation-induced male breast cancer, patients with hereditary cutaneous malignant melanoma, and patients from a family prone to a diversity of bony and soft-tissue sarcomas, brain and breast cancers, and leukemia. The availability of specimens on multiple generations of affected families have proven especially valuable for evaluating the suspected genetic mode of transmission of susceptibility in some cases. Cells from one family proved useful in defining the presence of a variant of human non-muscle tropomyosin.

Proposed Course: The new laboratory will continue to provide support to the Family Studies Section and has added to the capability to develop epithelioid cell lines and store tumor lines from the repository. A complete inventory and updating of clinical background material is proceeding satisfactorily.

Current Funding Level: $80,000.

Man Years: 2.0

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04411-06 EEB

PERIOD COVERED

October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Cancer and Related Conditions in Domestic Animals: Epidemiologic Comparisons with Man

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	H.M. Hayes, Jr.	Staff Associate	EEB	NCI
OTHER:	R.N. Hoover	Head, Environmental Studies Section	EEB	NCI
	J.F. Fraumeni, Jr.	Chief, Environmental Epidemiology Branch	EEB	NCI
	L.W. Pickle	Staff Fellow	EEB	NCI
	R.J. Biggar	Staff Associate	EEB	NCI
	K.L. Milne	Staff Associate	EB	NIEHS

COOPERATING UNITS (if any)

Clinical Epidemiology Branch, NCI

LAB/BRANCH

Environmental Epidemiology Branch

SECTION

Environmental Studies Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.3	1.2	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) The continuing purpose of this project is to identify domestic animal models applicable to further research into the etiology of cancer in humans. As cases accumulate, it is likely that some types of spontaneous cancers in pet animals can be identified as representing the effects of low-level environmental exposure to carcinogenic agents. The frequency of cancer in these animals would serve as a warning of general environmental hazard(s) to people in the same locale. The topics of current investigations are: (1) environmentally influenced cancer in pet dogs relative to the level and type of industry in their county of residence (e.g., bladder, nasal, and oral cancers); (2) morbidity among pet dogs living in Michigan, potentially exposed to PBB's; (3) morbidity among military working dogs who had considerable exposure to Agent Orange; (4) the epidemiologic features of canine transmissible venereal tumor, colorectal cancer, prostatic cancer, and gastrointestinal cancer in pet dogs; and (5) a case-control study of the long-term effects of Promone and Ovaban in female dogs.

Project Description

Objectives: To investigate the distribution of cancer and related conditions in domestic animals in order to 1) clarify etiologic factors in humans, 2) identify animal models useful in research, and 3) identify sentinels which may act as early predictors of environmental hazards to man..

Methods Employed: Animals with the disease under investigation are identified when possible from the medical abstract records in the Veterinary Medical Data Program. A comparison population-at-risk is constructed from all patients seen by participants during the same time period under study. The diseased animals are characterized by relative risk techniques for various factors (i.e., age, breed, sex, and various enviornmental variables). Other analytical techniques employed may include case-control comparison for factors associated with disease in man. Other animals are studied whenever another resource is available (i.e., military working dog autopsy file of the Armed Forces Institute of Pathology.

Major Findings:

1. The proportional morbidity of various cancers in the dog was correlated to the amount of industry in the locale of the dog's home (assessed by postal zip code). Bladder cancer had the strongest positive association of any canine cancer with industrial activity; nasal and oral cancers also showed strong but lesser association with industry activity. Further analyses showed similar correlations between human mortality from bladder cancer and industrial activity in the same geographic locales, suggesting the pet dog may be a sentinel for general low-level environmental hazards to companion humans.

2. Investigation of canine biliary carcinoma showed no apparent genetic predisposition, although a sex differential was suggested. Evidence was available suggesting that past infestation with bloodletting intestinal parasites occurs more frequently in cases of cholangiocarcinoma than expected. The geographic distribution of analogous human parasites is consistent with a presently unexplained high incidence of human cholangiocarcinoma.

3. Investigation of canine nasal carcinoma could not establish a direct relationship between the size or length of the dog's nose and risk for cancer. Several breeds were identified with excessive risk; finding mongrel dogs having the same risk as all purebred dogs combined further suggests the influence of environmental factors in the etiology.

4. Preliminary analysis demonstrated that the frequency of canine transmissible venereal tumor (TVT) in North America is inversely correlated with latitude. Review of the epidemiology indicates Kaposi's sarcoma and TVT share many common features, suggesting that the latter could serve as a model for this sarcoma.

Significance to Biomedical Research and the Program of the Institute: Canine bladder cancer in pet animals appears to be strongly associated with increasing amounts of industrial activity in U.S. counties where data are available from veterinary teaching facilities (none of these counties are sites for large chemical plants). We believe that spontaneous bladder cancer in the pet dog generally represents the effects of low-level environmental carcinogens; monitoring the frequency of canine bladder cancer nation-wide could serve as a sentinel to emerging hazards to companion humans. The epidemiology of canine transmissible venereal tumor suggests an infectious disease possibly associated with insect vectors. The similarities between TVT and Kaposi's sarcoma are numerous: both are much more aggressive in immunosuppressed hosts, both are sarcomas of uncertain histogenesis, both have been reported to be endemic in tropical Africa, and Kaposi's sarcoma seems associated with latitude and rainfall in Africa similar to our findings about TVT in North America. New directions in the laboratory research of TVT could provide the clues to the mechanism(s) of the presently unexplained epidemics of Kaposi's sarcoma in male homosexual populations.

Proposed Course: The methods employed and projects listed will be continued next year.

Publications:

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Wilson, G.P., and Hayes, H.M., Jr.: Developmental and Traumatic Diaphragmatic Hernias. In Slatter, D.H. (Ed.): Textbook of Small Animal Surgery. Philadelphia, W.B. Saunders Co. In press.

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Wilson, G.P., and Hayes, H.M., Jr.: Ovariohysterectomy in the Dog. In Bojrab, M.J. (Ed.): Current Techniques in Small Animal Surgery. Philadephia, Lea & Febiger. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04412-06 EEB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Carcinogenic Effects of Therapeutic Drugs

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R.N. Hoover	Head, Environmental Studies Section	EEB	NCI
OTHER:	J.F. Fraumeni, Jr.	Chief, Environmental Epidemiology Branch	EEB	NCI
	J.D. Boice, Jr.	Head, Radiation Studies Section	EEB	NCI
	M.H. Greene	Clinical Investigator	EEB	NCI
	L.A. Brinton	Staff Fellow	EEB	NCI
	M.A. Tucker	Clinical Investigator	EEB	NCI
	R.A. Kleinerman	Epidemiologist	EEB	NCI
	A.F. Kantor	Staff Fellow	EEB	NCI
	E.J. Trapido	Staff Fellow	EEB	NCI

COOPERATING UNITS (if any)
Kaiser Foundation Research Institute, Portland, OR, Los Angeles, CA, and Oakland, CA; Department of Epidemiology, Harvard School of Public Health; Division of Cancer Therapy, NCI

LAB/BRANCH
Environmental Epidemiology Branch

SECTION
Environmental Studies Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	2.0	0.5

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☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) The purpose of this project is to study the long-term health effects of therapeutic drugs as they may relate to carcinogenicity. Cohort studies of exposed groups are conducted, as well as case-control studies of selected cancer sites which involve lifetime drug use histories. Emphasis in the past year has been on the evaluation of various types of estrogenic preparations, immunosuppressive drugs, and cancer chemotherapeutic agents. Long-term use of menopausal estrogens is related to increased risk of breast cancer. In general, oral contraceptive use is not related to increased risk of breast cancer except, perhaps, for use during premenopausal periods or among certain subgroups. Use of immunosuppressive drugs is associated with markedly elevated risk of non-Hodgkin's lymphoma and certain other tumors. Alkylating agents used for treatment of cancer and some non-neoplastic conditions are associated with large excess risks of leukemia that seem to be dose-related.

Project Description

Objectives: (1) To study the long-term effects of therapeutic drugs in humans in order to identify drugs affecting risk of malignancy, and the characteristics of these risks. (2) To review what is known about the carcinogenic potential of drugs in order to identify those requiring study in humans.

Studies and Methods Employed:

1. A retrospective cohort study of 1,891 women treated with conjugated equine estrogens for menopause. This is a study of women so treated sometime between 1940 and 1969 and followed-up through the end of 1972. This is being done in collaboration with a gynecologist at the University of Louisville. Currently, an update of the follow-up is being conducted in order to bring the cohort follow-up through 1979.

2. The data from a case-control study of breast cancer in one pre-paid health plan were analyzed for the relationship between the use of estrogens for the climacteric and the subsequent risk of breast cancer.

3. A case-control study of breast cancer among mammography screening program participants has included an evaluation of the influence of estrogens, oral contraceptives, and tranquilizers on breast cancer (see Project No. Z01-CP-04501-04). Current analysis is assessing the effect of antihypertensive agents and thyroid medications on risk of breast cancer.

4. Study of the long-term effects of the immunosupressive drugs described in detail in Project No. Z01-CP-0441-04-EEB, "Immunologic Factors in Cancer Etiology."

5. A population-based, record-abstract, case-control study of approximately 600 cases of ovarian cancer and 700 controls from two pre-paid health plans is currently under analysis to determine the relationship between the use of a number of therapeutic drugs and the risk of this malignancy. The drugs being evaluated include estrogens, oral contraceptives, major tranquilizers, and other drugs affecting the pituitary-ovarian axis.

6. A population-based, record-abstract, case-control study of cases of breast cancer was continued in women who had undergone a bilateral oophorectomy prior to breast cancer diagnosis and in control women who also had a bilateral oophorectomy. This is being done in two pre-paid health plans in order to evaluate the risk of breast cancer associated with the use of replacement estrogens by women having had a bilateral oophorectomy.

7. A "case-control" study, where the "cases" are NCI Medicine Branch breast cancer patients whose tumors have estrogen receptors and the "controls" are those breast cancer patients without receptors, is currently being analyzed. Breast cancer risk indications and history of exposure to estrogens and oral contraceptives are being related to the absolute level of estrogen receptors of the tumors.

8. A study of patients with Hansen's disease is currently under analysis. The predominant mode of therapy of these individuals for a number of years has been the drug dapsone. This drug has recently been implicated as a carcinogen in the bioassay program of the NCI. Patients with Hansen's disease are also of interest with respect to their risk of malignancy because of their altered immunologic state (see Project No. Z01 CP 04401-04 EEB).

9. A case-control study in four U.S. cancer registries and in Denmark is ongoing. Approximately 400 persons who developed endometrial cancer as a second cancer following breast cancer therapy are being evaluated along with matched controls. Detailed information is being collected on medical histories and estrogen exposures. The risk of endometrial cancer will be quantified in light of cumulative estrogen use.

10. A case-control study of invasive and in situ cervical cancer is currently being conducted that will allow evaluation of risk associated with use of oral contraceptives and other hormones (see project Z01 CP 04501-05 EEB).

11. A systematic evaluation of adjuvant drug therapy for cancer treatment has continued. To evaluate the potential carcinogenic effects of various modalities in the treatment of cancer, information from several NCI-supported cancer treatment protocols is being combined and anlyzed. This study is being done in collaboration with the Division of Cancer Therapy. From a survey of NCI-funded protocols, a number of cancer treatment trials were selected for evaluation. Protocol chairmen and statisticians were contacted, available data evaluated, and abstract forms designed to obtain information on second cancers not readily available from computerized data. Contact has been made with the following surgical adjuvant groups: The National Surgical Adjuvant Breast Project, the Gynecologic Oncology Group, the Veterans Administration Surgical Oncology Group, the Eastern Cooperative Oncology Group, the Gastrointestinal Tumor Studies Group and the Southwest Oncology Group. Drugs being evaluated include: thioTEPA, L-PAM, MeCCNU, Cytoxan, and others.

12. A follow-up study of mortality and cancer incidence in 900 patients treated with alkylating agents for rheumatoid arthritis is currently being analyzed.

13. A follow-up study was done on 517 patients with non-Hodgkin's lymphoma treated at the National Cancer Institute to evaluate the risk of second cancers.

14. A follow-up of patients with early stage ovarian cancer participating in three prospective randomized trials (GOG, Princess Margaret Hospital, and the MD Anderson Hospital) was conducted and analyzed for the frequency of second tumors. In an effort to obtain larger numbers of cases so that questions arising from this analysis (e.g., dose-response, radiation/chemotherapy interaction, etc.) can be investigated, data from an additional 6,000 women with advanced stage ovarian cancer are now being analyzed. These data will be added to those already collected. The final study group will include approximately 7,500 ovarian cancer patients.

15. A case-control study of 203 children with second malignant neoplasms, evaluating the relationship between the therapy they received for their first malignant neoplasm and the development of their second, is currently under analysis.

16. A study of mortality and the frequency of second cancers related to drug and radiation treatments for Hodgkin's disease is under way using Connecticut Tumor Registry data.

17. A study of benign breast disease conducted in conjunction with epidemiologists at Oxford University allowed evaluation of the relationship of oral contraceptives to risk (see project no. Z01 CP 04501-05 EEB).

Major Findings:

1. Among 345 women with breast cancer and 611 controls, all of whom were members of one pre-paid health plan, the relative risk (RR) associated with ever having used conjugated estrogens was 1.4 (p=0.03). There was evidence of dose-response relationship with three different measures of dose, rising to two-fold for those with long-term use.

2. Oral contraceptive use was evaluated among 963 breast cancer patients and 858 controls identified through the Breast Cancer Detection Demonstration Project. Overall, there was no association between use and risk of disease (RR=1.1). In addition, there was no indication of increasing risk with years of use or years since initial use, despite slight excess risk among users of high dose preparations. However, non-significant excess risks associated with pill use were seen among premenopausal women who reported a family history of breast cancer in a sister (RR=3.6) or previous biopsies for benign breast disease (RR=3.2).

3. Use of immunosuppressive drugs by kidney transplant recipients is associated with a 25-fold excess risk of lymphoma, and lesser excess risks of lung cancer, lower urinary tract cancers, soft-tissue sarcomas, cancers of the liver and bile ducts, and malignant melanoma. (See Project No. Z01 CP 04401-06 EEB for a more complete description.)

4. Evaluation of the risk of leukemia following alkylating agent use in ovarian cancer patients participating in randomized trials indicates a high risk that apparently follows a dose-response relationship.

5. The risk of leukemia following adjuvant chemotherapy with methyl CCNU, a nitrosouria, was found to be significantly elevated in persons receiving this drug during NCI supported collaborative clinical trials. Nine separate trials were evaluated, and an independent hematology review panel evaluated all slides of leukemia or preleukemia; manuscript preparation is nearly complete.

6. Preliminary analysis of the follow-up of 517 patients treated for non-Hodgkin's lymphoma at the NCI suggests excesses of acute non-lymphocytic

15. A case-control study of 203 children with second malignant neoplasms, evaluating the relationship between the therapy they received for their first malignant neoplasm and the development of their second, is currently under analysis.

16. A study of mortality and the frequency of second cancers related to drug and radiation treatments for Hodgkin's disease is under way using Connecticut Tumor Registry data.

17. A study of benign breast disease conducted in conjunction with epidemiologists at Oxford University allowed evaluation of the relationship of oral contraceptives to risk (see project no. Z01 CP 04501-05 EEB).

Major Findings:

1. Among 345 women with breast cancer and 611 controls, all of whom were members of one pre-paid health plan, the relative risk (RR) associated with ever having used conjugated estrogens was 1.4 (p=0.03). There was evidence of dose-response relationship with three different measures of dose, rising to two-fold for those with long-term use.

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6. Preliminary analysis of the follow-up of 517 patients treated for non-Hodgkin's lymphoma at the NCI suggests excesses of acute non-lymphocytic

leukemia and lung cancer. The leukemia cases seemed to be related to therapy, particularly total body irradiation; whereas, the lung cancer excess was not related to a specific treatment. Of special interest is the fact that the leukemia excess was confined to patients with nodular lymphoma and lymphocytic lymphoma.

7. Evaluation of data from the Oxford Family Planning Association Contraceptive Study confirmed provided further support for oral contraceptives reducing the risk of benign breast disease.

Significance to Biomedical Research and the Program of the Institute:
Drug exposure has been one of the most fruitful areas for identification of carcinogens in man and subsequent opportunities for preventive programs and insights into the biologic mechanisms in cancer etiology. In addition, the studies of the long-term carcinogenic effects of anti-tumor drugs is an important part of the evaluation of the efficacy of treatment of various malignancies with these agents.

Proposed Course:

1. The retrospective cohort study of menopausal estrogen users will be continued and updated, and further analyses done on cancer incidence and mortality.

2. The analyses of the breast cancer case-control studies from the pre-paid health plans will be continued. Additionally, they will include the evaluation of stage and survival information as well as the risks associated with the use of drugs other than conjugated estrogens.

3. The study of ovarian cancers in pre-paid health plans will be analyzed, and potential for other drug evaluations in these plans will be assessed.

4. Another evaluation of the relationship between a number of drugs and the risk of breast cancer and benign breast disease will be analyzed using the data from the case-control interview study done in conjunction with the Breast Cancer Detection and Demonstration Projects (see Project No. Z01 CP 04501-05 EEB for a more complete description), and through the study of breast cancers in oophorectomized women in pre-paid health plans.

5. It is intended to continue the systematic monitoring of long-term toxic effects (including carcinogenicity) of a number of therapeutic agents used in the treatment of specific cancers. This will be done in collaboration with the Division of Cancer Treatment, as outlined in the methods. These effects will be supplemented, where appropriate, by observational case-control studies of specifically suspect constellations of double-primary malignancies. These evaluations will involve intensive record abstraction for therapy administered to patients who developed certain combinations of primary malignancies, compared with those with the same first primary who did not develop a subsequent malignancy.

6. Analyses will be done of the risk of cancer among patients with rheumatoid arthritis, who were treated with alkylating agents. In addition, other non-cancer patient groups who have received substantial amounts of immunosuppressive or cancer chemotherapeutic drugs will be sought, and their cancer risks evaluated, where possible.

7. The analyses of the mentioned ovarian cancer trials and the extended patient series will be completed, and the data from the NSABP will be obtained and evaluated. From this, and the prior studies noted, a decision will be made concerning a summary paper on the risk of leukemia following various dose levels of alkylating agents.

Publications:

Brinton, L.A.: Re: risk factors for benign breast disease The first author replies. Am. J. Epidemiol. In press.

Brinton, L.A., Hoover, R.N., Szklo, M., and Fraumeni, J.F., Jr.: Oral contraceptives and breast cancer. Int. J. Epidemiol. In press.

Hoover, R.N., Glass, A., and Finkle, W.D.: Conjugated estrogens and breast cancer risks in women. JNCI 67: 815-820, 1981.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 CP 04480-06 EEB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Studies of Occupational Cancer

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	A. Blair	Epidemiologist	EEB	NCI
OTHER:	W. Blattner	Clinical Investigator	EEB	NCI
	K. Cantor	Epidemiologist	EEB	NCI
	D. Grauman	Computer Systems Analyst	EEB	NCI
	S. Hoar	Epidemiologist	EEB	NCI
	J. Lubin	Health Statistician	EEB	NCI
	B. Miller	Epidemiologist	EEB	NCI
	R. Spirtas	Health Statistician	EEB	NCI
	T. Thomas	Epidemiologist	EEB	NCI
	J. Walrath	Epidemiologist	EEB	NCI
	D. Winn	Epidemiologist	EEB	NCI

COOPERATING UNITS (if any) Univ. of Minnesota, Univ. of Iowa, Univ of Kansas, Univ. of Southern California, New York State Dept. of Health, SSA, NIOSH, U.S. Coast Guard, USDA, Office of Personnel Management, U.S. Air Force, Veterans Administration

LAB/BRANCH
Environmental Epidemiology Branch

SECTION
Occupational Studies Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
11.3	7.8	3.5

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) Epidemiologic studies of occupational groups are valuable since workers often have heavy and prolonged exposures to carcinogens also found in the general environment. Completed during the past year were studies of 1) professional artists revealing an excess of leukemia and cancers of the bladder, kidney, brain, colon, prostate, and breast; 2) lung cancer among foundry workers showing excessive risks among younger workers employed in iron foundries; 3) embalmers exposed to formaldehyde where slightly increased mortality from cancers of the skin, kidney, and brain were noted; 4) workers in the petroleum industry which uncovered excess mortality from leukemia, multiple myeloma, lymphoma, and brain cancer; 5) a small number of chemical workers exposed to benzene who had a significant excess of leukemia; and 6) veterinarians whose mortality from leukemia was elevated particularly among those practicing during the late 1950's and early 1960's when radiologic procedures were less carefully controlled. Other investigations under way include proportionate mortality studies of plumbers, foresters, and millers; cohort mortality studies of formaldehyde workers, dry cleaners, furniture makers, shipyard workers, aircraft mechanics, potters, and chemists; and case-control studies of leukemia, lymphoma, soft-tissue sarcoma, and cancers of the brain and colon.

Project Description

Objectives: To identify and evaluate groups at high risk of developing cancer because of contact with carcinogenic materials in the work environment through analytic investigations of various designs.

Methods Employed: Cancer patterns are determined through long-term follow-up of persons employed in specific plants, industries, and occupations. Follow-up resources for cohort studies include the Social Security Administration, Office of Personnel Management, state motor vehicle bureaus, state vital statistics offices, city directories, and post offices. The cancer experience of study groups is usually compared to that of the general population (geographic-specific, if possible). In some instances, comparisons are made with other industrial populations. Proportionate mortality studies are conducted when population data are unavailable. Case-control studies of persons with particular cancers are carried out in certain geographic areas where industries or occupations of interest are concentrated. Occupational, demographic, and other information may be obtained on study subjects by personal interview or from available employment records.

Major Findings: Findings from completed studies include:

1. A retrospective cohort study of male chemists employed at a chemical company found the total number of cancer cases and cancer deaths to be fewer than expected. Chemists did appear to be at slightly elevated risk from melanoma, and cancer of the colon and prostate.

2. Disability between 1959 and 1977 among chemists was compared to that of other salaried employees from the company. Chemists, compared to other employees, experienced fewer absences lasting eight or more days due to disabilities, and fewer cases of benign and unspecified neoplasia, heart disease, peptic ulcer, and diseases of the urinary system, bones and joints, and skin. Exposure misclassification and socioeconomic status confounding may have contributed to these deficits.

3. Among a small number of chemical plant workers having contact with benzene, the only unusual finding was a significant excess of deaths from lymphoreticular cancers. Three deaths were due to leukemia and one to multiple myeloma.

4. An occupation and exposure linkage system was developed to facilitate categorization of study subjects by exposure. Classification systems based on occupation and industry currently available may misclassify subjects and reduce or obscure associations between specific exposures and disease.

5. Analysis of cause of death among professional artists revealed an excess of leukemia and cancers of the bladder, kidney, brain, colon, and prostate among males. The excesses for leukemia and cancer of the bladder were particularly striking among painters, while the excess for prostate cancer was limited to sculptors. Among females (particularly

female painters), higher than expected proportions of cancers of the rectum, lung, and breast were observed. These excesses may be due to pigments and dyes, metal fumes, and dusts used by professional artists in their work.

6. A proportionate mortality investigation of cancer among members of the International Molders and Allied Workers Union noted an excess of lung cancer. In a follow-up of this finding, a nested case-control study found an increased risk for lung cancer among younger (65 years at death) workers in iron foundries, but not among workers from steel and nonferrous foundries.

7. A case-control study among rubber workers was conducted to evaluate the role of solvents and complex hydrocarbons in the origin of brain cancer. Industrial hygiene assessments of individual exposures were not available; however, the risk of brain cancer was not unusual in any of the occupational categories evaluated.

8. Comparison of causes of death among veterinarians with the general population revealed significant elevations for cancers of the lymphatic and hematopoietic system, colon, brain, and skin. The leukemia excess may be related to the lax use of radiation protective equipment.

9. A case-control study of leukemia among Wisconsin farmers (1968-1976) showed increased risks for farmers born more recently, dying at younger ages, and residing in counties where fertilizer usage and dairy production were heavy. The risks of leukemia among study subjects specifically noted as dairy farmers were similar in pattern and magnitude to those among farmers in general.

10. A proportionate mortality study of cancer among petrochemical workers in Texas City, Texas, revealed excesses of deaths from cancers of the skin and brain from one company. Additional deaths from brain cancer have been identified among workers at this company who were not eligible for inclusion in this study.

11. When mortality data from an earlier report were supplemented with records on retired members of the Oil, Chemical, and Atomic Workers Union, excess mortality from certain cancers was observed. Proportionate mortality ratios were high for leukemia, multiple myeloma, and lymphoma, particularly among retired workers. Relative frequencies for brain tumor deaths were significantly elevated among active workers and slightly elevated among retirees. Case-control studies were designed to further evaluate this occupational association by summarizing information from work histories. Although the numbers are small, preliminary results showed a larger percentage of cases than controls in the occupations involved in moving crude oil and refinery products from one point to another. In addition, the mean length of employment in the motor oil category, involving second-step refinery operations such as the manufacture of lubricating oils, paraffin, and some solvents, was much longer for the cases than the controls.

12. The pattern of causes of death among tobacco workers revealed no striking elevations. More deaths from colon cancer occurred than expected among men and women, whites and blacks.

13. In a cohort mortality study of iron hematite miners, a significant excess of stomach cancer was noted in above and below ground miners. Lung cancer was elevated only among the foreign born.

14. Irritant effects of formaldehyde are well known, but recent laboratory experiments raised serious concerns regarding its carcinogenicity. To evaluate the potential hazards among humans, a proportionate mortality study of embalmers was undertaken. Increased frequencies of death from cancer of the skin, kidney, and brain were observed, particularly among those licensed for more than 35 years, or those who were first licensed before age 30. Although embalming fluids contain chemicals other than formaldehyde, these excesses suggest a need for additional studies to further clarify the potential risk associated with formaldehyde exposure.

15. A survey of mortality among copper smelter workers revealed that a respiratory cancer excess previously observed during the period 1938 through 1963 continued from 1964 to 1977. The risk was linked to work areas of the plant where airborne arsenic levels were high and increased with cumulative exposure.

16. Evaluation of the underlying cause of death among deceased members of the International Brotherhood of Potters and Allied Workers uncovered an elevated frequency of lung cancer among white men employed in the manufacture of ceramic plumbing fixtures. Heavy use of talc in the casting of these appliances may be involved in the origin of lung cancer in this occupational group.

17. A case-control study of non-Hodgkin's lymphoma was conducted using mortality listings from Wisconsin. Farming was more common among cases. Risks were increased among younger farmers residing in counties high for general agricultural activity, small grain acreage and acreage treated with insecticides, and wheat acreage. Relative risks increased over the 1968-1976 study period, 20 to 25 years after the large-scale introduction of many chemical pesticides, including herbicides.

18. Servicemen in chemical processing companies during World War II had contact with tetrachloroethane and other chlorinated compounds while impregnating clothing against mustard gas. The cancer mortality experience of these servicemen was elevated with slightly increased risks for leukemia, lymphoma, and cancers of the genital organs.

Significance to Biomedical Research and the Program of the Institute: Studies of the cancer experience of working populations have provided much of the information known about chemical carcinogenesis in man. Occupational groups may be regarded as indicators in evaluating possible hazards to the general population because exposures are often heavy and well defined.

Proposed Course: An increasing number of industrial populations will require epidemiologic investigation as leads are developed from clinical and laboratory observations. New studies are being initiated to meet this need. In addition, findings from completed studies suggest areas and methodologies where further research is needed to identify more accurately carcinogenic agents associated with the workplace and to better quantify the cancer risks.

Publications:

Blair, A.: Cancer Risks Associated with Agriculture: Epidemiologic Evidence. In Fleck, R., and Hollaender, A. (Eds.): Genetic Toxicology: An Agricultural Perspective. New York, Plenum Publishing Company. In press.

Blair, A., Berney, B.W., Heid, M.F., and White, D.W.: Causes of death among workers in the tobacco industry. J. Occup. Med. In press.

Blair, A., and Hayes, H.M.: Mortality patterns among U.S. veterinarians, 1947-1977: An expanded study. Int. J. Epidemiol. In press.

Blair, A., and Spirtas, R.: Use of Occuptional Cohort Studies in Risk Assessment. In Richmond, C.R., Walsh, P.J., and Copenhaver, E.D. (Eds.): Health Risk Analysis: Proceedings of the Third Life Sciences Symposium. Philadelphia, Franklin Press, 1981, pp. 95-108.

Blair, A., and White, D.W.: Death certificate study of leukemia among farmers from Wisconsin. JNCI 66: 1027-1030, 1981.

Cantor, K.P.: Farming and mortality from non-Hodgkin's lymphoma: A case-control study. Int. J. Cancer 29: 239-247, 1982.

Decoufle, P., Blattner, W.A., and Blair, A.: Mortality among chemical workers exposed to benzene and other chemicals. Environmental Res. In press.

Egan-Baum, B., Miller, B., and Waxweiler, R.: Lung cancer and other mortality patterns among foundrymen. Scan. J. Work Environ. Health 7: 147-155, 1981.

Hoar, S.K.: Epidemiologic Studies of Chemists. In Fawcett, H.H. (Ed.): American Chemical Society Symposium on Epidemiology. In press.

Hoar, S.K.: Epidemiology and Occupational Classification Systems. In Peto, R., and Schneiderman, M. (Eds.): Banbury Report 9: Quantification of Occupational Cancer. New York, Cold Spring Harbor Laboratory, 1981, pp. 455-470.

Hoar, S.K.: Job Exposures Matrices: A Point of View from the United States. In Acheson, E.D. (Ed.): Proceedings of Conference on Job Exposure Matrices. University of Southampton, England. In press.

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Miller, B., Blair, A., and McCann, M.: Mortality Patterns among Professional Artists: A Preliminary Report. In Proceedings of the Conference on Health Risks in Arts, Crafts, and Trades, April 2, 1981. Chicago, IL., Pathotox Publishers. In press.

Norman, J.E., Robinette, C.D., and Fraumeni, J.F., Jr.: The mortality experience of Army World War II chemical processing companies. J. Occup. Med. 23: 818-822, 1981.

Reeve, G.R., Thomas, T.L., Straussman, V.F., and Waxweiler, R.J.: A proportionate mortality study of an OCAW local in Texas City, Texas. Ann. NY Acad. Sci. In press.

Spirtas, R., and Fendt, K.: An Algorithm for Linking Job Titles with Individual Exposures in Occupational Epidemiology Studies. In Acheson, E.D. (Ed.): Proceedings of Conference on Job Exposure Matrices. University of Southampton, England. In press.

Symons, J.J., Andjelkovich, D., Spirtas, R., and Herman, D.R.: Brain and central nervous system cancer mortality in U.S. rubber workers. Ann. NY Acad. Sci. In press.

Thomas, T.L.: A preliminary investigation of mortality among workers in the pottery industry. Int. J. Epidemiol. In press.

Thomas, T.L., Waxweiler, R.J., Crandall, M.S., White, D.W., Moure-Eraso, R., Itaya, S., and Fraumeni, J.F., Jr.: Brain cancer among OCAW members in three Texas oil refineries. Ann. NY Acad. Sci. In press.

Thomas, T.L., Waxweiler, R.J., Moure-Eraso, R., and Fraumeni, J.F., Jr.: Mortality patterns among workers in three Texas oil refineries. J. Occup. Med. 24: 135-141, 1982.

Walrath, J., and Fraumeni, J.F., Jr.: Proportionate Mortality among New York Embalmers. In Gibson, J.E. (Ed.): Proceedings of Conference on Formaldehyde Toxicity, Research Triangle Park, NC. Washington, D.C., Hemisphere Publishing Corp., 1981. In press.

CONTRACTS IN SUPPORT OF THIS PROJECT

ABT, ASSOCIATES (N01-CP-11019)

Title: Support Services for a Case-Control Study of Brain Cancer and Occupation.

Objectives: This contract is to provide NCI with the necessary field support to conduct a case-control interview study of brain cancers to evaluate the role of occupation (particularly employment in the petroleum industry) and other factors in the origin of this cancer.

Methods Employed: Six hundred cases and 600 controls among adult males will be selected from geographic areas where at least 10 percent of the males are employed in the petroleum industry. Cases and controls will be identified from mortality records at State Vital Records offices. Next-of-kin of cases and controls will be interviewed to determine salient factors regarding the origin of this tumor.

Major Findings: This project has been under way for an insufficient period of time for a significant report.

Proposed Course: Continue the project as planned.

Current Annual Level: $183,165.

Man Years: 4.0

GENERAL SERVICES ADMINISTRATION (Y01-CP-20514)

Title: Retrieval of Civilian Personnel Records.

Objectives: To review civilian personnel records of individuals formerly employed at Hill Air Force Base, Ogden, Utah, as airplane maintenance workers.

Methods Employed: NCI submits to GSA magnetic tapes with Social Security numbers or listings with name and date of birth. GSA determines whether the personnel files are stored at the National Personnel Records Center in St. Louis, Missouri. The files which are present are pulled, given to on-site NCI contractor personnel for review and then refiled.

Major Findings: This Interagency Agreement provides NCI with the ability to review Official Personnel Folders which contain very detailed occupational histories of epidemiology study subjects.

Proposed Course: Expiration of the present agreement is September 30, 1982. The Project Plan for this effort has been approved through 1983, and it is anticipated that the work will continue according to the methodology outlined above.

Current Annual Level: $45,000.

Man Years: 1.0

GENERAL SERVICES ADMINISTRATION (Y01-CP-20515)

Title: Retrieval of Military Personnel and Health Records and Civilian Personnel Records.

Objectives: To review (1) military personnel and health records of officers and enlisted men who were formerly U.S. Coast Guard marine inspectors, (2) civilian personnel records of individuals formerly employed at the U.S. Coast Guard Shipyard in Curtis Bay, Maryland, and (3) civilian personnel records of individuals formerly employed by the U.S. Department of Agriculture.

Methods Employed: NCI submits to GSA magnetic tapes with Social Security numbers or listings with name and date of birth. GSA determines whether the personnel files are stored at the National Personnel Records Center in St. Louis, Missouri. The files which are present are pulled, given to on-site NCI contractor personnel for review and then refiled.

Major Findings: This Interagency Agreement provides NCI with the ability to review Official Personnel Folders which contain very detailed occupational histories of epidemiology study subjects.

Proposed Course: Expiration of the present agreement is September 30, 1982. The Project Plan for this effort has been approved through 1983, and it is anticipated that the work will continue according to the methodology outlined above.

Current Annual Level: $45,000.

Man Years: 0.67

KANSAS, UNIVERSITY OF (N01-CP-01027)

Title: A Case-control Study of Lymphoma and Soft-tissue Sarcoma: Association with Herbicide Exposure.

Objectives: Epidemiologic studies in Sweden have suggested that exposure to herbicides may increase the risk of lymphoma and soft-tissue sarcoma. This study will evaluate the risk of herbicide exposure among farmers from Kansas.

Methods Employed: A case-control interview study has been designed. Cases will be obtained from the Kansas Tumor Registry, and compared for herbicide exposure to a set of randomly selected controls drawn from the general population.

Major Findings: Cases and controls are being selected. Additional time is necessary to produce a significant report.

Proposed Course: Continue the contract as planned.

Current Annual Level: $446,000.

Man Years: 5.0

SOCIAL SECURITY ADMINISTRATION (Y01-CP-10502)

Title: Determination of Vital Status and Personal Data of Epidemiological Study Cohorts.

Objectives: To determine the vital status and obtain such demographic information as sex, race, and date of birth for members of epidemiology study groups.

Methods Employed: NCI provides the Social Security Administration (SSA) with magnetic tapes containing Social Security numbers and the first six letters of the last name. SSA then matches these tapes with its files and provides NCI with data which are used for further follow-up.

Major Findings: This Interagency Agreement provides the critical information needed for each retrospective cohort mortality study, namely the vital status of members of the study group. This information is then used to obtain death certificates (in the event of death), or to conduct further follow-up efforts.

Proposed Course: Expiration of the present agreement is September 30, 1982. The Project Plan for this effort has been approved through 1986, and it is anticipated that the work will continue according to the methodology outlined above.

Current Annual Level: $46,199.

Man Years: 1.0

WESTAT, INC. (N01-CP-01020)

Title: Mortality among Airplane Maintenance Workers.

Objectives: To compare the mortality experience of workers exposed to solvents and other hazardous substances with that of internal and external control groups.

Methods Employed: A cohort of 15,000-20,000 civilian workers at the Hill Air Force Base maintenance facility in Utah during 1952-57 is being assembled from personnel records. Occupational exposures will be estimated from job titles and departments. The present-day vital status of each member will be

ascertained and cause-specific mortality rates calculated for comparison with expected rates from the general U.S. population and unexposed civilian workers at the air base.

Major Findings: Results are not yet available. Data collection is under way. Additional time is necessary to produce a significant report.

Proposed Course: The contract will continue as planned.

Current Annual Level: $254,000. (Funds provided by the Department of Defense through an Interagency Agreement.)

Man Years: 4.0

WESTAT, INC. (NO1-CP-11006)

Title: Support Services for a Mortality Study of Workers Exposed to Formaldehyde.

Objectives: To provide technical and managerial support for data collection for a cohort mortality study of formaldehyde-exposed workers.

Methods Employed: The contractor will assemble a cohort of 20,000 workers from 10-20 companies whose workers have had exposure to formaldehyde. Data will be abstracted from personnel records, coded, edited, and entered in the computer. All study subjects will be traced to determine their present-day vital status.

Major Findings: Results are not yet available. The cohort is being assembled, work histories are being abstracted, and estimates of workplace exposure to formaldehyde are under way.

Proposed Course: The expiration date for this contract is June 24, 1984 and it is expected to continue as planned to that date.

Current Annual Level: $209,081.

Man Years: 4.0

WESTAT, INC. (NO1-CP-91034)

Title: Support Services for Occupational Studies.

Objectives: To provide technical, managerial, and clerical support for occupational studies conducted by the Environmental Epidemiology Branch.

Methods Employed: Westat, Inc. provides personnel to assist NCI in data collection for cohort, proportionate mortality, and case-control studies. This includes developing abstracting forms and questionnaires, administering

these instruments, maintaining quality control of data collection, tracing study subjects, editing and updating data, and preparing final data files.

Major Findings: This contract provides support for some 25 different projects. Significant contributions were made to items 3, 5, 8, 9, 11, 12, and 14 under Major Findings for this Intramural Research Project.

Proposed Course: Expiration date for this contract is September 29, 1983 and is expected to continue to that date in accordance with above methodology.

Current Annual Level: $753,979.

Man Years: 12.0

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04481-06 EEB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Studies of Radiation-Induced Cancer

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	J.D. Boice, Jr.	Head, Radiation Studies Section	EEB	NCI
OTHER:	C.E. Land	Health Statistician	EEB	NCI
	D.A. Hoffman	Epidemiologist	EEB	NCI
	N. Hayakawa	Visiting Scientist	EEB	NCI
	M. Tokunaga	Visiting Scientist	EEB	NCI
	R.A. Kleinerman	Epidemiologist	EEB	NCI
	E.B. Harvey	Epidemiologist	EEB	NCI
	B.W. Beebe	Health Statistician	CEB	NCI
	W.J. Blot	Head, Analytical Studies Section	EEB	NCI
	M.H. Greene	Clinical Investigator	EEB	NCI
	R. Curtis	Statistician	BB	NCI
	L. Pottern	Epidemiologist	EEB	NCI
	J. Scotto	Demographer	BB	NCI

COOPERATING UNITS (if any)
Clinical Epidemiology Branch, NCI; Radiation Effects Research Foundation, Japan; Bureau of Radiological Health, FDA; Medical Follow-up Agency, NAS; Biometry Branch, NCI; Department of Energy

LAB/BRANCH
Environmental Epidemiology Branch

SECTION
Radiation Studies Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
9.0	6.0	3.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) The objectives of this project are to examine cancer incidence and mortality among populations exposed to ionizing and non-ionizing radiation; to characterize the risk of radiation-induced cancer in terms of tissues at risk, dose response, radiation quality, temporal distribution of dose, time since exposure, age at exposure and at observation, and possible modifying influences of other environmental and host factors; and in particular, to quantify excess cancer risk at low dose levels. Groups studied include the Japanese A-bomb survivors, several large populations with documented therapeutic, diagnostic, and occupational exposures to ionizing radiation, and resident populations of SEER reporting areas with different ambient levels of solar UV radiation. Other biological effects, such as chromosomal abnormalities in circulating lymphocytes, are studied for possible insights into radiation carcinogenesis. Close liaison is maintained with experimental scientists concerned with radiation carcinogenesis. Project members serve on committees and task forces advising the government as well as national and international agencies.

Project Description

Objectives: (1) To plan and conduct studies of cancer risk and related health outcomes in populations exposed to ionizing radiation (e.g., x rays, gamma rays, neutrons, alpha and beta particles) and nonionizing radiation (e.g., ultraviolet light); (2) to evaluate excess cancer risk in exposed populations in terms of tissues at risk, amount, quality, and temporal and spatial distribution of radiation dose, latency, and possible modifying influences of host factors (e.g., age, sex, hormonal status) and external factors (e.g., drugs, other carcinogens); (3) in particular, to improve estimates of cancer risks associated with exposure to low doses of ionizing radiation; (4) to examine possible analogs of radiation carcinogenesis in man, such as the induction by radiation of benign tumors and cytogenetic abnormalities in man, or cancer and other effects in experimental systems, for insights into mechanisms of radiation carcinogenesis that may also result in improved theoretical models for the estimation of risk; and (5) to advise and collaborate with other government agencies involved in radiation research and the safety of activities involving radiation exposure.

Methods Employed:

1. Studies of Japanese A-Bomb Survivors. Investigations based on the Life Span Study sample of 82,000 A-bomb survivors and 26,000 nonexposed controls are carried out at the Radiation Effects Research Foundation (RERF) in Hiroshima and Nagasaki, Japan. All studies involving new or unpublished data are collaborative, and include investigators from NCI, RERF, and outside organizations as required; collaboration is facilitated by personnel exchanges between RERF and NCI. Protocols for collaborative studies undergo formal review at RERF and NCI.

   a. Data collection was completed for a case-control study of breast cancer in the LSS sample and available records for deceased cases and their controls. Dr. Norihiko Hayakawa of Hiroshima University's Institute for Nuclear Medicine visited Bethesda in April and May, bringing with him data needed for preliminary tabulations. The study was designed to investigate possible interactive effects of radiation with other environmental and host factors in the etiology of female breast cancer.

   b. Data collection for a second case-control study, having similar aims with respect to the etiology of lung cancer, continued in Hiroshima and Nagasaki.

   c. Case finding, data analysis, and manuscript preparation were completed for a cohort-based study of breast cancer incidence in the LSS sample during the period 1950-1980. Dr. Masayoshi Tokunaga of Kagoshima University visited NCI during May-August to work on this and other projects.

d. A manuscript was prepared and submitted for publication describing the findings of a 1979 U.S.-Japan pathology review of diagnostic materials for breast cancer in the LSS sample.

e. A pathology study of breast tissue samples from breast cancer cases and autopsy controls was conducted to investigate possible non-neoplastic changes related to radiation dose as suggested by a U.S.-Japan pathology review panel.

f. Preliminary plans were developed for a U.S.-Japan pathology review of diagnostic materials for thyroid cancer incidence in the LSS sample. The review would establish a series of cases that would not require further review in future studies of thyroid cancer incidence in the sample, and would investigate possible relationships between histological type and radiation dose and/or age.

g. The effect on dose-response analyses of random errors in estimated individual radiation doses was investigated, with emphasis on the Hiroshima-Nagasaki data.

h. A program of dose-response analyses was initiated in collaboration with members of RERF to determine one or more interim dosimetries that might be used for analyzing new RERF data, until a new dosimetry system can be developed and accepted by RERF. Essentially, the program considers dose-response analyses of survivors on whom present dose estimates were obtained in similar ways, and whose conversion to a new dosimetry presents relatively few problems with respect to the effects of shielding by materials such as buildings.

i. A collaborative study continued which involved parallel dose-response analyses of thyroid cancer incidence data from several different series: RERF, Tel Aviv University, New York University, and Michael Reese Hospital (Chicago). The study is modeled after a similar collaborative approach to breast cancer incidence among A-bomb survivors and patients receiving medical radiation during treatment for TB and acute postpartum mastitis.

2. Studies of patients with histories of medical exposure to radiation.

a. The follow-up of approximately 15,000 persons exposed to multiple fluoroscopic chest examinations in conjunction with pneumothorax treatment of tuberculosis is continuing. The TB-fluoroscopy studies are conducted in collaboration with Harvard University and Yale University, and TB patients treated between 1930-1954 in Massachusetts and Connecticut are being studied. The risk of breast cancer, lung cancer, and leukemia in men and women is being evaluated. A pilot telephone interview case-control study of breast cancer to evaluate the interaction of radiation and host factors has been completed. These studies are of particular public health importance, since the exposures were to low doses of radiation, repeated every few weeks for many years.

b. A large international study of 30,000 women treated for cervical cancer in 30 radiotherapy centers in 9 countries is continuing. In addition to the evaluation of radiogenic leukemia, the study has been expanded to evaluate the risk of developing solid tumors following relatively low-dose irradiation. Doses to body sites outside the pelvis are low (1-100 rads) and can be accurately determined. In addition to this clinical sample, the study has been expanded to include cervical cancer patients reported in 20 cancer registries across the world. Approximately 200,000 women are under study. Case-control studies within the various cohorts are being conducted, in addition to straightforward cohort evaluations. Dosimetry, pathology, questionnaire, and blood studies are ongoing.

c. A study of 3,300 children treated for enlarged tonsils by irradiation or surgery has continued in collaboration with the Children's Hospital and the Peter Bent Brigham Hospital in Boston. Physical examinations are being performed on both irradiated and surgical patients to more accurately determine the risk of thyroid nodular disease, and to adjust for the potential detection bias in previous studies which only screened radiation-exposed persons. Blood studies will determine radiation effects on the parathyroid and thyroid; and chromosome aberrations in circulating lymphocytes will be evaluated.

d. In collaboration with the Department of Energy-supported Cytogenetics Laboratory at the Oak Ridge Associated Universities, an investigation has continued in which chromosomal aberration frequencies are ascertained for circulating lymphocytes in radiation-exposed and control subjects from three different patient populations under study, as described in items a-c above. The study is intended to calibrate the technique of chromosomal analysis as a biological dosimeter for partial-body radiation exposures, and to explore the analogy between the induction of chromosomal aberrations and carcinogenesis.

e. Using the resources of pre-paid health plans in California and Oregon (Kaiser-Permanente), cases of leukemia and lymphoma and controls are being identified and long-term histories of diagnostic x-ray exposures are being obtained. Approximately 2,000 subjects are being studied, and the association with diagnostic x-rays evaluated.

f. Data tapes from the Connecticut Tumor Registry and the Twin Registry were linked to identify childhood cancer cases between 1930-1970 in order to evaluate the risk of developing childhood cancer in twins. Subsequently, the cancer cases were matched with twin controls, and hospital records were abstracted to assess the risk of prenatal x-ray exposure. Physicians are currently being contacted to obtain further details on prenatal history. A similar collaborative study is planned in California and possibly New York.

g. A case-control interview study of all thyroid cancer cases diagnosed between 1978-1980 in Connecticut has been completed. Approximately

200 persons with thyroid cancer and 400 population controls have been interviewed to evaluate the influence of radiation, drugs, and diet on thyroid cancer risk.

h. The Surveillance, Epidemiology and End Results (SEER) registries were used to identify second primary cancers in persons treated for cancers of the breast, cervix, testes, thyroid, and salivary gland. In addition to evaluating the carcinogenicity of alkylating chemotherapeutic agents, radiation treatments are being evaluated as a possible cause of second primary cancers. Cohort and case-control studies are being conducted.

i. Patients receiving whole body irradiation at the National Cancer Insititute for non-Hodgkin's lymphoma were evaluated for excess leukemia risk.

j. A case-control study of over 200 children who developed second primary cancers following treatments for childhood cancer was completed at 13 childhood cancer centers in 6 countries.

k. A collaborative study is continuing with the Chaim Sheba Medical Center, Israel, to evaluate the risk of cancer in 10,000 children exposed to x-rays during their treatment of ringworm of the scalp, and 15,000 comparison persons. The methods are unique in that all pathology records of Israeli hospitals are being searched to identify tumors. Analysis of existing data has continued for cancer incidence and mortality.

l. The risk of malignancies following therapeutic doses of 131-I in the treatment of hyperthyroidism has been evaluated in collaboration with the Mayo Clinic, the Bureau of Radiological Health (FDA), and the Radiumhemmet in Stockholm.

m. Women irradiated for benign menstrual disorders are being studied to evaluate the risk of radiogenic cancers at radiation dose levels less than those in the international cervical cancer study. Collaborators include the Radiumhemmet and the Roswell Memorial Park Institute.

n. A study of the risks of cancer among patients diagnosed with idiopathic adolescent scoliosis is in the planning stages. Collaboration with the Scoliosis Research Society of the American Orthopedic Association is being sought. In particular, the risk of breast cancer among women who were exposed to repeated x-ray examinations during adolescence, a time when the breast is most sensitive to environmental insults, will be determined. The approximate size of the study population will be 10,000 patients. Reconstruction of radiation doses will be made based on information from the radiologic records, and on measurements, using the original x-ray machines, if available.

o. A pilot case-control medical record review study of testicular cancer patients diagnosed between 1935-1980 in Connecticut has been initiated. Data will be collected for 90 testicular cancer patients who developed a second cancer and for 180 testicular cancers without a second cancer to evaluate the risk of second cancers in relation to radiotherapy and/or chemotherapy, particularly cis-platinum.

3. Studies of patients with histories of occupational exposure to ionizing radiation.

a. A successful feasibility study was completed, and a full scale investigation was begun, to evaluate the risk of cancer among 170,000 x-ray technologists identified from registry records for the years 1926-1980. This is a collaborative study with the University of Minnesota.

b. Sputum cytology data on uranium miners, controlling for radiation exposure and cigarette smoking, were analyzed. This has been a collaborative effort with NIOSH and Dr. Geno Saccomanno, a private physician of Grand Junction, Colorado.

4. Population studies of environmental radiation exposures.

a. Regions in the Western U.S. with uranium mill tailing desposits were identified, and correlations with county cancer mortality data made.

b. In collaboration with the Biometry Branch, a study was continued to clarify the role of solar UV-radiation in the development of non-melanoma skin cancer by means of a demographic survey using SEER Tumor Registries, and a case-control study to clarify the influence of various host and environmental co-factors.

5. Investigations into statistical methodologies for estimation of risk.

a. Several expository articles were written about methodological problems of estimating cancer risk from low doses of ionizing radiation, including the effects of random error of individual dose estimates, especially for analyses of grouped data.

b. Formal Bayesian procedures, by which prior and experimental information about dose-response functions can be used to estimate low-dose risk from high-dose epidemiological data, are being investigated in collaboration with Oregon State University.

6. Consultant activities and services on expert committees. The Co-Principal Investigators have served as consultants or committee members for the National Council on Radiation Protection and Measurements, the Department of Energy, the Department of Defense, the Environmental Protection Agency, the DHHS subcommittee to coordinate federal radiation activities, the American Cancer Society National Committee on Cancer Prevention and Detection, the International Commission on Radiation

Protection, and the World Health Organization. Staff members were also involved in evaluating studies of radioactive fallout in Utah.

7. Conference on radiation carcinogenesis. A multidisciplinary conference on radiation carcinogenesis was held in May 1982. The purpose was to allow principal investigators who have conducted major human epidemiologic or laboratory studies of radiation and cancer to summarize their findings, present current work, and suggest areas for future research. The implication of radiation studies to models of carcinogenesis were stressed, and the proceedings will be published.

8. Review papers. Several review papers concerning health effects following exposure to ionizing radiation were written, including a general overview, a review of cancer following medical irradiation, the epidemiology of radiogenic bone cancer and thyroid cancer, the effect of radiation on the immune system, the statistical aspects of estimating cancer risks from low doses of ionizing radiation, the implications of radiogenic breast cancer studies for models of human carcinogenesis, the effects of fetal irradiation, the long-term effects of radiation upon children, and the risk of cancer following treatment with radioactive iodine.

Major Findings:

1. The International Radiation Study of Cervical Cancer is a program of individual studies designed to learn about carcinogenesis by radiation. The registry analyses suggest that (a) heavily and moderately irradiated sites (i.e., those likely to have received over 100 rads of radiation) show a consistent pattern of increased risk with time since exposure, that is probably radiation related; (b) in particular, cancers of the bladder, rectum, bone, connective tissue, uterine corpus, ovary, small intestine, kidney, and multiple myeloma may be associated with radiation in this study; (c) the relative risk of cancers of heavily and moderately irradiated organs was greatest among those under age 30 at exposure, but generally constant among older women, and suggests that radiation may interact in a multiplicative fashion with other factors that cause cancer; (d) substantial radiation doses to the stomach and colon do not appear to increase risk beyond normal expectation; (e) the radiation regimens used to treat cervical cancer are not so effective in inducing leukemia as are other radiation exposures that have been studied; however, a slight risk might be associated with the radiation received by bone marrow outside the pelvis; (f) radiation effects on the ovary may lower breast cancer risk at all ages of exposure; (g) a substantial excess of lung cancer among cervical cancer patients is probably not related to radiation, since the organ dose was low and cigarette smoking is a possible confounder; (h) a small excess of thyroid cancer might be associated with a relatively low-dose exposure; (i) second cancers of other sites that received relatively low doses of radiation are either not increased above expectation or are probably elevated due to exposures to other strong risk factors, such as cigarette or alcohol consumption;

and (j) after a minimum latent period of about 10 years, the risk of radiogenic cancer remains throughout life and does not decrease.

2. The latest breast cancer incidence data from the Hiroshima and Nagasaki A-bomb survivor studies resemble earlier data. The dose-response function continues to show no departure from linearity, the temporal distribution of excess risk is unrelated to dose, and women exposed in the second decade of life continue to show the largest radiation-related excess risk. An apparent anomaly in earlier data, in which women exposed at ages 40-49 seemed to exhibit a statistically significant decrease in risk with increasing dose, did not appear in the most recent data. Women 40-49 and 50+ at exposure had similar levels of excess risk, which were not statistically different from zero. For the first time in any exposed population, women who were exposed in their first decade of life showed a dose-related excess breast cancer risk.

3. The studies of TB patients who had multiple fluoroscopic examinations of the chest, mastitis patients given radiotherapy, and A-bomb survivors are being continued. Analyses suggest that the risk for radiogenic breast cancer is greatest for persons exposed as adolescents. Direct evidence of radiation risk at doses under 50 rads is apparent among A-bomb survivors. The interval between exposure and clinical appearance of radiogenic breast cancer may be mediated by hormonal or other age-related factors, but is unrelated to dose. It is as yet impossible to determine whether an absolute or relative risk model more correctly describes the relationship between spontaneous and radiation-induced breast cancer.

4. Radiation-related breast cancers appear morphologically similar to other breast cancers occurring in women of comparable age, according to an evaluation of pathological materials from breast cancer cases diagnosed among A-bomb survivors during the periods 1950-1978 and 1950-1980.

5. Random error in individual dose estimates tends to introduce additional negative curvature in plotted dose-response curves when individuals are assigned to dose intervals on the basis of estimated dose. Conversely, analyses which correct for this tendency introduce additional positive curvature. Unless the amount of uncertainty exceeds 30 percent or so, however, the effect of curvature is slight.

6. Total lactation period, number of children, and age at first delivery are strongly related to breast cancer risk among A-bomb survivors, a finding consistent with earlier studies of this and other populations. Analyses of interactions between these factors and radiation are too preliminary to be reported.

7. Hiroshima-Nagasaki differences in dose response for certain radiation effects, which have been ascribed to differences in the quality of radiation received from the A-bombs dropped on the two cities, persist and are difficult to explain according to a proposed new dosimetry in which city differences in radiation quality are much less marked. The A-

bomb survivor data have less relevance to questions about the shapes of dose-response curves according to the proposed new dosimetry.

8. Existing (and forseeable) epidemiological data are unlikely to yield precise estimates of risk from low-dose radiation exposures unless arbitrary assumptions are made that severely restrict the possible forms taken by the fitted dose-response function, or unless relevant information can be incorporated from experimental data. Bayesian methods allow uncertainties of assumptions and variability of experimental data to be taken into account when incorporating such information into curve-fitting analyses of epidemiological dose-response data.

9. The second mail questionnaire follow-up of women who received multiple chest fluoroscopies during pneumothorax treatment of tuberculosis reaffirms that repeated, relatively low radiation doses pose some future risk of breast cancer, that the risk may be cumulative, and that a woman's lifetime risk of breast cancer is likely determined in large part during early adult life. Exposures around menarche and during first pregnancy appear especially hazardous, so that proliferating breast tissue may be particularly sensitive to the carcinogenic effects of radiation. Nulliparous women appear to be at higher radiation risk than women who have had children.

10. Additional studies have been conducted on tuberculosis patients in Massachusetts exposed to repeated fluoroscopic chest examinations. The estimation of radiation doses for the breast, lung, stomach, pancreas, and active bone marrow was refined and a mortality analysis performed. No excess of total cancer deaths, leukemia, lung cancer, or lymphoma was seen among fluoroscopically examined men or women. These findings indicate that the carcinogenic effect of multiple low-dose x-ray exposures is unlikely to be greater than currently assumed, and may be less for lung cancer and leukemia.

11. Subsequent cancers were examined in 517 patients with non-Hodgkin's lymphoma treated at the National Cancer Institute. A significant excess of acute nonlymphocytic leukemia (ANL) was found (9 observed, 0.08 expected, O/E = 105). The risk was greater in patients treated with both radiation and chemotherapy than in patients who received single modality therapy. A positive correlation between radiation dose to the bone marrow and risk of ANL was demonstrated, independent of chemotherapy duration.

12. Among 6,000 women with cervical cancer and treated with radiation in Connecticut, 449 developed second primary cancers compared to 313 expected (O/E = 1.4). The excess incidence was attributable to cancers of the lung, bladder, kidney, uterine corpus, and rectum. An excess of corpus cancer and ovarian cancer occurring 15 years post treatment is consistent with a radiation etiology, although metastatic lesions might have contributed somewhat to this excess. A deficit of breast cancer was observed, possibly due to radiation-induced menopause or to reproductive

factors peculiar to this group of women (e.g., early first pregnancy) that may be protective.

13. In a study of 1,005 women treated with radioactive iodine and 2,141 women treated with surgery for hyperthyroidism at the Mayo Clinic, no increased cancer mortality was observed in the 131-I treated women, although an overall excess mortality was apparent. The patterns of mortality by age, treatment, year of treatment, and time after treatment suggest that the increased overall mortality was a consequence of women with poor survival expectation being selected for 131-I therapy, and not as a result of the radiation exposure. In addition to mortality there was no increased risk of cancer in the 131-I group compared with the surgery treated women (RR=1.0). However, a significant risk of thyroid cancer in 131-I treated women (RR=9) was observed and also for organs of high 131-I exposure (RR=1.8), although the numbers are relatively small; the risk was limited to the first 5 years after treatment and no dose-response relationship was observed. In addition, no increased risk of breast cancer was observed in any subgroup of women, either those treated by thyroid replacement therapy, or radioactive iodine, or surgery. The absence of an effect among the radiation exposed women may be attributable to the relatively low breast dose from 131-I (40 rads), the small number of exposed women, and, possibly, the advanced age of the 131-I treated women.

14. A man with gynecomastia and a family history of diverse cancers developed adenocarcinoma of the breast 30 years following childhood thymic irradiation. His cultured skin fibroblasts displayed abnormal in vitro sensitivity to ionizing radiation, indicating impaired ability to repair damaged DNA.

15. Children with cancer treated by radiation and/or chemotherapy appear to be at substantial risk of developing a second cancer, especially leukemia and cancers of connective tissue, bone, thyroid, and possibly the brain.

Significance to Biomedical Research and the Program of the Institute:
Information about the carcinogenic effects of radiation is required in order to balance risks and benefits in medical, economic, and recreational activities. Epidemiologic data relating cancer incidence and mortality to radiation exposure, especially with good information on dose and timing of exposure, can influence theories of carcinogenesis and motivate experimental research.

The Radiation Studies program exists to investigate and clarify the established causal link between cancer risk and exposure to ionizing radiation and certain types of non-ionizing radiation. An immediate practical need is for risk estimates on which to base regulatory and other decisions about the use of nuclear and radiological technology in industry and medicine, and with which to assess the value of exposure avoidance as a means of cancer prevention. A particularly cogent reason for a high level of epidemiologic effort in studies of radiation carcinogenesis, however, is that the study of radiation carcinogenesis appears to be a particularly promising approach to

understanding carcinogenesis in general. In many exposed populations, quantitative and qualitative descriptions of exposure to affected tissues are straightforward, an advantage not available for most other carcinogens; furthermore, epidemiologic studies can draw upon the background of a vast literature of experimental and theoretical radiobiology, including radiation carcinogenesis in experimental animals as well as studies at the cellular and subcellular levels.

The importance of radiation studies for understanding carcinogenesis is illustrated by a series of breast cancer incidence studies carried out by the Radiation Studies Section and others. With remarkable consistency, these studies indicate (1) that sensitivity to radiation carcinogenesis can be heavily dependent on developmental factors but relatively independent of factors responsible for international differences in population rates, (2) that there may be wide variations among tissues with respect to the form of the dose-response curve, and (3) that while there is a recognizable "wave" in time of excess leukemia risk following radiation exposure, for many other radiation-induced cancers the time from exposure to diagnosis simply reflects age-specific population rates--that is, risk is increased, but the timing of that risk is unaffected by radiation dose.

The Radiation Studies program seeks information on 1) the relative sensitivities of various tissues to radiation carcinogenesis, 2) the influences of host factors and other exposures on the carcinogenic action of radiation, 3) the distribution over time of the excess cancer risk following radiation exposure, and 4) the influence of various dimensions of exposure, including dose, radiation quality, and fractionation and protraction of dose. A particular goal, pursued in response to a widely felt public health and regulatory need, is to provide information about the risk of cancer following exposure to low doses of sparsely ionizing radiations such as gamma and x-rays.

The approaches taken in the Radiation Studies program are, first, to assure that maximum benefit is derived from existing epidemiologic resources for investigating cancer risk in exposed populations; second, to aggressively develop data resources from exposed populations that have not been studied previously, but which offer unusual potential for new information; and third, to encourage the development, at NCI and elsewhere, of epidemiologic and statistical expertise in radiation carcinogenesis. The first of these approaches involves extending the follow-up of exposed populations already studied by project members and others (e.g., the Japanese A-bomb survivors, the Massachusetts series of former tuberculosis patients exposed as young women to multiple chest fluoroscopies, the international clinical series of women given radiotherapy for cervical cancer, the Israeli series of persons given radiation treatment for tinea capitis during childhood, and persons treated with 131-I therapy) and conducting ad hoc studies to investigate particular hypotheses. A major effort involves financial support for collaborative studies and extended visits by project members to the Radiation Effects Research Foundation (RERF) in Hiroshima, Japan. In addition, visits by Japanese scientists to NCI are encouraged, and NCI plans to station permanently a RSS member at RERF in order to increase participation by program

members in studies of the Japanese A-bomb survivors. Finally, authors of published studies relating risk from a particular cancer to radiation dose in different populations are encouraged to collaborate in a re-analysis of the combined material, using identical methods and assumptions for all data sets, so that differences and similarities can be highlighted and inferences strengthened. This has been done for breast cancer and is currently being done for thyroid cancer.

Investigations of previously unstudied populations account for, by far, the greater part of the effort and money expended under the project. Of these, the case-control studies of adult leukemia among members of prepaid health plans, and case-control studies of childhood cancer in relation to in utero exposure to x-ray pelvimetry among twins in Connecticut, have only short-term implications for the future of the program, since it is unlikely that further useful information can be obtained once the studies have been completed. Other exposure cohorts, on the other hand, can be followed as long as there are cohort members remaining at risk of radiation-induced cancer. Thus, the future of the Radiation Studies program is to a great extent being determined by cohort studies currently under way or under development, of cancer patient populations treated by radiation (of which the population of cervical cancer patients identified from tumor registries in different countries is by far the largest), men and women who received multiple fluoroscopic exposures during treatment for tuberculosis, persons exposed to diagnostic x-rays, such as scoliosis patients, and patient populations given radiation therapy for benign disease. Since much of the expense of epidemiologic studies of new cohorts is for identification and location of cohort members and gaining access to records, later follow-up studies of these cohorts should be easier and less expensive. The international cohort of 200,000 cervical cancer patients treated by radiation and/or surgery, as identified from records in 30 clinics and 20 cancer registries in over 20 countries, is expected to be a resource for future study comparable in value to the LSS sample of Japanese A-bomb survivors and the British series of patients given x-ray therapy for ankylosing spondylitis. Development of this resource, including dosimetry and current epidemiologic and pathology studies, is the most extensive project in the program.

Studies of cancer risk in populations exposed to low doses of ionizing radiation avoid problems of extrapolation of risk from high to low doses, but tend to be of little value because impracticably large sample sizes are required for adequate statistical power. Studies of large populations exposed to intermediate doses, on the order of 10-50 rads, offer the possiblities of adequate power and straightforward extrapolation of risk to lower doses. The feasibility of such a study depends on special circumstances, such as the existence of a common membership in a professional organization, which facilitates information gathering. Studies of cancer incidence among some 170,000 registrants in the American Registry of Radiation Technologists, whose occupational exposures to radiation, accumulated over many years, are large enough for acceptable statistical power with respect to the detection of at least some carcinogenic effects, according to currently accepted risk estimates, and will, thus, fill an important role in the program. Another approach to the low-dose risk estimation problem is to study populations

exposed to high cumulative doses received in small fractions. According to current radiobiological theory, the effect of such exposures should be proportional to cumulative dose, and only minimal assumptions are needed to extrapolate risk estimates from such data to very low-dose levels. Since cumulative doses are high, acceptable statistical power can be obtained with samples of only a few thousand. The population of patients exposed to multiple chest fluoroscopies during treatment for tuberculosis, therefore, will continue to be a major resource of the program.

Dosimetry is a crucial aspect of radiation studies, especially when non-uniform or partial-body exposures are involved. Dosimetry for current studies of medically-irradiated populations involves the collaboration of radiation physicists from the Bureau of Radiological Health, the M.D. Anderson Memorial Hospital and Cancer Institute, the Harvard Joint Center for Radiation Therapy, and collaborating institutions in other countries. They are developing sophisticated dosimetric procedures using experimental measurements with anthropometric phantoms and a Monte Carlo simulation algorithm developed at Oak Ridge National Laboratory. The current program is creating an expanded capacity for epidemiologic dosimetry which has already facilitated future studies.

The epidemiology of radiation carcinogenesis is concerned with questions that go far beyond the identification of radiation as a cancer risk factor for certain tissues. In particular, estimates of cancer risk from low-dose exposures to low-LET radiation must eventually depend on epidemiologic data obtained at higher dose levels. However, elucidation of the nature of that dependence probably will come from other radiobiological considerations, including the results of studies of experimental carcinogenesis, microdosimetry, and radiation effects, like chromosomal abnormalities, that, unlike cancer, can be easily studied at low doses. Therefore, the development of statistical and epidemiologic expertise in the area of radiation carcinogenesis depends, to a large extent, on closer association between persons involved in epidemiologic, experimental, and theoretical approaches to the problem. Another likely benefit of such association would be to encourage experimental investigations of questions arising from epidemiologic studies, such as the relative sensitivities to radiation carcinogenesis of pre- and post-pubertal, and pre- and post-menopausal, breast tissue. For these reasons, increased collaborative research is planned involving experimental radiobiologists at the National Laboratories of the Department of Energy and elsewhere, such as the current study of chromosome aberration frequencies in three exposed populations also under study for cancer risk, and possible re-analyses by project members of experimental dose-response data. In this regard, an interdisciplinary symposium on radiation carcinogenesis, sponsored by the Radiation Studies Section, was also held this year.

Proposed Course: Ongoing cohort studies of populations exposed to medical and occupational radiation will continue. The large studies of irradiated cervical cancer patients and atomic bomb survivors will receive major emphasis. Selected case-control studies will be initiated, as will additional cohort investigations. Studies under consideration include evaluating (a) the risk of contralateral breast cancer in women treated with radiotherapy for

primary breast cancer, (b) the risk of cancer in patients who received diagnostic x-ray exposures for scoliosis as adolescents, and (c) a feasibility study of the risk of cancer among nuclear power reactor workers. A more thorough discussion of specific studies is presented in the previous section.

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CONTRACTS IN SUPPORT OF THIS PROJECT

PETER BENT BRIGHAM HOSPITAL (NO1-CP-11008)

Title: Cancer Following Tonsil Irradiation: Physical Examinations and Blood Studies

Objectives: (1) To determine by physical examination whether there is any increase in thyroid nodules and head and neck cancer in persons irradiated for enlarged tonsils during childhood; (2) to determine whether there has been a radiation effect on the parathyroid as determined by blood tests of calcium levels; and (3) to evaluate the impact of intense screening on the detection of radiation-related thyroid tumors and the assessment of radiation risk.

Methods Employed: the Environmental Epidemiology Branch has been collaborating with clinicians at the Children's Hospital Medical Center and, in addition, will now be collaborating with the physicians at the Peter Bent Brigham Hospital where the exposed and non-exposed study subjects will be examined. The Brigham Medical Group will provide the examination rooms, nurses, physicians, laboratories, freezers for blood studies, and all other

necessary items for the conduct of the physical examinations and blood studies.

Major Findings: This project has been under way for an insufficient period of time for a significant report.

Current Annual Level: $147,268

Man Years: 1.5

TEXAS, UNIVERSITY OF, M.D. ANDERSON HOSPITAL (NO1-CP-01047)

Title: Studies of Iatrogenic Cancer and Radiation Dosimetry.

Objectives: To provide radiation dosimetry neccesary to estimate organ doses received during radiotherapy for cervical cancer. These measurements are essential to the assessment of radiation risks for our International Radiation Study of Cervical Cancer.

Methods Employed: Physics measurements are being made for x-ray machines and intracavitary isotopes (radium). These include orthovoltage, betatron, megavoltage x-ray machines, Van de Graaf machines, and cobalt-60 units, in addition to radium and cesium intracavitary sources. Abstracted data from all participating radiotherapy centers are also being evaluated with regard to dosimetry.

Major Findings: The contractor has rapidly and efficiently developed a measurement program to obtain organ specific doses following treatment for cervical cancer. Detailed progress reports have been written. Calculations of active bone marrow dose and measurements have been performed and compared with the results from a Monte Carlo computer technique in a mathematically described anthropomorphic phantom. Visits have been made to several radiotherapy centers in the U.S.A. and Europe, and questionnaires have been prepared for radiotherapists and medical physicists to determine treatment methods.

Proposed Course: To apply the developed dosimetry techniques to women identified from all hospitals and cancer registries collaborating in the international study of cervical cancer, and to apply the developed dosimetry to other groups of women similarly irradiated for metropathia and other conditions.

Current Annual Level: $105,000.

Man Years: 2.0.

WESTAT, INC. (NO1-CP-01011)

Title: Support Services for Radiation Studies.

Objectives: To obtain technical (nonprofessional), managerial, and clerical support for epidemiologic studies. The contractor functions in a supportive role carrying out specific tasks and does not engage in independent research.

Methods Employed: All phases of support services are being supplied, including: (1) preparing data collection forms; (2) preparing manuals for abstracting, coding, interviewing, and tracing; (3) tracing individuals to determine their vital status; (4) obtaining their consent to be interviewed; (5) interviewing or sending mail questionnaires; (6) obtaining death certificates; (7) abstracting, keying, editing, updating, and coding of data; (8) occasionally transporting biological specimens; (9) assessing exposure information; and (10) creating and manipulating data files.

Major Contributions: The contractor has provided support services for the following studies: (1) international radiation study of cervical cancer clinical follow-up; (2) registry case-control studies for the cervical cancer study; (3) questionnaire preparation for the x-ray technologist study; (4) the thyroid case-control interview study in Connecticut; (5) the Veterans Administration adjuvant drug study evaluations; (6) the clinical trial evaluations of MeCCNU; (7) the telephone questionnaire and second mailing for the TB-fluoroscopy breast cancer study in Massachusetts; (8) case-control study of second cancers following childhood cancer; (9) the study of second cancers following treatment for ovarian cancer; and (10) the case-control study of leukemia and lymphoma following diagnostic x-rays.

Proposed Course: Continue support for ongoing studies and newly developed studies.

Current Annual Level: $548,723

Man Years: 8.0

WESTAT, INC. (NO1-CP-11018)

Title: Support Services for Radiation and Related Studies

Objectives: Same as WESTAT (NO1-CP-01011), but studies under this contract are different from those in NO1-CP-01011.

Methods Employed: Same as WESTAT (NO1-CP-01011).

Major Contributions: The contractor has provided support services for the following studies: (1) risk of head and neck cancers following irradiation in childhood for enlarged tonsils; (2) TB-fluoroscopy study of males in Massachusetts; (3) case-control study of endometrial cancer following hormonal therapy for breast cancer; (4) case-control study of childhood cancer in twins associated with prenatal x-ray in Connecticut; (5) coordinating the physical examinations and blood studies for the tonsil irradiation study; and (6) coordinating the chromosome study of irradiated cervical cancer patients.

Proposed Course: Continue support for ongoing studies and newly developed studies.

Current Annual Level: $525,000

Man Years: 4.0

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04500-05 EEB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Methodologic Studies of Epidemiology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W.J. Blot	Head, Analytical Studies Section	EEB	NCI
OTHER:	J.H. Lubin	Senior Staff Fellow	EEB	NCI
	L.W. Pickle	Senior Staff Fellow	EEB	NCI
	C.E. Land	Health Statistician	EEB	NCI
	J.D. Boice, Jr.	Head, Radiation Studies Section	EEB	NCI
	E.J. Martin	Cancer Expert	EEB	NCI
	A.F. Kantor	Staff Fellow	EEB	NCI
	P. Hartge	Epidemiologist	EEB	NCI

COOPERATING UNITS (if any)

Biometry Branch, Clinical Epidemiology Branch, NCI; University of Washington

LAB/BRANCH
Environmental Epidemiology Branch

SECTION
Analytical Studies Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objectives of this project are to develop, adapt, expand, and evaluate methodological procedures useful in epidemiologic studies of cancer. Methods of design and analysis of cohort studies were given particular emphasis, with attention focusing on procedures for analysis of standardized mortality ratios, including regression methods and tests of equality and trend. Also considered were the adaptation and evaluation of multivariate epidemiologic models for analysis of case-control data. Other work focused on evaluating surrogate responses and assessing quality in case-control interview studies. Investigation of general epidemiologic techniques for studying environmental cancer continued, including the second edition of a text on biostatistical methods and review of methods to evaluate the magnitude of low-dose radiation risks.

Project Description

Objectives: To develop, adapt, expand, and evaluate methodological procedures useful in epidemiologic studies of cancer.

Methods Employed: Basic research is undertaken on statistical techniques which are useful in a variety of epidemiologic settings. Computational algorithms are developed as necessary, and the methods are applied to epidemiologic data generated and collected by investigators in the Environmental Epidemiology Branch and elsewhere.

Major Findings: Several Branch members contributed to the adaptation and development of statistical methods useful in epidemiologic studies. A widely used general text which features a library of programs for epidemiologic analysis using a programmable calculator was updated and expanded into a 2nd edition.

Research continued on methods of design and analysis of case-control data. A new method based on a recursion formula was developed which allows rapid computation of exact conditional likelihood estimates of the relative risk, extending the applicability of this useful technique. The extent to which unconditional logistic analyses overestimate odds ratios from matched data sets was examined in a simulation study. One report described the features of design and methods of execution that helped to reduce bias and assure quality control in a large case-control study. A review of a new text on analytical methods for case-control studies was prepared.

Several reports expanded methodology for use in occupational and other cohort studies. One report demonstrated a bias with the serially additive expected dose (SAED) method for assessing occupational exposure risks and proposed a correcting modification. Other reports presented new tests for equality of, and trends in, standardized mortality ratios (SMRs). A detailed method of regression analysis for SMRs was presented and linked to a Cox analysis for both cohort and case-control studies, thus unifying the various analytic techniques. Guidelines for sampling a case-control study from a cohort study suggested that a matching ratio of 10 or 20 to 1 is needed for risk estimation. Methodologic issues were also explored with respect to descriptive and correlational studies, especially using the county-by-county mortality resource.

Several methodologic issues related to the study of radiation-induced cancers were reported. Epidemiologic studies into the effects of low-dose radiation were reviewed, while the necessity of undertaking studies in high-dose rather than low-dose populations was stressed. Parametric families for dose-response curves were used to incorporate information on aspects of exposure other than dose, such as radiation quality and protraction and fractionation. Bayesian procedures were explored to incorporate uncertainties of assumptions and variability in experimental data in assessing human dose-response data.

Work has continued on the development of, and accessibility to, the Branch computer program library. SAS pre-programmed subroutines to estimate and test

relative risks and to facilitate conditional regression modeling with matched case-control data were expanded.

A guide to sample size requirements for use in familial studies of cancer was prepared. Data from several Branch case-control interview studies were combined in order to assess the comparability of information from surrogate respondents. Sibs were best able to respond to questions about the subject family or about events that occurred early in life, while spouses were best able to describe events that occurred during adult life.

A procedure for calculating HLA phenotype frequencies from two and three locus frequencies was described and formulas derived.

Significance to Biomedical Research and the Program of the Institute: Research in statistical methodology will help provide means for adequate analyses of the epidemiologic studies carried on by members of the Branch, as well as by epidemiologists in other institutions.

Proposed Course: Methods development and adaptation will continue, with particular emphasis on techniques applicable to the Branch's analytical epidemiologic studies program.

Publications:

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Blot, W.J. and Fraumeni, J.F., Jr.: Geographic Epidemiology of Cancer in the United States. In Schottenfeld, D. and Fraumeni, J.F., Jr. (Eds.): Cancer Epidemiology and Prevention. Philadelphia, W.B. Saunders, 1982, pp. 179-193.

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Boice, J.D., Jr., and Day, N.E.: Second Cancer in Relation to Radiation Treatment for Cervical Cancer: Result of a Cancer Registry Collaboration. IARC Monographs. Lyon, France, International Agency for Research on Cancer. In press.

Breslow, N.E., Lubin, J.H., and Marek, P.: Models and the analysis of cohort data. J. Am. Statist. Assoc. In press.

Gail, M.H., Lubin, J.H., and Rubinstein, L.V.: Likelihood calculations for matched case-control studies and survival studies with tried death times. Biometrika 68: 703-707, 1981.

Hartge, P., Cahill, J.I., West, D., Hauck, M., Austin, D., Hoover, R., and Silverman, D.: Design and methods to assess and assure quality in a large case-control interview study. Am. J. Epidemiol. In press.

Henderson, D.J., and Blattner, W.A.: A Macro for Two Population Non-Parametric Univariate Discriminant Analysis with Extensions to Higher

Dimensional Spaces. In Proceedings of the SAS Users Group International Sixth Annual Conference. Cary, North Carolina, SAS Institute, Inc. In press.

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Land, C.E.: Low Levels of Ionizing Radiation and Cancer - Are We Understanding the Risk? (Contributions to the discussion of a paper by S.C. Darby and R.A. Reissland). J. Royal Statist. Soc. A. 114: 326-327, 1981.

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Pickle, L.W. and Mulvihill, J.J.: An example of sample size estimation for family studies. Am. J. Epidemiol. 114: 299-303, 1981.

Rothman, K.J., Boice, J.D., Jr. and Austin, H.: Epidemiologic Analysis with a Programmable Calculator. Chestnut Hill, Massachusetts, Epidemiologic Resources, Inc. In press.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04501-05 EEB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Case-Control Studies of Selected Cancer Sites

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R.N. Hoover	Head, Environmental Studies Section	EEB NCI
OTHER:	J.F. Fraumeni, Jr.	Chief, Environmental Epidemiology Branch	EEB NCI
	W.A. Blattner	Head, Family Studies Section	EEB NCI
	P. Hartge	Epidemiologist	EEB NCI
	L.A. Brinton	Staff Fellow	EEB NCI
	T.J. Mason	Head, Population Studies Section	EEB NCI
	K.P. Cantor	Epidemiologist	EEB NCI
	L.M. Pottern	Epidemiologist	EEB NCI
	W.J. Blot	Head, Analytical Studies Section	EEB NCI
	A.F. Kantor	Staff Fellow	EEB NCI
	S.K. Hoar	Staff Fellow	EEB NCI

COOPERATING UNITS (if any) Dept. of Health of the State of New Jersey; Biometry Branch, NCI; Division of Cancer Control & Rehabilitation; Medicine Branch, NCI; 28 Breast Cancer Detection & Demonstration Project Centers; 10 SEER Centers in Continental U.S.; University of Oxford and Birmingham University, England

LAB/BRANCH
Environmental Epidemiology Branch

SECTION
Environmental Studies Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
10.0	6.0	4.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords) The purpose of this project is to utilize the case-control interview methodology to do in-depth evaluations of specific cancer sites. By the nature of this method, the evaluations include many of the exposure categories under numerous etiologic hypotheses to be tested, or because a tumor may require in-depth evaluation in an attempt to generate hypotheses, these types of studies cover a number of different exposure categories. Fifteen separate studies are in various phases and include evaluations of breast cancer, bladder cancer, melanoma, cervical cancer, nasal cancer, mycosis fungoides, and various childhood malignancies. Exposures being assessed include occupation, diet, smoking, reproductive and sexual characteristics, drugs, personal habits, and various host factors.

Project Description

Objectives: (1) To identify tumor sites for which there are a number of unusual demographic laboratory or clinical associations indicating the necessity to evaluate a broad range of potential exposures. (2) To identify populations in which these in-depth case-control evaluations can be most efficiently carried out. (3) To design, conduct, and analyze these intensive case-control studies.

Methods Employed: During this year the project has included 15 studies using the case-control method: 2 of breast cancer, 3 of bladder cancer, 1 of non-Hodgkin's lymphoma, 1 of mycosis fungoides, 1 of childhood bladder cancer, 1 of intraocular melanoma, 1 of multiple myeloma, 1 of cervical cancer, 1 of kidney cancer, 1 of testicular cancer, 1 of ovarian cancer, and 1 of nasal cancer.

1. Breast cancer patients (1,554) identified by the Breast Cancer Detection and Demonstration Project (BCDDP), women with benign breast disease (1,574), and normal screenees (1,391) were interviewed in their homes to collect information about risk factors for breast cancer and use of oral contraceptives, other exogenous estrogens, antihypertensive agents, thyroid medications, and major tranquilizers. Histological and clinical data were collected from BCDDP records. Analyses of these data are currently under way.

2. A continuation of the breast cancer study noted above is being conducted. Breast cancer patients, approximately 2,500, identified by the BCDDP, equal numbers of women with benign breast disease, and women with no breast disease will be interviewed at home to collect information about a range of potential risk factors. Histological and clinical data will be collected from BCDDP records.

3. All bladder cancer patients (4,000) who were diagnosed in 1978 in five states and five metropolitan areas were identified, and controls (7,000) were drawn from the general population of the 10 geographic areas. Subjects were interviewed in their homes to collect data about saccharin use, smoking habits, occupational history, sources of drinking water, hair dye use, coffee-drinking, and medical history. Histological data were collected from pathology reports. Analyses of these data are currently under way. Reports from six such analyses have been published or submitted for publication.

4. All bladder cancer patients (150) who were diagnosed in 1979 in greater Atlanta, and controls (150) from the general population, have been interviewed, following the protocol described in 3. The additional cases and controls will permit assessment of exposures stemming from work with textiles and dyes. Such analyses are currently under way.

5. Male residents of 3 Georgia counties (56) who were diagnosed with kidney cancer from 1975 to 1978 were interviewed using an abbreviated version of the questionnaire described in 3. Exposures to be analyzed include occupational history, drinking water sources, use of tobacco, artificial

sweeteners, coffee and pain relievers, and history of diabetes and urinary problems. Matched controls will be selected from the population-based controls interviewed during 1978 and 1979 as part of the bladder cancer study.

6. According to mortality rates for 1950-1969, among the 48 contiguous states, New Hampshire has the second highest bladder cancer mortality rates for both white men and women. Vermont has similar high rates, especially for white women. A case-control study of bladder cancer is being conducted in this area to look for environmental associations in both sexes. Project personnel identified 364 New Hampshire and Vermont residents who died from bladder cancer and 756 residents who died from other causes during 1975-1979. Successful in-person interviews with the next-of-kin of 89 percent of the study subjects were obtained. The interview consisted of questions about occupation, residence, smoking habits, and other aspects of lifestyle. The questionnaires and the death certificate abstracts have been coded and are currently being edited via range and logic checks. Information has also been collected describing the type and location of business establishments in the leather, textile, and paper and pulp industries during the past forty years.

7. A case-control study of cutaneous T-cell lymphomas (CTCL) is under way in a series of 300 patients who are being treated for CTCL at the Skin and Cancer Hospital of Temple University in Philadelphia, Pennsylvania. The study has been designed to determine whether there is an association between CTCL and several variables possibly related to its etiology, many of which have in common exposures of the host to chronic antigenic stimulation. The influence of environmental agents as carcinogens will also be explored.

8. Data from a study of non-Hodgkin's lymphoma patients treated at the NIH Clinical Center and their sibling controls are currently being analyzed. The study consists of complete information on 91 cases and 121 controls. Risks for NHL by radiation exposure, occupational exposure, and past drug usage are now being evaluated.

9. Testicular cancer patients treated at the NIH Clinical Center, Walter Reed Army Hospital, and Bethesda Naval Medical Center, and controls treated in those hospitals for other cancers, have been interviewed in the hospital or by telephone to collect information about their occupational and environmental exposures, medical history with emphasis on genital tract abnormalities, family history, and lifestyle. Testicular cancer cases and controls were interviewed. Mothers of subjects were also interviewed by telephone, and their medical records abstracted to obtain data on subjects' prenatal and early childhood exposures to drugs, hormones, and radiation. Analyses of the data are just beginning.

10. Ovarian cancer patients (350) diagnosed between 1978 and June, 1981 in 25 Washington, D.C. area hospitals, and women hospitalized for other conditions (350), are being interviewed in their homes to collect

information about medical, family, reproductive and menstrual histories, use of exogenous estrogens, contraception, occupation, and smoking. Pathologic slides have been reviewed and questionnaires mailed to subjects' physicians to collect additional data. Data are now being coded and edited.

11. A case-control study of intraocular malignant melanoma was undertaken in collaboration with Wills Eye Hospital in Philadelphia. Data collection for the study has been completed. A total of 1,465 medical records was abstracted and 1,285 telephone interviews were completed. Analysis is under way.

12. A case-control study of invasive and in situ cervical cancer is being conducted in conjunction with five of the Comprehensive Cancer Centers whose rates of these diseases is excessively high. Home interviews are being conducted with approximately 500 patients with invasive disease, 500 with in situ cancer, and with 1,000 population controls, matched to the invasive cases on race, age, and geographic area and identified through random digit dialing techniques. Interviews focus on reproductive and menstrual history, sexual behavior, medical events, contraceptive usage, smoking and alcohol use, diet, and family history of cancer.

13. The Environmental Epidemiology Branch is conducting a case-control study of childhood bladder cancer in cooperation with investigators participating in the SEER Program. The study has been designed to determine whether childhood bladder cancer is associated with pre- or post-natal exposures to known or suspected bladder carcinogens such as artificial sweeteners and cigarette smoking. Interviewing is just about completed, and analysis of the data should begin soon.

14. A case-control study of nasal cancer is nearing completion in a series of 200 patients diagnosed at four hospitals in North Carolina and Virginia. Telephone interviews obtained from these patients or their next-of-kin and a series of 400 hospital controls, as well as death certificate controls, have focused on occupational exposures, residential history, medical history, and smoking and alcohol usage.

15. A review was conducted of risk factors for in situ and invasive cervical cancer.

16. A study of benign breast diseases was conducted in conjunction with epidemiologists at Oxford University, in order to assess similarities in patterns of risk for benign breast disease and breast cancer.

Major Findings:

1. The National Bladder Cancer Study has been under analysis in the past year. The issues being addressed include further evaluations of risks associated with artificial sweetener use, coffee drinking, hair dye use, work in the chemical and leather industries, cigarette smoking, drinking

water quality, urinary tract infection, and an in-depth analysis of possible occupational explanations for the high bladder cancer rates in Detroit. The preliminary findings have shown no obvious association of risk with use of hair dyes, a diminished risk associated with never having drunk coffee (but no dose-response relationship among coffee drinkers), and a two-fold risk associated with multiple urinary tract infections.

2. By nature of its large size, the breast cancer case-control study allowed evaluation of the interaction of familial and hormonal risk factors. A family history of breast cancer was associated with a two-fold excess risk, with greater elevations in risk among younger subjects and in those reporting both an affected mother and sister. The effect of family history on breast cancer risk was modified by age at menarche, but not by age at first birth or type of menopause. These findings suggest that familial susceptibility to breast cancer may be mediated through hormonal factors that operate early in a woman's life. In addition, a synergistic relationship was observed between family history and the occurrence of benign breast biopsies, a relationship that merits further investigation.

Significance to Biomedical Research and the Program of the Institute: The case-control methodology provides a rapid, relatively inexpensive, yet scientifically rapid way of assessing the relationship between a disease and a wide variety of potential causes of that disease (occupational, general environmental, lifestyle, genetic, etc.). This method is the usual one first employed by epidemiologists to test hypotheses that have come from clinical observations, laboratory experiments, or descriptive epidemiologic efforts. Because of the speed with which these studies can be performed, and the wide variety of potential causes that can be assessed simultaneously, these studies often provide the first sound scientific evidence of a preventable cause of malignancy. The evidence can then be acted upon through educational programs or regulatory actions. As such, this type of work is a key element in identifying preventable causes of malignancy in humans.

Proposed Course:

1. The second study of breast cancer in the BCDDP, and the study of invasive and in situ cancer of the uterine cervix, will both continue in the phase of data collection during the next year.

2. The first breast cancer study in the Breast Cancer Detection Demonstration Project, the bladder and kidney cancer studies in Atlanta, the non-Hodgkin's lymphoma study, the intraocular melanoma study, and the studies of testicular cancer, mycosis fungoides, bladder cancer in children, bladder cancer in New England, ovarian cancer, and nasal cancer have all had completed data collection and will undergo analyses during the next year.

3. Evaluations of a number of tumor sites will be conducted to identify those tumors for which intensive case-control studies would be the most

appropriate next step in evaluating potential etiologic hypotheses. Avenues for appropriately achieving these case-control evaluations will be explored. Particular attention will be paid to such opportunities for childhood cancers and for bile duct carcinomas.

4. The National Bladder Cancer Study will continue under analysis to assess exposures other than artificial sweeteners, with particular emphasis on tobacco use, drinking water, and occupational risk factors.

Publications:

Brinton, L.A.: Etiologic factors for cervical cancer. In Gold, E. (Ed): The Changing Risk of Disease in Women. Lexington, MA, D.C. Heath and Co. In press.

Brinton, L.A., Hoover, R.N., and Fraumeni, J.F., Jr.: Interaction of familial and hormonal factors for breast cancer. JNCI. In press.

Cantor, K.P.: Case-control studies in risk assessment. In Proceedings of the ORNL Life Science Symposium on Health Risk Analysis. Philadelphia, Franklin Institute Press. In press.

Cantor, K.P., Kopfler, F.C., Hoover, R.N., and Strasser, P.H.: Cancer epidemiology as related to chemicals in drinking water. Toxicol. Environ. Chem. Rev. In press.

Hoover, R.N., and Hartge, P.: Non-nutritive sweeteners and bladder cancer. Am. J. Public Health. 72: 382-383, 1982.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 04779-06 EEB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Field Studies in High Risk Areas

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

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OTHER:	J.F. Fraumeni, Jr.	Chief, Environmental Epidemiology Branch	EEB NCI
	R. Hoover	Head, Environmental Studies Section	EEB NCI
	T.J. Mason	Head, Population Studies Section	EEB NCI
	B.J. Stone	Mathematician	EEB NCI
	L. Pickle	Senior Staff Fellow	EEB NCI
	L. Brinton	Research Epidemiologist	EEB NCI
	L. Pottern	Epidemiologist	EEB NCI
	L. Brown	Epidemiologist	EEB NCI
	J. Lubin	Senior Staff Fellow	EEB NCI
	R. Ziegler	Cancer Expert	EEB NCI
	M. Greene	Clinical Investigator	EEB NCI

COOPERATING UNITS (if any) Cross Cancer Institute, Lehigh Univ., Louisiana State Univ., New Jersey State Department of Health, Univ. of Miami, Univ. of Minnesota, Univ. of Texas, Univ. of North Carolina

LAB/BRANCH
Environmental Epidemiology Branch

SECTION
Analytical Studies Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
7.5	6.5	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The objectives of this project are to identify and describe environmental and host determinants of cancer in areas of the U.S. at high risk of cancer through the use of analytical epidemiologic techniques, particularly case-control studies of specific cancers. Completed during the year were interview studies of (a) breast cancer in Alberta, Canada, which revealed higher risks among women with diets high in intake of beef and pork, and found that patterns for other risk factors often differed between pre- and post-menopausal women; (b) oral cancer among women in North Carolina, which did not confirm a previously reported link to textile manufacturing, but showed an association with mouthwash use limited to abstainers from tobacco and alcohol; (c) lung cancer in Pennsylvania, which found increased risks among long-term steel workers, suggesting that previous estimates of lung cancer among workers may have been underestimated; and (d) renal pelvis cancer in Minnesota showing a strong, causal association with cigarette smoking.

Project Description

Objectives: To identify and describe the environmental determinants of cancer in areas of the United States where cancer rates are high.

Methods Employed: Field studies are conducted in areas of the country where cancer rates are high and etiologic hypotheses can be tested. The studies are generally case-control investigations whereby cancer patients and controls, or their next-of-kin in the event they had died, are interviewed regarding lifetime histories of residence, occupation, tobacco consumption, diet, and medical or other factors. Comparison of responses between the cases and controls are then made by analytical epidemiologic techniques to identify, estimate, and evaluate cancer risk factors. When a particular suspect environmental or occupational exposure among a well-defined population group is recognized, cohort investigations may be initiated to determine the group's cancer experience. Often both the case-control interview and the cohort studies are preceded by reviews of appropriate death certificates and medical records for cancer cases and controls, for comparisons of available information.

Major Findings: A series of case-control investigations is ongoing in areas of the U.S. where mortality rates for particular tumors are high. A major effort continued to evaluate risk factors for lung cancer, the leading cause of cancer death among men in the United States. Previous Branch investigations of lung cancer in coastal areas of Georgia and Florida, and lung cancer and mesothelioma in the coastal Tidewater area of Virginia, found significantly increased risks associated with employment in the shipbuilding industry, particularly during World War II. A summary analysis combining data from more than 2,500 interviews in these surveys placed the relative risk, adjusted for smoking, for employment in the industry in the 1940's at about 1.4, suggesting that as many as 100,000 extra lung cancer deaths may eventually result among the cohort of some 4.5 million Americans who worked in ship construction and repair during World War II.

Also completed during the year was a case-control study of lung cancer in eastern Pennsylvania. A significantly increased risk was found among men who had worked in the steel industry, the area's major employer. The excess was primarily among long-term employees, particularly those who began work before 1935, but was not confined to a single trade within the industry. Adjusted for cigarette smoking, the relative risk associated with career steel employment was 1.8 (95 percent confidence limits 1.2 to 2.8). No significant associations were found for other industries, although a 60 percent increase was noted for zinc smelter workers employed at least 15 years. The findings implicate occupation as a cause of cancer in the area, and suggest that exposure within the steel industry may contribute to an extent greater than that previously recognized.

A study of respiratory cancer continued in collaboration with the University of Texas School of Public Health to evaluate the high risk of this cancer among both sexes in coastal Texas, and interviewing continued to obtain detailed information on characteristics of cancer patients in Louisiana, as

part of a collaborative (with the EPA and Louisiana State University) case-control interview study in Louisiana for lung, pancreatic, and stomach cancers. Lung and bladder tumors were also studied in a broad-based epidemiologic study in New Jersey in collaboration with the State Department of Health. Bladder cancer is also the focus of a study begun in rural New England to evaluate the unusually high rates in both sexes in this area of the country.

A correlation study previously published by the Branch revealed that nasal cancer mortality was high in counties with furniture manufacturing industries. Subsequent examinations of death certificates from North Carolina, where the industry is most heavily concentrated, showed a 4-fold excess of this tumor associated with individuals for whom furniture manufacturing was listed on the certificate as the usual occupation. Although nasal cancer is rare and the number of cases small and spread over a fairly wide geographic area, interviews (by telephone) of cases diagnosed in Virginia and North Carolina over the past ten years were conducted to further quantify risk factors for this cancer.

Reported during the year were results from a study of esophageal cancer in Washington, D.C. Esophageal cancer is relatively rare among whites, yet this tumor accounts for more deaths than any, except lung and prostate cancers, among black men in Washington. The study showed alcohol consumption to be the strongest risk factor, with the risk rising to about eight-fold among heavy consumers (one pint or more per day). Cigarette smoking, after controlling for alcohol consumption, was linked to esophageal cancer in this series, but the association was much weaker (relative risk = 1.5). One of the more interesting findings related to nutrition, with decreased intake of fruits and vegetables, fresh meats, and dairy products and eggs among the cases.

Analyses of occupational data from a case-control study of cancer of the mouth and throat among women in North Carolina showed no evidence of an increased risk associated with employment in the textile industry, a primary employer of women and an industry previously implicated in oral and pharyngeal cancer risk. There was an increased risk associated with electronics manufacture, but the numbers of individuals involved were small. Use of mouthwash was implicated as an oral cancer risk factor, but only among abstainers from alcohol and tobacco, a finding consistent with reports from two other surveys of this cancer.

Renal cancer mortality and incidence rates are high in the north central part of the U.S. An interview study involving 590 cases of renal cancer and 1,180 controls was conducted in collaboration with the University of Minnesota. The response rate was remarkable, interviews being completed with over 97 percent of those contacted. Analyses under way are focusing on diet and beverage intake, smoking, occupation, and ethnic background among other characteristics. Initial results show smoking to be the major risk factor for cancer of the renal pelvis, with greater than 5-fold excesses among smokers of both sexes, and to account for about a 50 percent increased risk of renal adenocarcinoma. A systematic review of all cancer death certificates in Minnesota for 1970-76 revealed that a high proportion were of German origin,

suggesting an ethnic contribution to the area's high rates. HLA antigen typing from patients under age 45, patients with bilateral tumors, and patients with a family history of renal cancer showed some unusual patterns which seem, at least in part, to reflect ethnic determinants.

Anaylses were completed of data from a case-control study of colorectal cancer in areas of rural Nebraska, where mortality rates were high. Elevated risks were found among persons of Czechoslovakian descent, among whom diets high in fat intake were implicated.

Working with the Centers for Disease Control's Bureau of Alaskan activities, Branch members conducted studies to uncover reasons for the high rates of certain tumors in Alaskan natives. Updated incidence statistics prepared during the year showed that rates for nasopharyngeal cancer continued to be high. Field studies are focusing on familial aggregations of this cancer, as well as on the laboratory testing of native foods in high-risk villages for mutagens, aflatoxins, and nitrosamines.

Staff members of the Branch collaborated with the W.W. Cross Cancer Institute in a large case-control study of breast cancer in Edmonton, Canada, in the province of Alberta, where rates for breast cancer are among the highest in the world. The study is unique in that nearly all cases throughout the province are interviewed by the Institute. With the cooperation of the Alberta Health Care Insurance Commission, population-based controls were identified and nearly 1,000 interviews of adult female residents were conducted. Reported during the year were contrasts in risk factors according to age when breast cancer was diagnosed. Differences were occasionally striking, e.g., among women over age 45, combined late age at first birth and low parity were associated with a 7-fold increased risk while no excess was seen with these factors among women below this age. A link to diet was also seen, with risks higher among women with higher intakes of beef and pork.

Significance to Biomedical Research and the Program of the Institite: These studies allow the testing of hypotheses regarding the etiology of cancer in the United States. Answers obtained may lead to the recognition of cancer hazards, and may directly suggest actions that need to be taken to prevent the exceptional rates of cancer occurrence in the high-risk areas of the country.

Proposed Course: Field studies in areas where cancer rates are high will continue. Analysis of the information collected in North Carolina, Virginia, and Minnesota will be completed in the coming year, with interviews continuing in Louisiana, Texas, and rural New England. Results from these ongoing studies will help suggest where further epidemiologic research will be worthwhile.

A new study of colorectal cancer may be started in retirement areas of Florida, focusing on identifying reasons for the unusually low mortality of this cancer among males and females, pending results of a preliminary telephone survey conducted during the year to evaluate the possibility of selective migration factors. Case-control studies of rare cancers in several

urban areas of the country are also planned. Also beginning is an interview study of esophageal cancer among blacks in southeast Atlantic coastal areas, particularly Charleston, South Carolina, where rates have been historically high. Under consideration are epidemiologic studies in collaboration with the Cancer Institute of the Peoples's Republic of China to identify causes for the exceptional incidence of esophageal tumors in certain rural areas, including the initiation of a population-based nutrition intervention trial to evaluate the potential protective effects of carotene, ascorbic acid, and riboflavin supplements.

Publications:

Blot, W.J., Davies, J.E., Morris, L.E., Nordwall, C.W.,. Buiatti, E., Ng, A., and Fraumeni, J.F., Jr.: Occupation and the high risk of lung cancer in northeast Florida. Cancer. In press.

Blot, W.J., and Fraumeni, J.F., Jr.: Cancer among Shipyard Workers. In Banbury Report 9. Quantification of Occupational Cancer. New York, Cold Spring Harbor Laboratory, 1981, pp. 37-50.

Blot, W.J., and Fraumeni J.F., Jr.: Changing patterns of lung cancer in the United States. Amer. J. Epidemiol. In press.

Blot, W.J., and Fraumeni, J.F., Jr.: Geographic Epidemiology of Cancer in the United States. In Schottenfeld, D. and Fraumeni, J.F., Jr. (Eds.): Cancer Epidemiology and Prevention. Philadelphia, W.B. Saunders, Co., 1982, pp. 179-193.

Blot, W.J., and Fraumeni, J.F., Jr.: Lung cancer following employment in the steel and zinc smelting industries. Amer. J. Epidemiol. In press.

Blot, W.J., Winn, D.M., and Fraumeni, J.F., Jr.: Oral cancer and mouthwash. JNCI. In press.

Kantor, A.F., McLaughlin J.K., Blattner, W.A., Bach, F.H., Blot, W.J., Schuman, L.M., and Fraumeni, J.F., Jr.: HLA antigens in renal cell cancer. Int. J. Cancer. In press.

Lanier, A.P., Bender, T.R., Blot, W.J., and Fraumeni, J.F., Jr.: Cancer in Alaskan natives 1974-78. JNCI. In press.

Lubin, J.H., Blot, W.J., Burns, P.E., Ziegler, R.G., Lees, A.W., and Fraumeni, J.F., Jr.: Dietary factors and breast cancer risk. Int. J. Cancer 28: 685-689, 1981.

Lubin, J.H., Burns, P.E., Blot, W.J., Lees, A.W., May, C., Morris, L.E., and Fraumeni, J.F., Jr.: Risk factors for breast cancer in northern Alberta as related to age at diagnosis. JNCI 68: 211-217, 1982.

McLaughlin, J.K., Blot, W.J., Mandel, J.S., Schuman, L.M., Mehl, E., and Fraumeni, J.F., Jr.: Cancer of the renal pelvis: cigarette smoking and other risk factors. Lancet. In press.

Pickle, L.W., Greene, M.G., Ziegler, R.G., Toledo, A., Hoover, R., Lynch, H.T., and Fraumeni, J.F., Jr.: A case-control study of colorectal cancer in rural Nebraska. JNCI. In press.

Pottern, L.M., Morris, L.E., Blot, W.J., Ziegler, R., and Fraumeni, J.F., Jr.: Esophageal cancer among black men in Washington, D.C. I. Alcohol, tobacco, and other risk factors. JNCI 67: 777-783, 1981.

Winn, D.M., Blot, W.J., and Fraumeni, J.F., Jr.: Snuff dipping and oral cancer. N. Engl. J. Med. 305: 230-231, 1981.

Winn, D.M., Blot, W.J., Shy, C.M., and Fraumeni, J.F., Jr.: Occupation and oral cancer among women in the south. Amer. J. Ind. Med. In press.

Ziegler, R.G., Blot, W.J., Hoover, R.N., Blattner, W.A., and Fraumeni, J.F., Jr.: Protocol for a study of nutritional factors and low risk of colon cancer in southern retirement areas: A study protocol. Cancer Res. 41: 3724-3726, 1981.

Ziegler, R.G., Morris, L.E., Blot, W.J., Pottern, L.M., Hoover, R.N., and Fraumeni, J.F., Jr.: Esophageal cancer among black men in Washington, D.C. II. The role of nutrition. JNCI 67: 1199-1206, 1981.

CONTRACTS IN SUPPORT OF THIS PROJECT:

WESTAT, INC. (NCI-CP-01044)

Title: Support Services for Epidemiologic Studies.

Objective: To provide technical, managerial, and computer support for epidemiologic studies of cancer, including those in high risk areas.

Methods Employed: A management system has been established to facilitate direct communication between NCI scientific investigators and the Westat program director, project managers, computer specialists, and field supervisors so that all phases (study design, forms preparation, conduct, quality control, and analysis) of field studies can progress efficiently.

Major Contributions: Field studies were completed for oral cancer in North Carolina, esophageal cancer in Washington, D.C., breast cancer in Alberta, Canada, and lung cancer in Virginia and Florida. Interviewing and/or computational support were conducted for studies of renal cancer in Minnesota, bladder cancer in New England, Louisiana and Georgia, nasal cancer in North Carolina and virginia, eye cancer in Pennsylvania, and colon cancer in Florida retirement areas.

Proposed Course: Support for a variety of epidemiologic investigations will continue as needed to enable the Branch to answer critical questions about the environmental and host determinants of cancer.

Current Annual Level: $1,500,000.

Man Years: 45.0

LEHIGH UNIVERSITY (NO1-CP-81038)

Title: Support Services for Epidemiologic Studies of Lung Cancer in Communities with Non-ferrous Smelters.

Objectives: To provide interviewing, data preparation, and environmental measurement support services for a case-control study of lung cancer in eastern Pennsylvania.

Methods: An interviewing and medical records abstract team was established to carry out the field data collection phase of this case-control study and assemble information on area environmental pollutants.

Major Contributions: Eleven support services were completed.

Proposed Course: This contract was terminated upon completion of the support services.

Current Funding Level: Funding completed.

Man Years: 2.8

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 CP 05128-03 EEB

PERIOD COVERED
October 1, 1981 to September 30, 1982

TITLE OF PROJECT (80 characters or less)

Nutritional Factors in Cancer Etiology

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	R.G. Ziegler	Cancer Expert	EEB	NCI
OTHER:	A.G. Ershow	Staff Fellow	EEB	NCI
	R.N. Hoover	Head, Environmental Studies Section	EEB	NCI
	W.J. Blot	Head, Analytical Studies Section	EEB	NCI
	L.W. Pickle	Staff Fellow	EEB	NCI
	D.M. Winn	Staff Fellow	EEB	NCI
	G. Gridley	Health Statistician	EEB	NCI
	J.H. Lubin	Staff Fellow	EEB	NCI
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	A.E. Blair	Acting Head, Occupational Studies Section	EEB	NCI
	E.S. Pollack	Chief, Biometry Branch	BB	NCI
	L.E. Morris	Epidemiologist	EEB	NCI
	L.A. Brinton	Staff Fellow	EEB	NCI

COOPERATING UNITS (if any)

National Center for Health Statistics, National Institute on Aging, New Jersey Department of Health

LAB/BRANCH
Environmental Epidemiology Branch

SECTION
Environmental Studies Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	2.3	0.2

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☒ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Dietary exposures being assessed in human populations include consumption of specific food groups and food items, such as meat, fruits and vegetables, ethnic dishes, and coffee; macronutrient and micronutrient intake, such as fat, vitamin A, carotene, vitamin C, or folacin; general nutritional status; anthropometry; biochemical indices, such as serum cholesterol, serum vitamin A, or serum uric acid; and cooking practices. Cancers being studied include those of the colon, rectum, breast, esophagus, pharynx and oral cavity, lung, cervix, pancreas, stomach, and kidney. Case-control studies have been initiated in high-risk areas with unusually high mortality from cancers conceivably related to diet and among migrants whose changing cancer rates appear related to new lifestyles, such as Japanese-Americans. Selected cohorts with relevant dietary or biochemical data already collected, such as HANES I participants, are being followed-up. Data from HANES I and II and the USDA Food Consumption Survey are being analyzed to test specific hypotheses, such as the relationship of age at menarche to diet, and to provide descriptive information on U.S. dietary patterns, diet variation, and the determinants of nutrient intake.

Project Description

Objectives:

1. To assess in human populations specific hypotheses concerning the relationship of diet and cancer that have been suggested by biochemical, animal, clinical, and epidemiological studies. Such hypotheses may concern specific food groups, food items, macro or micronutrients, general nutritional status, food additives, cooking and processing practices, biochemical measures related to diet, anthropometric parameters, etc. Cancer may be initiated, promoted, or inhibited by such exposures.

2. To search for relationships between diet and specific cancers in high or low risk areas, identified by the U.S. cancer maps, where diet could conceivably be involved in generating the observed geographical patterns.

3. To search for relationships among dietary patterns, age at migration, and specific cancers in migrants whose changing cancer rates appear related to changing lifestyles.

4. To develop and utilize national nutrition data resources that might contribute to cancer epidemiology. One example is HANES I, the first Health and Nutrition Examination Study of the American people, conducted on a national sample of 23,000 people.

5. To develop methods for nutritional epidemiology, including dietary questionnaires, protocols for laboratory tests, and analytic frameworks.

6. To elucidate the basic biology of carcinogenesis through studying the influence of diet on cancer in human populations.

Methods Employed: Studies numbered 1, 2, 3, 5, 6, 7, 13, 14, and 17 represent the nutrition-related components of cancer epidemiology studies described in more detail in Project Z01 CP 04779-06 EEB, Field Studies in High Risk Areas.

1. A death certificate-based, case-control study of esophageal cancer was initiated among black male residents of Washington, D.C., the U.S. metropolitan area with the highest rate of esophageal cancer among black males. Next-of-kin of 120 black men who died of esophageal cancer in 1975-77 and of 250 black men of similar age who died in 1975-77 of other causes were interviewed. Information was collected on dietary patterns (usual adult frequency of consumption of 31 food items, prior to 1974), unusual substances eaten, cooking practices, alcohol consumption, and other relevant exposures.

2. A population-based, case-control study of breast cancer was conducted in Alberta, Canada in collaboration with Cross Cancer Institute, Edmonton. Breast cancer rates in Alberta are among the highest in the world.

Interviews were conducted with 577 breast cancer cases, aged 30-80 and diagnosed during 1975-77, and with 826 disease-free population controls. Questions were asked about the usual adult frequency of consumption of eight food items.

3. A case-control study of colorectal cancer was conducted in two rural farming counties in eastern Nebraska with unexpectedly high rates of colon cancer. Many people of Czechoslovakian ancestry live in this area; and Czechoslovakians, when compared to other immigrants to the U.S., show elevated rates of colon cancer. Since their traditional ethnic diet might be involved, emphasis was placed on assessing diet. Interviews were completed for 58 colon cancer cases and 28 rectal cancer cases, all diagnosed during 1970-77, and for 176 hospital controls of similar age, sex, county of residence, and place and year of hospitalization. Next-of-kin constituted 56 percent of the interviews. Information was collected on dietary patterns (usual adult frequency of consumption of 57 food items, including ethnic foods), cooking and preserving practices, wine and beer consumption, and other relevant exposures.

4. A death certificate-based, case-control study of colorectal cancer was initiated in the three regions of Florida with high rates of immigration from the Northeast and North Central states. The U.S. cancer maps showed that colorectal cancer mortality rates for white men and women were lower in the South, by about 50 percent, than in the Northeast or North Central states. This regional gradient in risk could not be explained by differences in income or population density between North and South. Close examination of the age-specific cancer mortality rates for those counties in Florida to which many Northerners move at retirement revealed that in these counties colorectal cancer rates were as low as in southern counties of comparable population and did not rise toward the northern rates at the older retirement ages.

   This preliminary study will define the characteristics of this apparent reduction in colorectal cancer risk on migration, quantify it, and determine whether it might be due to some change in lifestyle, possibly diet or drinking water, or whether it might be due to the migrants being a self-selected healthy subset of Northerners. The case series, selected from the 1979 Florida mortality tape, consisted of 1,160 white colon cancer deaths and 205 white rectal cancer deaths, 25-79 years of age, whose usual place of residence was one of the eleven Florida counties with heavy inmigration. The two control series, also drawn from the 1979 Florida mortality tape, consisted of 1,021 controls who died of a cancer other than colon, rectum, or breast and 700 controls who died of a cause other than cancer. Both control series were frequency-matched to the case series on age, sex, and usual county of residence. Questions focused on residential history; medical history; social, economic, and demographic characteristics; and a few indicators of general dietary patterns (usual adult frequency of consumption of 11 food items, before and after migration to Florida).

5. A case-control study of oral and pharyngeal cancer was conducted among women in central North Carolina since the U.S. cancer maps revealed excess mortality from these two cancers among white women in the Southeast U.S. Interviews were completed for 232 cases, who were diagnosed or died in 1975-78, and 410 controls individually matched on age, race, county of residence, and source of ascertainment. Next-of-kin provided 60 percent of the interviews. Information was collected on dietary patterns (usual adult frequency of consumption of 22 food items), unusual foods eaten, methods of cooking, and alcohol consumption.

6. A population-based, case-control study of lung cancer, with a dietary component, was initiated in collaboration with the New Jersey State Department of Health in those areas of New Jersey showing unusually high lung cancer mortality rates on the U.S. cancer maps. Since many animal and cell culture studies have indicated that pharmacologic doses of retinoids can protect against development of cancer, vitamin A has been postulated to reduce cancer risk. The few relevant epidemiological studies of dietary patterns and cancer have suggested a broader association: fruit and vegetable intake in general was elevated among those at less risk. The dietary component of this study was designed to assess whether vitamin A activity, retinol, carotene, vitamin C, or all fruits and vegetables in general is associated with reduced risk of lung cancer. The study will also evaluate the interaction among smoking, occupational exposure, pollution, and diet, in order to evaluate the potential of diet to alter risk at a later stage of tumorigenesis. Usual adult frequency of consumption, prior to 1975, of 44 food items and a history of vitamin pill usage were collected. Approximately 900 lung cancer cases diagnosed in 1980-81 and 900 population controls of comparable age, sex, race, and residence were selected; approximately 40 percent of the interviews were with next-of-kin.

7. A parallel case-control study of lung cancer was initiated in collaboration with the University of Texas School of Public Health in those Gulf Coast areas of Texas showing unusually high lung cancer mortality rates on the U.S. cancer maps. Approximately 1,050 lung cancer cases diagnosed in 1976-82 and 1,050 population controls of comparable age, sex, race, and residence, as well as 250 laryngeal cancer cases and 250 similarly matched population controls, were selected. Approximately 33 percent of the interviews that could be conducted on the subjects themselves included questions on the usual frequency of consumption, four years earlier, of 37 food items and usual vitamin pill usage.

8. HANES I, the Health and Nutrition Examination Survey, conducted in 1971-74 by the National Center for Health Statistics, collected dietary, biochemical, clinical, and anthropometric information on the nutritional status of a national sample of the U.S. population comprising 23,000 persons. With data from HANES I, regional differences in vitamin A and vitamin C and fruit and vegetable intake are being assessed to see whether such differences could explain the North-South gradient in colon, rectal, and breast cancer mortality noted in the U.S. cancer maps. For these three cancers mortality rates were higher in Northeast and North Central states than in the South, and the difference could not be explained by socioeconomic status or population

density. Intake of vitamin A, retinol, carotene, and vitamin C based on 24-hour recalls; intake of vitamins A and C based on food frequencies; frequency of fruit and vegetable consumption; and serum vitamin A levels are being compared, after adjustment for sex, race, and age, with analysis of variance and regression techniques.

9. With the serum vitamin A data collected in HANES I on a national sample of 14,000 adults, possible determinants of serum vitamin A levels are being evaluated: sex, race, age, poverty status, pregnancy-lactation status, region, diet and individual variation. Regression and analysis of variance techniques are being used. Since several prospective studies have recently shown that mean serum vitamin A levels were lower, prior to disease, among those that eventually developed cancer, the determinants of serum vitamin A levels are of interest. A specific hypothesis being tested is whether within a population as well fed as that in the U.S., serum vitamin A levels are not significantly affected by vitamin A intake since intake is generally more than adequate.

10. With the HANES I dietary and anthropometric data collected for approximately 100 women between the ages of 12 and 18, various food groups, macronutrients, and body measurements are being evaluated as predictors of age at menarche. In international comparisons, age at maturation and age at menarche are inversely correlated with risk of breast cancer and may be indicators of the dietary patterns that promote this disease.

11. Using the HANES I 24-hour recalls, individual food items are being ranked by their contribution to total vitamin A intake for various age-sex-race-region subpopulations. This information will help to focus the dietary interviews being developed to measure usual vitamin A intake and assess its relationship to cancer risk.

12. In 1982-84 the Environmental Epidemiology Branch, in cooperation with the National Institute on Aging, several other Institutes, and the National Center for Health Statistics, will trace and re-interview, if still living, 14,000 adults examined in HANES I 8 to 13 years earlier. By collecting intervening cancer morbidity and mortality for this cohort, associations between dietary patterns prior to disease and the common cancers can be determined. Once these people are traced in 1982-84, further cancer mortality can be followed with the National Death Index. Questions specifically designed to estimate intake of those food groups and nutrients believed to be associated with cancer risk were incorporated into the re-interview to supplement the dietary data collected in 1970-74.

13. A population-based, case-control study of kidney cancer, categorized as renal cell or renal pelvis, was conducted in the Minneapolis-St. Paul SMSA because of the extremely high kidney cancer mortality rates in Minnesota. Approximately 600 cases and 1,200 controls were selected; half of the interviews involved next-of-kin. Information collected included dietary patterns (usual adult frequency of consumption of 30 food items, five years earlier), cooking practices, alcohol consumption, and an extensive beverage history.

14. A case-control study of lung, pancreas, and stomach cancer was initiated in 1979 in southern Louisiana in collaboration with Louisiana State University because of the relatively high mortality rates for these three cancers in this region. The study sample consists of approximately 1,200 lung, 300 stomach, and 275 pancreatic cancer patients and an equal number of hospital controls, individually matched by age, sex, race, parish of residence, and hospital. Next-of-kin are providing approximately 15 percent of the interviews. Information is being collected on dietary patterns (usual adult frequency of consumption of 57 food items, prior to disease), food preparation and storage practices, beverages consumed, spices used, source of drinking water, and alcohol consumption. Ascertainment of stomach and pancreatic cancer cases will continue through 1982; the data on lung cancer are now being analyzed.

15. A case-control study of breast cancer was carried out in Israel by the Department of Clinical Epidemiology, Chaim Sheba Medical Center. The study was undertaken to investigate the relationships between dietary patterns and breast cancer, with an emphasis on variations in risk attributable to ethnic background. International comparisons show that breast cancer rates are correlated with availability of fat and meat, and are higher in Western Europeans and Americans than in other nationalities. The case group consisted of 818 women, newly diagnosed between 1975-79 and examined at one of nine hospitals in the Tel Aviv area. Two controls were matched to each case by age and ethnic background: one selected from a surgical ward at the time of case ascertainment and the other selected from the same residential neighborhood as the case. Data on the almost 300 food frequency questions are being analyzed by the Israeli researchers in collaboration with the EEB.

16. A case-control study involving 500 newly diagnosed cases of invasive cervical cancer, 500 cases of in situ cervical cancer, and 1,000 neighborhood controls, of comparable age and race to the invasive cases, will be initiated in the summer of 1982 in five Comprehensive Cancer Centers with especially large numbers of cervical cancer patients (Philadelphia, Chicago, Miami, Birmingham, and Denver). Risk factors to be assessed include sexual practices, reproductive-contraceptive-gynecological history, smoking, and diet. This study will be the first to evaluate dietary exposures in a large number of patients with clearly invasive cervical cancer. Low intake of several micronutrients--vitamin A, carotene, folacin, vitamin C, and vitamin E--has been hypothesized to increase the risk of cervical cancer or cancer in general. A number of epidemiological studies have demonstrated inverse associations between the foods that contribute much of the vitamin A in the diet and several epithelial cancers; preliminary analysis of one case-control study suggests that cervical dysplasia-cervical cancer risk is doubled in low vitamin A consumers. Limited dietary data make it difficult for these studies to assess whether it is carotene or vitamin A, or fruits and vegetables in general, that is primarily associated with reduced risk. In a small clinical trial of women with cervical dysplasia, all of whom were using oral contraceptives and had low red blood cell folates, oral folate supplementation led to improved biopsy scores. In a case-control interview study of women with cervical dysplasia and carcinoma in situ, vitamin C was the nutrient, of the 19 examined, that was most strongly and

consistently associated with reduced risk. Laboratory evidence suggests that vitamin E, an antioxidant, may prevent lipid peroxidation and subsequent carcinogenesis.

The dietary interview includes 71 food frequencies and a supplemental vitamin history in order to estimate the usual adult intake of the micronutrients of interest and to characterize dietary patterns and nutritional status. To complement the dietary interview, blood samples will be collected with which to measure serum levels of retinol, carotene, vitamin C, folacin, and tocopherol and red blood cell folate, and possible serum cholesterol and ferritin. Blood will be drawn from the cases several months after completion of treatment, with the minimal elapsed time to be based on preliminary studies, and prior to treatment whenever possible. The unused serum will be stored in aliquots at -70°C for any other micronutrient determinations that might be deemed appropriate at some time in the future. Case ascertainment and interviewing for this study will continue through the summer of 1983.

17. A case-control study of esophageal cancer in males is being started in 1982 in Charleston, Savannah, and Jacksonville--three major cities in the region of elevated esophageal cancer mortality along the Southeast coast. Black males, in particular, have especially high rates of esophageal cancer in this area. Approximately 300 cases, diagnosed between 1982 and 1985, and 600 hospital controls, matched on age, race, county of residence, and hospital of initial diagnosis, will be interviewed directly; no next-of-kin interviews are anticipated. The dietary interview will include 63 food frequencies (usual adult consumption, disregarding any recent changes in diet), food cooking and preparation practices, ethnic food items, beverage use, alcohol consumption, and a supplemental vitamin history.

Major Findings:

1. In the study of esophageal cancer among Washington, D.C., black males, five indicators of general nutritional status--fresh or frozen meat and fish consumption, dairy product and egg consumption, fruit and vegetable consumption, relative weight (wt/ht^2), and number of meals eaten per day--were each significantly and inversely correlated with the relative risk of esophageal cancer. Associations with other food groups were not apparent. The least nourished third of the study population, defined by any of these five measures, was at twice the risk of the most nourished third. None of these associations was markedly reduced by controlling for ethanol consumption, (the other major risk factor in this population), smoking, socioeconomic status, or the other nutrition measures. When the three food group consumption measures were combined into a single overall index of general nutritional status, the relative risk of esophageal cancer between extremes was 14. Estimates of the intake of vitamin A, carotene, vitamin C, thiamin, and riboflavin were inversely associated with relative risk; but each micronutrient index was less strongly associated with risk than were the broad food groups that provide most of the micronutrient. Thus, no specific micronutrient deficiency was identified. Instead, generally poor nutrition was the major dietary predictor of risk and may partially explain the susceptibility of urban black men to esophageal cancer.

2. In the study of breast cancer in Alberta, Canada, significant increasing trends were found with more frequent consumption of beef (Relative Risks [RRs] by tertiles of consumption were 1.0, 2.3, 1.5; test for trend, $p < 0.001$), pork (RRs of 1.0, 1.6, 2.2; test for trend, $p < 0.001$), and sweet desserts (RRs of 1.0, 1.3, 1.5; test for trend, $p = 0.01$). Elevated risks were also noted for use of butter at the table and for frying with butter or margarine, as opposed to vegetable oils. The association of total beef and pork consumption with breast cancer was not materially affected by controlling for age at first birth, family history of breast cancer, previous benign breast biopsy, or socioeconomic status. Nor was the association reduced by controlling for ages of menarche and menopause even though within the control series the intake of beef and pork reported in adult life was higher among those with a lower age at menarche or an older age at natural menopause.

3. In the study of colorectal cancer in rural Nebraska, the excess risk was primarily among persons of Czechoslovakian ancestry, with those from Bohemia and Moravia predominating in this area. An elevated risk among Bohemians was associated with diets high in fat (meat and dairy products) and sweets. The colon cancer risk was elevated among commercial beer drinkers regardless of their ethnic background, although Bohemians were particularly heavy consumers. An excess risk was also associated with intestinal polyps, reported more often by Moravians, and with familial occurrence of gastrointestinal and other cancers. Since 1969 the mortality and incidence rates for colon cancer in this area have declined, presumably as a consequence of acculturation of the American-born descendants of Czech immigrants.

4. In the study of oral and pharyngeal cancer among North Carolina women, major findings included a protective effect of a usual adult diet high in fruits and vegetables. The relative risk of 0.65 for high and 0.52 for moderate consumption relative to the least frequent consumption of fruits and vegetables was statistically significant and consistent throughout subgroups within the study population. A protective influence with bread consumption also was observed. In addition, heavier adult relative weights were associated with diminished risk. However, these findings could not be attributed to a generalized benefit from better nutritional status since fish/shellfish and pork consumption were related to increased risk of oral and pharyngeal cancer, and dairy and egg consumption was unrelated to cancer risk. The reduction in risk from greater fruit and vegetable consumption is consistent with a protective effect of beta-carotene on oral and pharyngeal mucosal tissue.

Significance to Biomedical Research and Program of the Institute: General dietary patterns, nutritional status, specific foods and food groups, and food additives are being recognized as possible causes of cancer. The American people seek guidance on diets to minimize their risk of cancer, and Congress and the Executive Branch seek advice on what to advocate and regulate. Epidemiological studies of diet and cancer can contribute to a rational basis for public policy and individual decisions. It is necessary to test and quantify in human populations those hypotheses about the role of diet in carcinogenesis that have resulted from animal studies, in vitro experiments, and clinical observations. In addition, exploratory nutritional epidemiology can suggest correlations between

dietary patterns and cancer, which then serve as the basis for further laboratory research and further analytic epidemiology.

Certain diets and foods seem able to initiate carcinogenesis, others promote it, while still others seem to reduce cancer risk. Their mechanism of action can be direct through interaction with DNA or indirect through alteration of metabolic pathways or cell regulation or even more indirect through modification of the endocrine or immune systems. Further research on diet and cancer, in which both epidemiology and laboratory science must cooperate, could yield insights into these mechanisms and the biology of carcinogenesis.

Proposed Course: If the preliminary death certificate-based study of colorectal cancer in Florida indicates that the apparent reduction in risk among Northerners moving to Florida is not due simply to their being a self-selected, especially healthy, subset of Northerners, then a more comprehensive case-control study of incident colorectal cancer in Florida retirement areas will be initiated. This study will help to identify the attributes of the Southern environment or lifestyle that might be involved in reducing cancer risk--possibly increased consumption of fruits and vegetables, more vitamin A or C, or the quality of the drinking water. If the preliminary study indicates a migrant effect, then another study focused on the etiology of colorectal cancer--possibly one based on the risk differential between North and South--will be designed.

A large case-control study of breast cancer among women of Japanese descent now living in Hawaii and the West Coast is currently being developed. When Japanese women migrate to the U.S., their low rates of breast cancer rise toward American rates over a period of several generations. The age between menarche and first pregnancy is hypothesized to be particularly sensitive to exposures that lead to breast cancer. Thus, this study will attempt to identify that period of the lifespan diet which is operative in promoting breast cancer risk and to assess which aspects of the Western diet are involved.

The study of lung cancer and diet in New Jersey will be continued in black men and in white women during 1982-83 in order to compare the results with those obtained for white men in the first phase of the study. The study on blacks will help to determine whether poor diet accounts for the apparently higher lung cancer rates in black men than in white men. The female population will be utilized to assess the association between diet and lung cancer in nonsmokers and to refine, with more extensive dietary information, the basic associations between diet and lung cancer identified in the first study.

Uric acid, an antioxidant, may protect cells from lipid peroxidation and subsequent carcinogenesis. To test this hypothesis, a cohort of 7,000 Japanese men living in Hawaii, followed for the last 20 years and with serum uric acid and other biochemical indices measured at onset of the study, will be analyzed. A second prospective cohort study, in collaboration with the Kaiser Foundation Research Institute in the San Francisco Bay Area, will evaluate the observed relationship between low serum cholesterol and elevated risk of cancer. Within this reasonably representative and large American cohort of about 200,000 individuals, the possibility that preclinical cancer itself could be the cause of the low serum cholesterols, the spectrum of anatomic sites that are involved and the relationship of serum cholesterol to both cancer incidence and prognosis will be assessed.

A collaborative arrangement between Chinese scientists and the EEB is being developed to facilitate epidemiologic studies of cancer in China. The tentative diet-related components include case-control studies of esophageal and nasopharyngeal cancers in their respective high-risk areas and a case-control study of the relationship of diet to lung cancer risk.

Publications:

Lubin, J.H., Burns, P.E., Blot, W.J., Ziegler, R.G., Lees, A.W., and Fraumeni, J.F, Jr.: Dietary factors and breast cancer risk. Int. J. Cancer 28: 685-689, 1981.

Pottern, L.M., Morris, L.E., Blot, W.J., Ziegler, R.G., and Fraumeni, J.F., Jr.: Esophageal cancer among black men in Washington, D.C. I. Alcohol, tobacco, and other risk factors. JNCI 67: 777-783, 1981.

Ziegler, R.G., Morris, L.E., Blot, W.J., Pottern, L.M., Hoover, R., and Fraumeni, J.F., Jr.: Esophageal cancer among black men in Washington, D.C. II. Role of nutrition. JNCI 67: 1199-1206, 1981.

ENVIRONMENTAL EPIDEMIOLOGY BRANCH

CONTRACT INDEX

CENTERS FOR DISEASE CONTROL (YO1-CP-00500)

Title: Epidemiologic Studies of Cancer in Alaskan Natives.

Contractor's Project Director: Anne P. Lanier M.D.

Project Officer (NCI): William J. Blot, Ph.D.

Objectives: To study the incidence of cancer in Alaskan natives from 1974 onward and the mortality from cancer in Alaskan natives from 1960 to the present; to investigate several striking familial aggregations of cancer in Alaskan natives, particularly nasopharyngeal cancer (NPC) and hepatocellular carcinoma (PHC) to search for environmental determinants of nasopharyngeal cancer; and to conduct analyses of native foods for the presence of mutagens, aflatoxins, and nitrosamines

Methods Employed: Newly-diagnosed cancers and cancer deaths in Alaskan natives are ascertained through the facilities of the Public Health Service Hospitals in which virtually all Alaskan natives receive their health care. The data are collected in a format compatible with the SEER Registries to permit comparison with other U.S. populations Cancer-prone families will be studied in collaboration with professionals from the Family Studies Unit of NCI's Environmental Epidemiology Branch, utilizing clinical and laboratory protocols tailored to the malignancies to be evaluated. This will include hepatitis B and Epstein-Barr virus assays, immunologic and genetic tests, and other serologic markers aimed at uncovering mechanisms of disease susceptibility. A case-control study of NPC is planned, which will focus on dietary exposures of possible etiologic interest. Samples of suspect native food items will be assayed for the presence of nitrosamines, aflatoxins, and mutagenic activity.

Major Findings: The updated cancer mortality and incidence statistics show that rates continue to be elevated among Alaskan natives for cancers of the nasopharynx, liver and gallbladder, kidney, and uterine cervix, and that the rates of lung, colorectal, and breast cancer are now approaching those of the U.S. white population. Familial aggregation of both NPC and PHC have been identified. Preliminary Ames-testing has shown mutagenic activity in several native fish samples, leading to more detailed sampling and testing to identify the sequence in preparation or storage where mutagens are formed.

Significance to Biomedical Research and the Program of the Institute: The projects in this proposal are directed toward investigating the unusual patterns of cancer in the culturally and geographically unique population that Alaskan natives represent, and to identify risk factors for these cancers.

Proposed Course: Trends in cancer rates will be analyzed, assays of biological specimens from familial cancer clusters will continue, and laboratory tests of native foods will be expanded.

Date Contract Initiated: December 17, 1979.

Current Annual Level: $174,093.

CHAIM-SHEBA MEDICAL CENTER, ISRAEL (NO1-CP-01042)

Title: Radiation Risk Estimation in Israeli Children Irradiated for Tinea Capitis.

Contractor's Project Director: Baruch Modan, M.D.

Project Officer (NCI): John D Boice Jr., Sc.D.

Objectives: To determine the incidence of cancer in 10,000 Israeli children irradiated for ringworm of the scalp, in 10,000 nonexposed persons selected from the general population, and in 5,000 nonexposed siblings.

Methods Employed: The study cohorts were previously identified from immigration records (1949-60) and the risk of thyroid cancer evaluated. Medical records in all Israeli hospitals and records available in the Central Tumor Registry are being searched to determine malignant and benign tumors that have occurred in the exposed and comparison cohorts. Detailed dosimetry data are being abstracted. Death certificates will be obtained for those who have died, and vital status as of 1981 will be determined for all enrolled persons. Sites of particular interest include: thyroid, brain, parotid gland, breast, bone, lung, esophagus, larynx, skin, leukemia, and lymphoma.

Major Findings: Record abstraction and follow-up of the populations are ongoing, and results will be reported when available.

Significance to Biomedical Research and the Program of the Institute: The study of patients irradiated for benign diseases has been an important area for evaluating biological mechanisms for carcinogenesis in man. The minimal confounding effects of other carcinogenic influences, such as smoking or occupation, and the possible greater susceptibility of young people to environmental carcinogens, enhance the chances of detecting radiogenic effects and provide an opportunity for life-time studies of cancer incidence. This particular study has already reported excess thyroid nodular disease following low-level exposure (9 rad) and further follow-up will be informative for clarification of this finding. This project has direct relevance to the Institute and the Federal commitment to evaluate the adverse health effects of ionizing radiation.

Proposed Course: Data collection will proceed for approximately two more years followed by analysis and publication of study findings.

Date Contract Initiated: September 25, 1980.

Current Annual Level: $130,000.

CHILDREN'S HOSPITAL (NO1-CP-91049)

Title: Late Effects of Treatment for Cancer in Childhood.

Contractor's Project Director: Anna T. Meadows, M.D.

Project Officer (NCI): John D. Boice, Jr., Sc.D.

Objectives: The primary objective is to evaluate the potential carcinogenic effects of various modalities (radiation and chemotherapy) used in the treatment of childhood cancers. Other objectives are to evaluate the interaction of therapy and genetic predispositions to cancer, determine incidence and histopathology of second cancer, and evaluate survival.

Methods Employed: Using the resources of the Late Effects Study Group, an international collaboration involving 13 major centers for childhood cancer therapy, a case-control study of approximately 200 individuals with second primary cancer was completed. Rosters of long-term survivors of childhood cancer were prepared, controls randomly selected from this roster, therapy information abstracted from records, and detailed radiation dosimetry performed. The distribution of chemotherapeutic agents and radiation treatments between cases and controls will be compared.

Major Findings: Preliminary analyses suggest a risk of second cancers among persons treated as children for cancer. Radiation may be associated with elevated risks of leukemia and cancers of the bone, connective tissue, thyroid, and possibly brain.

Significance to Biomedical Research and the Program of the Institute: The study of the carcinogenic effects of anti-tumor agents and radiation therapy should provide insights into the biological mechanisms of cancer etiology. The minimal confounding effects of other carcinogenic influences, such as smoking or occupation, and the possible greater susceptibility of young people to environmental carcinogens, enhance the chances of detecting increased risks due to therapy. Assessment of the carcinogenic potential of anti-tumor agents is part of the Institute's overall investigation of possible carcinogenic actions of therapeutic drugs.

Proposed Course: Analysis will continue to evaluate whether radiation or chemotherapy can account for the observed excesses of second cancers.

Date Contract Initiated: September 30, 1979.

Current Annual Level: $47,103.

E.I. DUPONT DE NEMOURS & CO., INC. (NO1-CP-01038)

Title: Study of the DuPont Chambers Works Bladder Cancer Screening Program.

Contractor's Project Director: W.J. Vogler.

Project Officer (NCI): Thomas J. Mason, Ph.D. and Philip C. Prorok, Ph.D.

Objectives: The primary objective of the study is to evaluate the effectiveness of urinary cytology as it is currently utilized in detecting early bladder cancer among employees at DuPont's Chambers Works facility.

Methods Employed: Certain data are abstracted from DuPont employee medical and employment records, including complete work histories, every urine cytology reading, the results of every urine blood test, information from physical exams, and information concerning the clinical course of bladder cancer among this work force.

Major Findings: This contract is in its data collection and editing phase, and no analysis of data has as yet been undertaken.

Significance to Biomedical Research and the Program of the Institute: This study provides an extensive re-examination of the DuPont bladder cancer screening program as recommended by the State-of-the-Arts-Conference on Bladder Cancer Screening in December, 1977. Thus, it will aid NCI in formulating policy and research priorities with respect to bladder cancer screening and populations at high risk as a consequence of occupational exposure.

Proposed Course: This contract will be extended with funds to permit the completion of data collection on all bladder cancer cases among this work force.

Date Contract Initiated: September 5, 1980.

Current Annual Level: $25,000.

ENERGY, DEPARTMENT OF (YO1-CP-10504)

Title: Studies on Radiation-Induced Chromosome Damage in Humans

Contractor's Project Director: L. Gayle Littlefield, Ph.D.

Project Officer (NCI): Charles Land, Ph.D.

Objectives: (1) To calculate dose-response curves for frequency of chromosomal aberrations of various types; (2) within study populations, to analyze these curves for variation by dose and age at exposure, and to compare them with similar curves obtained for cancer incidence; and (3) to compare curves among study populations to assess the influence of different exposure modalities.

Methods Employed: Chromosomal aberrations will be determined and analyzed in 450 subjects selected from among 3 populations exposed to diagnostic and therapeutic radiation during the period 1930-1970, and which are currently under study by the EEB for late health effects in relation to individual dosimetry. These populations are cervical cancer patients given radiotherapy, tuberculosis patients given multiple chest fluoroscopies, and persons irradiated for lymphoid hyperplasia during childhood. About fifty non-exposed persons from each of these populations will be selected as controls. Blood specimens, drawn at the hospitals where these persons were treated, will be analyzed at the DOE-supported cytogenetic laboratory at the Oak Ridge Associated Universities.

Major Findings: This project has been under way for an insufficient period of time for a significant report.

Significance to Biomedical Research and the Program of the Institute: The project is very likely to yield data that will improve our understanding of the relationship between radiation exposure and the frequency of chromosomal aberrations, and therefore to improve our ability to assess the analogy to radiation carcinogenesis. The populations to be studied are all being monitored for cancer risk, and there is a high probability that dose-response relationships will be obtained for radiation-induced cancer, which can be compared to the dose-response curves for chromosomal aberrations. Also, the project should improve the usefulness of chromosomal aberration frequency as a biological dosimeter for partial-body exposures to ionizing radiation.

Proposed Course: To continue cytogenetic evaluations on the irradiated populations selected for study.

Date Contract Initiated: September 21, 1981.

Current Annual Level: $107,047

FOOD AND DRUG ADMINISTRATION (YO1-CP-20511)

Title: Case-Comparison Study of Childhood Cancer.

Contractor's Project Director: Richard P. Chiacchierini, Ph.D.

Project Officer (NCI): Robert Spirtas, Dr. P.H.

Objectives: To add information on parental occupation to the existing data based on childhood cancer in Great Britain. Recent studies, based on relatively small sample sizes, have suggested a relationship between childhood cancer and father's occupation in industries with lead and hydrocarbon exposures. The proposed study will provide a much larger data base for evaluating the relationship between childhood neoplasms and parental employment.

Methods Employed: The project has a case-control design. Since 1955 all deceased cases of childhood cancer in England have been matched with a live control child on date-of-birth, sex, and place of residence. Data have been obtained by questionnaire on about 80 percent of the subjects and consist of over 300 variables including information on family, medical, occupational, and radiation exposure histories.

Major Findings: Numerous publications have resulted from the data collected in this study, but evaluation of parental occupation still remains to be done.

Significance to Biomedical Research and the Program of the Institute: This contract will provide the largest data base for evaluating the effect of parental employment on childhood cancer. The current hypotheses provided by small scale studies emphasize the importance of the large-scale study to confirm or refute the results reported to date.

Proposed Course: Transfer funds to FDA via Interagency Agreement. FDA will add these funds to its ongoing contract with the University of Birmingham in England.

Date Interagency Agreement Initiated: August 1982.

Current Annual Level: $50,000.

GEORGE WASHINGTON UNIVERSITY (NO1-CP-81051)

Title: Study of Ovarian Cancer in Greater Washington, D.C.

Contractor's Project Director: Larry McGowan, M.D.

Project Officer (NCI): Robert N. Hoover, M.D.

Objectives: To investigate the role of exogenous hormones and other factors in the etiology of ovarian cancer.

Methods Employed: All cases of ovarian cancer being diagnosed in 25 hospitals in the greater Washington (approximately 13 per month) were identified as they occurred during the 3-year period August 1, 1978-July 31, 1981. For each case, a control was drawn of the same age and race, and discharged from the same hospital at approximately the same date. After the subject and her physician gave permission, the subject was interviewed in her home. The questionnaire elicited information about the few factors known to relate to risk of ovarian cancer (marital status, gravidity, age at first pregnancy, parity, infertility) and many factors under investigation including hysterectomy, use of menopausal estogens, oral contraceptives and other exogenous hormones, occupational exposures, family history of diseases, and childhood illnesses.

In addition to the personal interview, questionnaires mailed to subjects' physicians and independent review of the tumor tissue provided information for analysis. The mailed questionnaires permitted verification of exposures to exogenous estrogens. The independent slide reviewer is valuable because of the variety of ovarian cancer types, differing in cellular origin, histologic appearance, survival patterns, and risk factors.

Major Findings: The case and control ascertainment and interviewing portion of the survey are complete and the data are being coded and edited.

Significance to Biomedical Research and the Program of the Institute: This study directly addresses the possible role of exogenous hormones in ovarian cancer. These agents are widely used and have been linked to malignancies of reproductive organs, so it is imperative to examine their long-range effects on risk of cancer of other reproductive organs. In addition, any elucidation of the etiology of ovarian cancer would be of value. Although this form of malignancy is relatively common (17,000 cases per year in the U.S.) and often fatal (10,000), very little is known of its causes.

Proposed Course: Analysis of the data will be performed in the Environmental Epidemiology Branch.

Date Contract Initiated: October 1, 1978.

Current Annual Level: 0 (No cost extension.)

HARVARD UNIVERSITY (NO1-CP-81058)

Title: Follow-up of Fluoroscopically Examined Tuberculosis Patients in Relation to Incidence of Cancer.

Contractor's Project Director: Richard R. Monson, M.D.

Project Officer (NCI): John D. Boice, Jr., Sc.D.

Objectives: Approximately 7,000 former tuberculosis patients, treated between 1930-52, are being evaluated; 48 percent received pneumothorax and associated multiple chest fluoroscopies. The objectives are: (1) to evaluate the risk of radiation-induced breast cancer in older women, (2) to evaluate the risk of radiogenic lung cancer and leukemia in men and women, (3) to evaluate the association between Isoniazid and liver disease, and (4) to evaluate the interaction between radiation and biological modifiers of risk.

Methods Employed: Records of former tuberculosis patients are available in Massachusetts and are being reviewed. Fluoroscopic exposures, medical treatments, and demographic information have been abstracted. Follow-up activities include the use of HSPH Town Lists, Motor Vehicle Bureau records, Department of Vital Statistics records, outpatient records, and other sources. Questionnaires are being sent to persons known to be alive to ascertain current medical status. Death certificates are being obtained for all who have died. Analysis is being done using a general computer program.

Major Findings: The abstraction of data has been finished. Death certificates have been obtained for persons who died. The questionnaire has been sent to those patients who are living. Preliminary mortality analyses suggest an increase of cancer mortality among all tuberculosis patients, but the excess appears concentrated in the nonexposed group. Among the exposed population, no statistically significant excess of cancer deaths was found for sites that might have been expected to be elevated, i.e., the breast, lung, and leukemia. Results for cancer incidence should be available shortly.

Significance to Biomedical Research and the Program of the Institute: One of the National Cancer Institute's most controversial programs has been the Breast Cancer Detection and Demonstration Project (BCDDP) which has enrolled 280,000 women for annual mammographic x-ray examinations to detect breast cancer early in asymptomatic women. Guidelines for cancer screening and detection by mammography have been undergoing major changes, and it is important that the guidelines be validated. Specifically, it is important to determine (1) whether women 35 to 49 years of age at screening are likely to develop radiation-induced breast cancer from repeated low-level exposures and (2) whether women at high natural-risk of developing breast cancer because of

underlying host conditions (such as benign breast disease or family history of cancer) are at especially repeated low-dose radiation exposures have the same effect as a single large exposure in inducing leukemia and lung cancer. Because population exposures a in large part to low dose, cumulated over many years, it is important to estimate risks from irradiated populations receiving similar, though much larger, radiation exposures.

Proposed Course: Specific plans for the last year of this contract include (1) cancer incidence evaluation, (2) dosimetry evaluation, and (3) final report.

Date Contract Initiated: September 29, 1978.

Current Annual Level: $24,900.

HAWAII, UNIVERSITY OF (NO1-CP-71006)

Title: Occupational Cancer Risk in Hawaii.

Contractor's Principal Investigator: Ming Pi Mi, Ph.D.

Project Officer (NCI): Thomas J. Mason, Ph.D.

Objectives: This project is designed to provide data which will permit the linkage of official records: Civil Defense file, birth records, and death certificates for the population of 1942-78, with specific emphasis on occupation and/or place of employment.

Methods Employed: From a Civil Defense file with detailed information on the Hawaiian population of 1942-43, occupational data have been abstracted for the Oahu cohort, as well as on individuals residing in the remaining counties (islands of Kauai, Maui, Molokai, Lanai and Hawaii) during the war years. These data are important because of the number of individuals who resided and worked on sugar, pineapple, and rice plantations, and might have been exposed to carcinogens. Occupational data will be abstracted from all death certificates for the period in question.

Major Findings: The abstracting of occupational data has been completed for all persons registered in 1942-43. Vital records files have been expanded to include all deaths throughout 1978. Linkage programs have been written, and the population of 1942-43 has been followed through 1978. We are currently pursuing both proportionate mortality and proportionate cancer mortality analyses for each of the major races with emphasis on occupation. Several manuscripts are in their final stages of preparation.

Significance to Biomedical Research and the Program of the Institute: The collection of occupational data fits in well with the specific interest in the geographic distribution of malignancy in the United States that the Environmental Epidemiology Branch has developed over the past several years. The ability to link records over this time span will greatly facilitate the investigation of occupational factors related to malignancy.

Proposed Course: This contract will expire in September, 1982.

Date Contract Initiated: April 11, 1977.

Current Annual Level: $132,670.

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER (NO1-CP-11017)

Title: International Radiation Study to Evaluate the Risk of Radiation Exposure in Cervical Cancer--European Segment.

Contractor's Project Director: Allen Linsell, M.D.

Project Officer (NCI): John D. Boice, Jr., Sc.D.

Objectives: To quantify the risk of high and low-level radiation doses and to evaluate the influence of host factor (such as age) on subsequent radiogenic risk.

Methods Employed: More than 22,000 patients treated for cervical cancer in 22 European clinics will be evaluated for the occurrence of second cancers subsequent to radiotherapy. Organ doses to sites outside the pelvis will receive low-level doses (under 100 rad) and will be accurately characterized. Detailed dosimetry information will be abstracted from hospital records. Morbidity and mortality will be determined through active follow-up. Fifteen cancer registries have been conducting cohort analyses on the risk of second cancers among 200,000 former cervical cancer patients; case-control studies in these registry areas will be conducted to evaluate the influence of cancer risk factors, such as smoking, on subsequent risk, and to provide detailed dosimetry information for risk assessment.

Major Findings: The cancer registry cohort analyses are almost complete and will be published as a monograph. Findings indicate excess risks, related to radiation, of cancers of the rectum, kidney, ovary, and corpus uteri as well as acute nonlymphocytic leukemia. A deficit of breast cancer, possibly related to ovarian ablation, was also observed.

Significance to Biomedical Research and the Program of the Institute: The project will estimate the risk of radiogenic cancers following low-dose and high-dose irradiation. The project has the potential to elucidate mechanisms of carcinogenesis and is directly relevant to the Institute and Federal commitment to evaluate the possible adverse health effects of low-level ionizing radiation. This project will provide information useful in formulating preventive measures and in setting radiation protection guidelines for occupational, medical, and public exposure to radiation.

Proposed Course: Data collection will proceed for approximately three years, followed by analysis and publication of study findings. A monograph of the cancer registry cohort analyses should be published this year.

Date Contract Initiated: September 1, 1981.

Current Annual Level: $864,110.

IOWA, UNIVERSITY OF (NO1-CP-11020)

Title: A Study of Environmental Factors in the Origin of Leukemia and non-Hodgkin's Lymphoma among Adult White Males from Rural Areas.

Contractor's Project Director: Peter Isacson, M.D.

Project Officer (NCI): Aaron Blair, Ph.D.

Objectives: To collect information to evaluate the role of environmental determinants (particularly agriculturally related) in the origin of leukemia and non-Hodgkin's lymphoma. This contract will supplement NO1-CP-01033 by providing additional study subjects needed to obtain sufficient statistical power for this project.

Methods Employed: The project is a case-control design. Three hundred histologically confirmed cases for each tumor and 600 matched controls among adult white males will be selected and interviewed to determine personal habits and occupational exposures. Controls (meeting age, race, and sex requirements) will be randomly selected from the general population.

Major Findings: No findings are available. Interviewing of cases and controls is under way. Additional time is necessary to produce a significant report.

Significance to Biomedical Research and the Program of the Institute: This contract will provide needed data on the origin of leukemia and non-Hodgkin's lymphoma. Geographic studies of these cancers by the Branch have suggested new leads, particularly in the area of farm-related exposures, that urgently need to be evaluated. The increase in non-Hodgkin's lymphoma in recent years and the limited effort devoted to the epidemiology of this cancer, further underscore the need for this project.

Proposed Course: Continue with methodology as described above.

Date Contract Initiated: June 1, 1981.

Current Annual Level: $241,026.

KAISER FOUNDATION RESEARCH INSTITUTE, LOS ANGELES, CALIF. (NO1-CP-11038)

Title: Studies on Environmental Cancer Utilizing a Pre-Paid Health Plan

Contractor's Project Director: Harry K. Ziel, M.D.

Project Officer (NCI): Robert N. Hoover, M.D.

Objectives: (a) To evaluate hypotheses concerning environmental causes of cancer by analysis of information in a pre-paid health plan which has been recorded over many years on large groups of patients having particular cancers, and to compare the data to those on individuals without the disease. (b) To follow up this analysis by extensive studies on those individuals who have had known exposures to the particular environmental factors which are suspect in the etiology of the cancers concerned. This is one of three Kaiser Foundation Research Institute collaborating contracts (see NO1-CP-11009 and 11037)

Methods and Major Findings: The surgical record books from the Southern California Permanente Medical Group from 1952 through 1970 were reviewed manually to identify a group of health plan members who had oophorectomy during this time period. These lists were compared with a list of breast cancer patients identified by the health plan's tumor registry during the years 1972-1977 in order to identify patients with a history of oophorectomy who subsequently developed breast cancer. From the same surgical record books and the files of health plan members, four controls for each case were drawn. These controls are women having undergone an oophorectomy in the same year as the case, matched on age at which this operation occurred, and duration of health plan membership (to the date of diagnosis of the case). Breast cancer risk factors and hormonal replacement therapy information are being abstracted from these patients' charts. An additional study of estrogens and other risk factors as they relate both to breast cancers and abnormal mammographic findings utilizing data from a large screening program in this plan is also being conducted. The potential teratogenicity of estrogens is also being evaluated via a case-control evaluation of drug exposures during pregnancy and subsequent development of limb-reduction and cardiac defects in the fetus.

Significance to Biomedical Research and the Program of the Instititue: The objective of the Environmental Epidemiology Branch is to generate and test ideas concerning the environmental and host determinants of cancer by a broad range of epidemiologic studies based on knowledge and application of clinical medicine and oncology, statistical methodology, new developments in carcinogenesis, and resources available at various levels. This project is immediately relevant to this broad objective because it attempts to focus directly on environmental factors which occur during an individual's life and to which he or she may be exposed at various time periods.

Proposed Course: The investigators in Southern California will analyze their own data, and in addition will send a copy of the material to NCI so that the data can be merged with similar information obtained from the two other Kaiser Permanente Health Plans. New evaluations in the project will include assessment of drugs in relationship to endometrial carcinoma and further evaluation of potential teratogenicity of various medications.

Date Contract Initiated: Sept. 30, 1981.

Current Annual Level: $176,254.

KAISER FOUNDATION RESEARCH INSTITUTE, OAKLAND, CALIFORNIA (NO1-CP-11037)

Title: Studies on Environmental Cancer Utilizing a Pre-Paid Health Plan.

Contractor's Project Director: Gary Friedman, M.D.

Project Officer (NCI): Robert N. Hoover, M.D.

Objectives: (a) To evaluate hypotheses concerning environmental causes of cancer by analysis of information in a Pre-Paid Health Plan which has been recorded over many years on large groups of patients having particular cancers, and to compare the data to those on individuals without the disease. (b) To follow up this analysis by extensive studies on those individuals who have had known exposures to the particular environmental factors which are suspect in the etiology of the cancers concerned. This is one of three Kaiser Foundation Research Institute collaborating contracts (see NO1-CP-11009 and 11038).

Methods and Major Findings: In Northern California data collection was completed for a case-control study of ovarian cancers similar to that in Portland. Following a protocol for a case-control study of patients who developed breast cancer after having had a bilateral oophorectomy data collection was undertaken and is nearing completion. The major exposure of interest being assessed in this study, in addition to the usual breast cancer indicators, is the use of menopausal estrogen replacement therapy. Both the Northern California and the Portland Plans have submitted information on malignancies occurring in the health plans, and various other demographic information concerning the potential hypotheses to be explored in these health plans. There have been extensive evaluations of cancer incidence among various industrial groups covered by the pre-paid plan, and a number of analytic studies based on these evaluations are currently being designed. Two new evaluations were begun: a study of the relationship of serum cholesterol levels among men attending a multiphasic screening exam and their subsequent risk of malignancy, and a case-control study of patients with leukemia and lymphoma which will evaluate the risk associated with diagnostic irradiation.

Significance to Biomedical Research and the Program of the Institute: The objective of the Environmental Epidemiology Branch is to generate and test ideas concerning the environmental and host determinants of cancer by a broad range of epidemiologic studies based on knowledge and application of clinical medicine and oncology, statistical methodology, new developments in carcinogenesis, and resources available at various levels. This project is immediately relevant to this broad objective because it attempts to focus directly on environmental factors which occur during an individual's life and to which he or she may be exposed at various time periods.

Proposed Course: New studies which are planned include evaluation of the interrelationships between oral contraceptive use and benign and malignant breast disease, drug use and reproductive risk factors for endometrial cancer, and follow-up studies based on findings from the cholesterol, occupational, and radiation studies currently being conducted or analyzed.

Date Contract Initiated: Sept. 30, 1981

Current Annual Level: $160,000

KAISER FOUNDATION RESEARCH INSTITUTE, PORTLAND, OREGON (NO1-CP-11009)

Title: Studies on Environmental Cancer Utilizing a Pre-Paid Health Plan

Contractor's Project Director: Andrew Glass, M.D.

Project Officer (NCI): Robert N. Hoover, M.D.

Objectives: (a) To evaluate hypotheses concerning environmental causes of cancer by analysis of information in a Pre-Paid Health Plan which has been recorded over many years on large groups of patients having particular cancers, and to compare the data to those on individuals without the disease. (b) To follow up this analysis by extensive studies on those individuals who have had known exposures to the particular environmental factors which are suspect in the etiology of the cancers concerned. This is one of three Kaiser Foundation Research Institute collaborating contracts (see NO1-CP-11037 and 11038).

Methods and Major Findings: Time trend analyses of cancer incidence in this plan are currently under way. The data collection phase of a case-control study of ovarian cancer has been completed. A common protocol for studying ovarian cancer has been worked out between the Portland region and the Northern California region under the direction of the NCI Project Officer. The focus of this study is on therapeutic drugs and medical conditions which alter the pituitary-ovarian hormonal axis. Data on stage of disease and survival information have been abstracted and computed for the series of breast cancer patients included in a prior study done in this plan. This information has been forwarded to NCI. Case-control studies of cholesterol level and risks of colon and lung cancer in men, and diagnostic irradiation and the risk of leukemia and lympyhoma, are in the data-collection phase.

Significance to Biomedical Research and the Program of the Institute: The objective of the Environmental Epidemiology Branch is to generate and test ideas concerning the environmental and host determinants of cancer by a broad range of epidemiologic studies based on knowledge and application of clinical medicine and oncology, statistical methodology, new developments in carcinogenesis, and resources available at various levels. This project is immediately relevant to this broad objective because it attempts to focus directly on environmental factors which occur during an individual's life and to which he or she may be exposed at various time periods.

Proposed Course: New studies which are planned include evaluations of the interrelationships between oral contraceptive use and benign and malignant breast disease, drug use and reproductive risk factors for endometrial cancer, and follow-up studies based on findings from the cholesterol, occupational, and radiation studies currently being conducted or analyzed.

Date Contract Initiated: Sept. 30, 1981.

Current Annual Level: $295,000.

LOUISIANA STATE UNIVERSITY (NO1-CP-91023)

Title: Cancer in Southern Louisiana: A Case-Control Study of Lung, Pancreas, and Stomach Cancers.

Contractor's Project Director: Pelayo Correa, M.D.

Project Officer (NCI): Linda W. Pickle, Ph.D.

Objectives: To identify risk factors responsible for the high rates for lung, pancreas, and stomach cancers in southern Louisiana.

Methods Employed: A case-control interview study among residents of southern Louisiana parishes will provide information on lifetime histories of residence, occupation, tobacco and alcohol consumption, diet, and ethnic and social factors for approximately 3,000 cancer cases and controls.

Major Findings: Sample identification and interviewing of cases and controls is under way.

Significance to Biomedical Research and the Program of the Institute: This investigation generates data to uncover reasons for the exceptional occurrence of cancer in Louisiana and thus provide information useful in formulating measures aimed at prevention.

Proposed Course: Interviewing of lung cancer cases and controls was completed during 1981. Preliminary analysis of these data are under way. Interviewing of stomach and pancreas cases and controls will continue until 1983. Analysis will be completed by May 1, 1983.

Date Contract Initiated: March 13, 1979.

Current Annual Level: $252,520.

MAYO FOUNDATION (NO1-CP-11023)

Title: Follow-up Study of Women Evaluated for Infertility.

Contractor's Project Director: George D. Malkasian, Jr., M.D.

Project Officer (NCI): Louise A. Brinton, Ph.D.

Objectives: To investigate the risk of cancer among women with varying types of infertility.

Methods Employed: The records of all Rochester, MN female residents evaluated for infertility at Mayo Clinic during the period 1935-1965 are being reviewed and abstracted according to a standardized protocol. Information being abstracted relates to demographic characteristics, reproductive and contraceptive histories, details of the clinical work-up for infertility, and information on therapy for the underlying cause of infertility. All study subjects are being followed through the present time to ascertain vital

status, and to collect information of subsequent reproductive and medical events. For deceased subjects, death certificates are being sought. Living subjects are sent a short questionnaire by mail which they are asked to complete and return to Mayo Clinic. All occurrences of cancer in this population are being documented by retrieval of pathology reports.

Major Findings: Subject ascertainment and follow-up is still in progress, and data will be analyzed later.

Significance to Biomedical Research and the Program of the Institute: Studying a cohort of women evaluated for infertility will allow comparison of disease incidence among subgroups with differing abnormalities, an analysis that may aid in understanding the mechanisms of carcinogenesis. If an abnormal hormonal milieu is the important factor in contributing to excesses of certain endocrine cancers, these women with identifiable hormonal problems will be of particular interest. Specifically, the proposed study will allow evaluation of an hypothesis regarding breast cancer etiology that has received recent interest, namely that women who exhibit luteal phase defects may be at increased risk due to their relatively unopposed state of endogenous estrogens. In addition, this study will allow examination of various infertility treatment effects, including radiation exposures to the pituitary and/or ovaries, and use of progestational agents.

Proposed Course: Data will be collected through 1982. Analysis of the data will be performed by the Environmental Epidemiology Branch.

Date Contract Initiated: October 1, 1981.

Current Annual Level: $98,000

MAYO FOUNDATION (NO1-CP-01057)

Title: Leukemia Following Chemotherapy for Ovarian Cancer.

Contractor's Project Director: George D. Malkasian, M.D.

Project Officer (NCI): Mark H. Greene, M.D.

Objectives: Identify, abstract, and follow up approximately 1,500 one-year survivors of ovarian cancer to document the occurrence of second tumors, particularly leukemia. Quantify the risk of subsequent malignancy in relation to the antineoplastic drugs employed for the ovarian cancer.

Methods Employed: Suitable cases will be culled from 6,000 women with ovarian cancer treated at the Mayo Clinic between 1950 and 1979. Data will be abstracted from hospital records using an instrument developed by NCI/EEB. Data collection will focus on a detailed summary of ovarian cancer treatment. All patients will be actively followed to identify those who develop second cancers. All leukemia cases will be reviewed by an independent pathology panel. Death certificates will be sought on all deceased patients. The data will be pooled with identical data being collected from three additional sources (the M.D. Anderson Hospital, the Gynecologic Oncology Group, and the

Princess Margaret Hospital of Toronto) to evaluate in detail: (a) the relation between ovarian cancer treatment and leukemia risk; (b) possible dose-response relationships; (c) possible chemotherapy-radiation therapy interactions in leukemia risk; (d) possible differences in leukemia risk of different chemotherapeutic agents; and (e) whether a leukemia-prone subset of ovarian cancer patients can be identified.

Major Findings: All data abstraction, patient follow-up and location of pathology material is complete. The data are now being keypunched and edited; analysis (to be done by the NCI Project Officer) will commence in the summer of 1982.

Significance to Biomedical Research and the Program of the Institute: This project is the linchpin of the NCI/EEB Late Effects of Cancer Therapy Program, which is designed to evaluate the potential carcinogenic effects of various modalities used in the cancer treatment. One of the main goals of this project is to identify specific agents which are particularly safe or hazardous. Considerable data suggest that melphalan, a first line agent for ovarian cancer, is leukemogenic in man. Cyclophosphamide is equally effective clinically, but has not been systematically studied. The Mayo Clinic is the only institution known to have used single agent cyclophosphamide extensively in the treatment of ovarian cancer. This will permit a comparison of this agent's late effects with those of melphalan. These studies will also elucidate mechanisms of carcinogenesis in general.

Proposed Course: This contract is now complete. It terminated December 30, 1981.

Date Contract Initiated: September 30, 1980.

Current Annual Level: $97,000.

MEMORIAL SLOAN-KETTERING CANCER CENTER (N01-CP-01050)

Title: Cell Proliferation and Susceptibility to Cancer of the Large Intestine.

Contractor's Project Director: Martin Lipkin, M.D.

Project Officer (NCI): William A. Blattner, M.D.

Objectives: This project was established to explore the possible utility of an in vitro cell proliferation assay involving colon biopsies in an epidemiologic context. Various high- and low-risk groups from specified age groups are to be studied.

Methods Employed: Colonic biopsies are to be collected by the contractor on various well-defined risk groups. These are to be placed in short-term tissue culture and studied by radioautography. Raw data are to be entered into a computer for statistical analysis.

Major Findings: This is the final year of a two-year study. Specimen collection has been completed. Over 524 samples have been collected, 75 percent of which have been processed, and computer data entry is under way. Sophisticated statistical approaches have been applied to groups of patients with positive family histories, and an improved discriminant for distinguishing between normal and high-risk patients has been developed. Using this statistical approach, high-risk individuals have been evaluated and the distribution compared to that expected if risk is 50 percent for an individual high-risk patient. A manuscript describing these results has been accepted for publication. Analysis of various high- and low-risk groups is underway and manuscripts concerning these studies are being prepared.

Significance to Biomedical Research and the Program of the Institute: The assay procedure being studied under this procurement may represent a final common pathway in the pathogenesis of the malignantly transformed colon cancer cell. The abnormalities detected by this procedure mirror those seen in experimental models where carcinogens induce similar changes in rat colons. Characterization of the factors that modulate this phenotype in humans may help validate this test as a tool for studying human colon cancer carcinogenesis.

Proposed Course: Data collected over the first 18 months of the study are now being processed and prepared for final analysis and publication.

Date Contract Initiated: September 26, 1980.

Current Annual Level: $98,000.

MINNESOTA, UNIVERSITY OF (NO1-CP-43384)

Title: Immunodeficiency -- Cancer Registry

Contractor's Project Director: John H. Kersey, M.D.

Project Officer (NCI): Robert N. Hoover, M.D.

Objectives: To study the frequency and possible determinants of malignancy in immunodeficient patients.

Methods Employed: Through the cooperation and good will of immunologists in many of the specialized immunology centers throughout the world, clinical information and occasionally biological specimens from immunodeficient patients who have developed malignancy are sent to the registry for tabulation and analysis. During the past year there has been continued monitoring for new cases of malignancy in patients with genetically determined immunodeficiency syndromes. In addition, selected clinical and pathological materials have been analyzed, particularly data on the surface marker characteristics of malignant lymphoblast cells in these patients. The registry has participated in the development of a large pool of individuals with one immunodeficiency disease, Wiskott-Aldrich syndrome. The emphasis in this study is to identify families who carry the gene for this syndrome, and to study in some detail the characteristics of risks of the individuals with

this syndrome, as well as the carriers of the gene. In addition, a detailed description of the malignancy experience of patients with ataxia-telangiectasis has been performed.

Major Findings: To date 450 cases have been reported to the the registry. Lymphoreticular malignancies predominate in almost all of the Genetically Determined Immunodeficiency Disease categories. Other tumors that appear to be excessive include stomach cancer (in adults only), malignant melanoma, soft tissue sarcoma, and hepatobiliary carcinomas. A special study of Wiskott-Aldrich families indicated a similar overall attach rate among the carriers of the gene and non-carriers, but an earlier age-at-onset among carriers (nine years). Computerization of the registry information has been completed.

Significance to Biomedical Research and the Program of the Institute: Patients with altered immunologic states have demonstrated excess risks of malignancy several hundred times that of the general population. Study of the determinants of these excesses has a high probability of shedding light on immunologic aspects of cancer etiology.

Proposed Course: Case findings will continue to assure a continuing increase in the amount of material available for analysis in the registry. The analysis of the study of the Wiskott-Aldrich families will be expanded, and the data will be analyzed to estimate a number of relative risks according to the presence of certain clinical characteristics. A case-control study of Wiskott-Aldrich patients who have developed malignancy will be instituted in order to test the hypothesis that the risk of malignancy may be related to the amount of immunostimulation experienced by these children. This contract will be transferred to the Carcinogenesis Extramural Program this year as a resource contract.

Date Contract Initiated: July 1, 1974.

Current Annual Level: $111,483

MINNESOTA, UNIVERSITY OF (NO1-CP-91014)

Title: Long-Term Mortality Study of Minnesota Iron-Ore Miners.

Contractor's Project Director: Leonard M. Schuman, M.D.

Project Officer (NCI): William J. Blot, Ph.D.

Objectives: To clarify the role of iron and its compounds in human cancer by studying the mortality experience of iron-ore miners in the Mesabi Range, Minnesota, where competing exposures from other environmental agents are minimal or nonexistent.

Methods Employed: Employee records of iron-ore mining companies were reviewed to assemble a large cohort of hourly-wage employees involved in hematite mining prior to 1965. General employment data were abstracted. Vital status of the cohort was determined, death certificates obtained, and age- and

calendar-specific mortality rates calculated. Mortality information was obtained for the years 1944 to 1978, excluding 1945, which was not available.

Major Findings: A cohort of 13,000 iron-ore miners has been assembled, relevant work histories abstracted, and follow-up for vital status completed. Preliminary analyses indicate no overall excess of lung cancer among the total cohort when compared to U.S. rates, but some excess was noted among foreign-born workers. Stomach cancer rates were also high.

Significance to Biomedical Research and the Program of the Institute: Studies of hematite miners in Great Britain have shown them to be at increased risk of dying from respiratory cancer; however, it is not clear whether iron-containing materials are the causative agents, since other potentially carcinogenic exposures were present. This project is intended to clarify the role of iron and its compounds.

Proposed Course: This contract was completed in January 1982.

Date Contract Initiated: July 1, 1979.

Current Annual Level: No cost extension.

MINNESOTA, UNIVERSITY OF (NO1-CP-21015)

Title: Risk of Cancer in X-Ray Technologists.

Contractor's Project Director: Leonard M. Schuman, M.D.

Project Officer (NCI): John D. Boice, Jr., Sc.D.

Objectives: To evaluate the long-term effects of chronic exposures to radiation experienced because of occupation, among 170,000 registered American radiological technologists.

Methods Employed: Using the resources of the American Registry of Radiologic Technologists, inactive members (30,000) will be located to determine vital status and cause of death. Living members will be contacted by mail questionnaire to determine cancer incidences, and to obtain information on the use of dosimeters and cancer risk factors such as smoking history.

Major Findings: A feasibility study was completed which determined that inactive members of the society could be located, and that it was possible to characterize radiation exposure based on length of employment, film badge readings, and questionnaire responses.

Significance to Biomedical Research and the Program of the Institute: The study will evaluate the effects of low-dose fractionated exposures received over a period of many years in a large group of occupationally exposed women. The two most sensitive organ sites for radiation carcinogenesis in women, the breast and thyroid, will be the focus of this investigation. This project is an important part of the overall Federal response to establish a broad-based program in radiation carcinogenesis and has direct relevance to cancer etiology and the setting of radiation protection guidelines. This study is

unique in that the average doses will be relatively low (5-30 rads), and extrapolation from high-dose data to estimate low-dose risks will not be necessary.

Proposed Course: Data collection will proceed for approximately 2 1/2 years followed by analysis and publication of findings. Afterwards, selected technologists may be sent mail questionnaires at 5-year intervals, and the National Death Index will be used for mortality follow-up.

Date Contract Initiated: February 22, 1982.

Current Annual Level: $257,596.

MINNESOTA, UNIVERSITY OF (NO1-CP-01033)

Title: A Study of Environmental Factors in the Origin of Leukemia and Non-Hodgkin's Lymphoma among Adult White Males from Rural Areas.

Contractor's Project Director: Leonard Schuman, M.D.

Project Officer (NCI): Aaron Blair, Ph.D.

Objectives: To collect information to evaluate the role of environmental determinants (particularly agriculturally related) in the origin of leukemia and non-Hodgkin's lymphoma.

Methods Employed: The project is a case-control design. Three hundred histologically confirmed cases for each tumor and 600 matched controls among adult white males will be selected and interviewed to determine personal habits and occupational exposures. Controls (meeting age, race, and sex requirements) will be randomly selected from the general population.

Major Findings: This contract was initiated September 30, 1980, and insufficient time has elapsed for major findings to become available. Since the award, hospitals have been contacted and their cooperation obtained, data collection instruments have been developed, and cases and controls are now being interviewed.

Significance to Biomedical Research and the Program of the Institute: This contract will provide needed data on the origin of leukemia and non-Hodgkin's lymphoma. Geographic studies of these cancers by the Branch have suggested new leads, particularly in the area of farm-related exposures, that urgently need to be evaluated. The increase in non-Hodgkin's lymphoma in recent years and the limited effort devoted to the epidemiology of this cancer further underscore the need for this project.

Proposed Course: The expiration date is September 29, 1983. During FY83, approximately 200 cases and 500 controls will be identified and interviewed.

Date Contract Initiated: September 29, 1980.

Current Annual Level: $181,069.

NATIONAL ACADEMY OF SCIENCES (NO1-CP-53573)

Title: Epidemiologic Studies in Etiology of Cancer in Veterans.

Contractor's Project Director: Seymour Jablon, M.A.

Project Officers (NCI): John D. Boice, Jr., Sc.D. and Judy Walrath, Ph.D.

Objectives: To develop and conduct a broad program of epidemiologic studies among veterans.

Methods Employed: The Epidemiology Branches of the National Cancer Institute and the Medical Follow-up Agency of the National Academy of Sciences have developed an epidemiology program designed to make efficient use of the military-veteran population, utilizing medical, demographic, and environmental observations of veterans ascertained through facilities of the Veterans Administration, and supplemented by mortality data. During the past year, efforts have focused on completion of follow-up studies of veterans who have certain conditions (e.g., epididymitis) that may influence the risk of cancer. Also completed is a study of occupational exposure to tetrachloroethane, a chlorinated hydrocarbon used for impregnating clothing against mustard gas during World War II. Evaluation of the large Dorn study on smoking regarding occupational risk has continued, as has the study of adjuvant drug carcinogenesis in VA clinical trials.

Major Findings: A survey of men serving in chemical processing companies during WWII revealed no excess mortality from cancer that could be attributed to tetrachlorethylene or dry cleaning solvents that have been carcinogenic in laboratory animals (JOM 23:818-822, 1981). As part of a large cooperative study, evaluation of a drug used as adjuvant therapy for gastrointestinal cancer, a nitrosourea, was found to increase the risk of subsequent leukemia.

Significance to Biomedical Research and the Program of the Institute: The Environmental Epidemiology Branch, NCI, is concerned with studies to identify and clarify environmental and host factors in cancer. Evaluation of the risk of developing second cancers following low-dose adjuvant chemotherapy has important implications regarding the NCI cancer therapy program, and may also provide insights into the mechanism of carcinogenesis. The VA-Surgical Onocology Group is a unique resource in this regard. The evaluation of the large veteran population in the Dorn study will be beneficial to the occupational studies program due to the unique combination of detailed data on smoking, occupation, and industry. The objectives can be more readily achieved by coordinating efforts with the unique resources and competent staff of the Medical Follow-up Agency.

Proposed Course: A study will continue of cancer mortality, among 300,000 U.S. veterans, by occupational and smoking habits, updating the follow-up of the cohort originally assembled by Dorn. A study of testicular cancer will continue to evaluate various risk factors, including mumps, orchitis, and genitourinary defects. The evaluation of the risk of developing a second cancer following adjuvant chemotherapeutic drugs will be analyzed in nine selected clinical trials.

Date Contract Initiated: June 28, 1971.

Current Annual Level: $329,655.

NATIONAL ACADEMY OF SCIENCES (NO1-CP-01012)

Title: Epidemiologic Studies of Cancer Among A-bomb Survivors.

Contractor's Project Director: Hiroo Kato, M.D.

Project Officer (NCI): Charles E. Land, Ph.D.

Objectives: The objectives of this collaborative study are to identify and quantify the possible interactive roles of radiation and other environmental and host risk factors in the development of certain cancers, and to carry out other studies of cancer risk among members of the A-bomb survivor population.

Methods Employed: Investigations based on the Life Span Study sample of 82,000 A-bomb survivors and 26,000 non-exposed individuals, and a clinical subsample of 12,000 survivors and controls, are carried out at the Radiation Effects Research Foundation (RERF) in Hiroshima and Nagasaki, Japan. All studies involving new or unpublished data are collaborative, and include investigators from NCI, RERF, and outside organizations as required; collaboration is facilitated by personnel exchanges between RERF and NCI. Methods include cohort studies of cancer incidence as determined from death certificates, tumor and tissue registries, and searches of hospital and clinical records, and case-control studies in which epidemiological factors other than radiation, as determined from existing records or by interview, are investigated. Reviews of diagnostic material by panels of pathologists may be employed in connection with the studies.

Major Findings: Interviews for breast cancer cases and controls identified through 1980 were completed, and preliminary analyses undertaken to examine major epidemiologic risk factors and their possible interactions with radiation dose in the causation of breast cancer. Dr. Norihiko Hayakawa of Hiroshima University participated in the preliminary analyses as a visitor to the Radiation Studies Section during March-April. Breast cancer incidence in the LSS sample was ascertained through 1980, and rates were analyzed for variation by age at the time of the bombings, radiation dose, and time after exposure. Dr. Masayoshi Tokunaga of Kagoshima University, who carried out the case ascertainment for the breast cancer incidence study, also did serial sections of breast tissue from the RERF autopsy series, and evaluated the slides histologically during a 3-month visit to NCI. Interviews of next-of-kin of lung cancer cases and age-sex matched controls were completed, and arrangements made to analyze these data next year, when Dr. Akiba will visit the Branch for several months.

The most noteworthy finding has been the appearance of a dose-related excess breast cancer risk among women who were under 10 years of age in 1945. Five hundred sixty-five cases of breast cancer were identified for the period 1950-1980, an increase of more than 200 over the previous survey covering the period 1950-1974. Twenty-four of these cases occurred among the 0-9 ATB cohort. The statistically significant increasing trend with increasing dose in this cohort is the first strong evidence that underdeveloped breast tissue

is susceptible to radiation carcinogenesis. Other findings of previous series, including linearity of dose response, similar temporal distributions for radiation-induced and other breast cancers, and increased risk among women exposed premenopausally were strengthened by the new data.

Significance to Biomedical Research and the Program of the Institute: The project should substantially contribute to our knowledge of whether and to what extent radiation may interact with other known risk factors in inducing certain cancers. Dose-response relationships, and the influence of such factors as age at exposure and at observation for risk on the response to radiation, should be clarified by more intensive investigation of certain established cancer effects. The existence of the collaborative agreement permits great flexibility in initiating investigations of theoretical and practical interest to the NCI program in the epidemiology of radiation carcinogenesis that probably would receive much lower priorities in the RERF research program.

Proposed Course: Increased emphasis will be placed on studies at the level of incidence, in which case ascertainment from all available sources will be combined with pathological reviews and with case-control interview studies aimed at the elucidation of risk factors other than radiation dose. Likely candidates for the coming year include thyroid cancer for which an incidence survey is already underway at RERF, and colon cancer. Analyses of the case-control interview data for breast cancer and lung cancer will be completed.

Date Contract Initiated: November 10, 1979.

Current Annual Level: $227,835.

NATIONAL CENTER FOR HEALTH STATISTICS (YO1-CP-10503)

Title: Follow-up of the Health and Nutrition Examination Survey Cohort (HANES).

Contractor's Project Director: Helen Barbano.

Project Officer (NCI): Aaron Blair, Ph.D.

Objectives: To follow up and interview the national sample of approximately 14,000 examinees who were 25 years or older at the first survey, in order to assess their current health status. These data will be extremely valuable in evaluating cancer/diet hypotheses.

Methods Employed: The contractor will trace all members of the cohort to determine their vital status. If deceased, death certificates will be obtained. Interviews will be conducted of survivors and next-of-kin of decedents, probing for dietary habits, occupational history, medical history, and other pertinent factors. These data will be coded, edited, and computerized, and made available to NCI for analysis.

Major Findings: Additional time is necessary to produce a significant report.

Significance to Biomedical Research and the Program of the Institute: NCI is vitally interested in the role of nutritional factors in the origin of cancer. This project provides a unique opportunity to evaluate the role of diet in cancer development in a population. The availability of dietary data on study subjects obtained before the clinical onset of cancer eliminates the problems of recall bias that may occur in case-control studies.

Proposed Course: Continue what is described above under Methods.

Date Contract Initiated: June 18, 1981.

Current Annual Level: $100,000.

NAVAL MEDICAL RESEARCH INSTITUTE (YO1-CP-00502)

Title: Immunologic and Immunogenetic Studies of High-Risk Cancer Families and Logistical Support Services.

Contractor's Project Director: Douglas Michael Strong, Ph.D.

Project Officer (NCI): William A. Blattner, M.D.

Objectives: This project was established to promote studies of the role of immunologic and immunogenetic factors in the etiology of cancer. As part of this effort, support was provided for maintaining a large repository of sera, plasma, white blood cells, and other biological materials obtained on members of high-risk families since 1974. Recently this aspect of the effort was transferred to a separate procurement.

Methods Employed: Biologic specimens on high-risk families are processed for cryopreservation. When materials on all members of a single family or group of families or study cohort are collected, specific immunologic studies are performed according to written protocol. Methods include HLA and B-cell alloantigen typing, PLT and MLC typing, in vitro immune studies of proliferative response, assays of regulatory cell function, natural killer cell activity, and quantification of subpopulations of immunoregulatory populations utilizing a fluorescence activated cell sorter (FACS-II).

Major Findings: This project has focused major attention on establishing the laboratory, purchasing equipment, and implementing study protocols. Several major tasks have been accomplished: (1) Large quantities of human T-cells from homozygous human donors were grown and sent to Dr. Jack Strominger, who is extracting the DNA from these cells for gene cloning experiments aimed at molecular study of histocompatibility genes. As a by-product, cell surface membrane preparations are being returned to the laboratory for use in attempting the establishment of hybridoma antibodies against Dr antigens. (2) Cell strains infected and not infected with a new human T-cell lymphoma virus were typed for HLA and B-cell alloantigens. Those cells known to contain the virus are untypable for HLA, while cell strains from the same patient are reproducibly typable. HLA-Dr typing is not affected by the presence of the virus, helping to confirm the genetic source of the various cell strains. A manuscript concerning these data has been submitted for publication. The

scrambling of HLA cell surface gene products by the virus, causing a nonsense antigen to be produced, may be related to the mechanism by which the virus-infected cells are successfully propagated in the host. These cells, bearing altered self-antigens, may accumulate in an abnormal way because the usual cell surface markers are unrecognized by the host. Molecular studies of the HLA genome and its alteration by virus are under way in collaboration with Dr. Strominger. (3) A familial chronic lymphocytic leukemia cluster was studied using the FACS-II. Previously-unrecognized cell surface markers, shared in a number of cases, were identified. A case previously known to have chronic lymphocytic leukemia (CLL) appears to have reverted to normal except for a slight perturbation in B-cell profile that may indicate a persistance. One sibling successfully treated for lung cancer has an altered percentage of suppressor cells. In vitro assessment of this abnormality is planned. (4) A major new tumor has been the study of immunologic perturbation in male homosexuals at high-risk for a variety of immune-related malignancies. These results have been published and a large case-control laboratory study is under way to follow up these results.

Samples have been collected on a number of families, including: (1) one with hairy cell leukemia, (2) one with CLL and multiple cases of unexplained lymphocytosis, (3) one family with multiple cases of non-Hodgkin's lymphoma and unexplained immunoglobulin abnormalities in close relatives, and (4) one family with multiple cases of mycosis fungoides.

Significance to Biomedical Research and the Program of the Institute: This interagency agreement represents the only Institute research project specifically targeted at evaluating immunogenetic factors in human neoplasia. In addition, the immunogenetic program provides support for a large segment of NIH research aimed at studying HLA and disease associations, especially in terms of newly-defined antigen systems, such as MT and MB.

Proposed Course: During the next year, we anticipate that research performed under the current agreement will proceed at a much more rapid pace since personnel and equipment problems have been solved. Specimens are now available on a number of families that will allow rapid implementation of several currently developed protocols. Collaboration in the study of the immunogenetic aspects and immunologic effects of a newly discovered candidate human leukemia virus affords a unique opportunity for clarifying etiologic relationships.

Date Contract Initiated: June 20, 1980.

Current Annual Level: $505,000.

NEW JERSEY, STATE OF, DEPARTMENT OF ENVIRONMENTAL PROTECTION (NO1-CP-91048)

Title: Environmental Health Data Base for New Jersey.

Contractor's Project Director: Thomas A. Burke, M.P.H.

Project Officer (NCI): Thomas J. Mason, Ph.D.

Objectives: To develop an environmental data base for New Jersey which will be utilized in their ongoing intramural research projects.

Methods Employed: The water supply questionnaire developed by NCI will define the basic water quality information to be obtained for each purveyor in New Jersey. Records of DEP will be examined to determine how much of the needed information is on record, and all relevant data will be abstracted. Information not available from DEP records will be acquired through personal interviews with representatives of water purveyors and through examination of purveyors' records. Additional information on toxic substances and carcinogens in raw and finished water will be provided by the Program on Environmental Carcinogens and Toxic Substances. Completed questionnaires will be forwarded to NCI for coding and keypunching. Additional information not covered by the questionnaire will be coded and keypunched by Rutgers University and forwarded to NCI on computer tapes.

Major Findings: A total of 307 NCI Water Supply Data Abstracting Forms was completed, covering all water purveyors serving over 1,000 people. This amounts to coverage of over 95 percent of the New Jersey population. Information collected on these forms supplied the foundation for the drinking water section of the data base project. This information includes: (a) definition of service areas, population served, and raw water sources for each purveyor; (b) determination of historical and current chlorination and treatment practices; and (c) identification of potential raw water pollution sources, including all upstream dischargers.

Altogether 304 of the purveyors were sampled for volatile organic compounds including the trihalomethanes and heavy metals. Metal analysis was performed by the EPA laboratory in Cincinnati, Ohio. Analysis for organic compounds was performed in Cambridge, Massachusetts. This sampling has provided NCI and New Jersey DEP with the following benefits: (a) quantitative water quality information on the drinking water of over 95 percent of the New Jersey population; (b) a better understanding of the relationship between chlorination practices and the amount of trihalomethane in finished drinking water; and (c) a better understanding of the relationship between water source pollution and finished water quality.

A 12-month subcontract was initiated with Rutgers University Department of Geography to assist in the compilation of the drinking water information. Rutgers was responsible for documenting all sampling and collecting current and historic water quality information which could not be obtained from the records of DEP.

All sampling and questionnaire information was submitted to the National Cancer Insitute for computerization. A computer tape containing all the information will be returned to New Jersey for inclusion in the data base. An investigation of seasonal variation of trihalomethanes in 15 selected drinking water supplies was conducted. The raw, treated, and delivered water of each supply was sampled at three different times over a six month period to measure variation. To ensure laboratory accuracy, samples were split and analyzed by the ERCO Labs and the DEP consultant laboratory at Rutgers. Results of these investigations will provide DEP and NCI with the seasonal variation of trihalomethanes in public drinking water supplies.

Significance to Biomedical Research and the Program of the Institute: This project will provide needed specificity concerning the exposure of persons in areas of the United States which have high rates of cancer mortality, and in which water is suspect as a source of carcinogenic chemicals.

Proposed Course: This contract will expire in September, 1982.

Date Contract Initiated: September 30, 1979.

Current Annual Level: $165, 000.

NEW JERSEY, STATE OF, DEPARTMENT OF HEALTH (NO1-CP-61031)

Title: Etiologic Studies of Cancer in New Jersey

Contractor's Project Director: Ronald Altman, M.D.

Project Officer (NCI): Thomas J. Mason, Ph.D.

Objectives: To examine the cancer mortality experience in the State of New Jersey with specific emphasis on quantifying risk factors for bladder cancer and lung cancer, as well as other anatomic sites with known or suspected occupational risk factors.

Methods Employed: Descriptive Studies: A system of computer programs has been developed which, with modification, can efficiently calculate sex- and -race-specific rates for any cause of death for the period of time 1962-75. Refinement of the data to municipalities and specific analyses to quantify biases in the approach will be pursued. Case-control Studies: The contractor will interview selected cancer case, and matched controls (or their families when appropriate) to ascertain occupational, environmental, and personal characteristics of the study population. These studies will be performed in areas which have been found to have exceptionally high mortality rates.

Major Findings: The contractor has concentrated on several projects during the July 1, 1981 to June 30, 1982 contract year. These activities included the publication of a two-volume atlas of cancer mortality in New Jersey, field operations for a population-based, retrospective case-control study of lung cancer, and analyses of data collected during retrospective, case-control bladder cancer studies.

The first volume of the atlas of the descriptive epidemiology of cancer mortality in New Jersey presents summary information, descriptive analysis, discussion of the results of statistical analysis, and graphic displays of basic data. The second volume is a statistical appendix. This atlas will facilitate the selection of areas within the state where further study would seem warranted.

The lung cancer study is in its data collection phase. Our current estimates of response rates are between 84-89 percent for cases and from 78-82 percent for controls. Several distinctive features of lung cancer mortality during the 1949-1976 period have come to light. First, cancers of the trachea,

bronchus, and lung are perhaps the most important causes of cancer mortality in New Jersey. Second, lung cancer mortality rates for county populations in New Jersey consistently exceeded national lung cancer mortality rates throughout the 1950-1975 period. Third, and perhaps most important, geographic distribution of lung cancer mortality rates is distinctly non-uniform throughout New Jersey. Refinement of the geographic patterns of lung cancer mortality rates to the municipality level showed several areas within the State with distinct concentrations of significantly high lung cancer mortality rates for white males. The retrospective case-control study which is ongoing is examining these high risk areas to determine occupational and environmental factors responsible for this geographic pattern. This study will permit the NCI to evaluate the possible preventive role of dietary vitamin A.

Significance to Biomedical Research and the Program of the Institute: Recent reports of excessive mortality from bladder cancer in New Jersey need to be followed up by means of a project such as this to assess the relative contribution to this excess from industrial (occupational) as well as common environmental exposures. This type of investigation also fits in well with the specific interest in the geographic distribution of malignancy in the United States to which the Environmental Epidemiology Branch is committed.

Proposed Course: Additional analyses of data collected during the National Survey of Environment and Health (a national bladder cancer study) will be conducted. One approach will involve refinement of the risks identified as statistically significant in the initial analyses by studying length and intensity of exposure and age at first exposure. The issues of interaction between smoking and occupation will be further investigated by stratification and multivariate techniques.

The interview phase of the retrospective study of male lung cancer in high risk areas will be completed for 900 cases and 900 controls during the current contract period. Analyses of risk associated with cigarette smoking, occupation, and dietary vitamin A will be evaluated. These factors will be studied in the total male population, and in subgroups of cases with specific histologic types.

Case findings in the study of lung cancer among New Jersey males will be continued in the proposed contract period for the nonwhite population to enable calculation of race-specific risks for cigarette smoking, employment and dietary vitamin A.

A study of lung cancer among females in New Jersey will include all incident cases diagnosed during a one-year period beginning July 1, 1982, and a population-based series of controls. Analyses of these data will follow those proposed for the study of males.

Date Contract Initiated: February 10, 1976.

Current Annual Level: $500,000.

NEW YORK STATE HEALTH DEPARTMENT (NO1-CP-11028)

Title: Epidemiologic Study of Mesothelioma Risk Factors Utilizing Population-Based Tumor Registries.

Contractor's Project Director: Nicholas Vianna, M.D.

Project Officer (NCI): Robert Spirtas, Dr. P.H.

Objectives: To collect information to evaluate the role of occupational exposure in the origin of mesothelioma. Asbestos miners, insulators, and shipyard workers are known to be at higher risk for mesothelioma, but information on other industries where exposures to asbestos and other fibrous products are lower is incomplete. This study is designed to fill this gap.

Methods Employed: The project has a case-control design. Cases of mesothelioma from tumor registries and their matched controls will be interviewed (next-of-kin will be interviewed if the cases are deceased) to obtain work histories and other information pertinent to the origin of this tumor.

Major Findings: Additional time is necessary to produce a significant report.

Significance to Biomedical Research and the Program of the Institute: This contract will provide data needed to estimate the risk of low-level asbestos exposures in a variety of industries and occupations. The rising incidence of this tumor and the widespread exposure to asbestos underscores the need to identify new exposure groups so that preventive action may be taken.

Proposed Course: Continue data collection.

Date Contract Initiated: September 30, 1981.

Current Annual Level: $96,805.

PENNSYLVANIA, UNIVERSITY OF (NO1-CP-91047)

Title: Special Projects in Hereditary Cutaneous Melanoma.

Contractor's Project Director: Wallace H. Clark, Jr., M.D.

Project Officer (NCI): Mark H. Greene, M.D.

Objectives: To perform ultrastructural (electron microscopic) analysis of precursor nevi and related pigmented lesions. To develop educational materials for members of melanoma-prone families, the physicians involved in their care, and the pathologists responsible for interpreting pigmented lesion biopsies from high-risk family members. To perform light microscopic evaluation of the pigmented lesions removed from persons at high-risk of malignant melanoma.

Methods Employed: Three separate educational programs are to be produced using the clinical, photographic, and histologic material collected during studies of melanoma-prone families. The production work has been subcontracted to E.J. Stewart, Inc. Selected pigmented lesions (including junctional nevi, compound nevi, spindle cell tumors, superficial spreading melanoma, and lentigo maligna melanoma) have been identified, fixed, and appropriately sectioned for electron microscopic study. Pigmented lesions removed from members of high-risk families will be collected and submitted to the contractor for standard evaluation.

Major Findings: The three educational videotapes are now in nationwide distribution under a free-loan program sponsored by NCI. These programs have been received with extraordinary enthusiasm by the medical community; it is estimated that 55,000 persons will have viewed these programs by the end of calendar year 1982. The routine light microscopy review of pigmented cutaneous lesions from persons at increased risk of melanoma has provided invaluable, objective data in support of EEB's Hereditary Cutaneous Melanoma Project. The electron microscopy study has strengthened the evidence suggesting an etiologic relationship between dysplastic melanocytes and the malignant melanocytes of radial growth phase melanoma. At the same time, some key differences between these two types of pigment cell abnormalities have been found, which should facilitate improved diagnostic accuracy in borderline melanocytic lesions.

Significance to Biomedical Research and the Program of the Institute. The dysplastic nevus syndrome has now been clearly identified as etiologically important in both hereditary and sporadic melanoma. The light microscopy studies have permitted classification of members of high-risk families into subgroups of patients who are at either high-risk or normal risk of melanoma. All of these findings will contribute to the eventual control of mortality from this potentially lethal tumor.

Proposed Course: This contract has been completed. Although it terminated in September, 1981, the Final Report was not received until March 1982.

Data Contract Initiated: September 27, 1979.

Current Annual Level: $98,000.

SOCIAL SECURITY ADMINISTRATION (YO1-CP-20513)

Title: Mesothelioma and Employment: Utilization of SSA Quarterly Earnings File.

Contractor's Project Director: Faye Aziz.

Project Officer (NCI): Gilbert W. Beebe, Ph.D.

Objectives: To study the feasibility of using particular SSA administrative records in the furtherance of epidemiological and other health-related studies, and to compare the quality and completeness of industrial employment history information obtainable through SSA vs. next-of-kin interviews.

Methods Employed: A computer file consisting of identification and work history information on an estimated 100 cases of mesothelioma and 100 controls will be supplied by NCI. SSA will (1) abstract lifetime employment history for each of these 200 persons from the SSA Quarterly Earnings File and translate Employer Identification Number into 4 digit SIC code; and (2) collaborate with NCI on statistical analysis of resulting data file.

Major Findings: Additional time is necessary to produce a significant report.

Significance to Biomedical Research and the Program of the Institute: SSA files may be used to build employment histories for individuals in case-control studies. These histories would be free of the retrospective bias that accompanies such studies when information on exposure is obtained after the fact. They also go back in time longer than the period of probably reliable recall of the next-of-kin. Because the hazards of the workplace are very real and SSA records cover most of the employed population of the U.S., SSA records should be highly informative as to those hazards, at least insofar as mortality and disability effects are concerned.

Proposed Course: Continue data collection.

Date Interagency Agreement Initiated: May 1, 1982.

Estimated Annual Level: $35,000.

SOUTHERN CALIFORNIA, UNIVERSITY OF (NO1-CP-11042)

Title: Epidemiologic Study of Meosthelioma Risk Factors Utilizing Population-Based Tumor Registries.

Contractor's Project Director: Brian Henderson, M.D.

Project Officer (NCI): Robert Spirtas, Dr. P.H.

Objectives: To collect information to evaluate the role of occupational exposure in the origin of mesothelioma. Asbestos miners and insulators and shipyard workers are known to be at higher risk for mesothelioma, but information on other industries where exposures to asbestos and other fibrous products are lower is incomplete. This study is designed to fill this gap.

Methods Employed: The project has a case-control design. Cases of mesothelioma from tumor registries and their matched controls will be interviewed (next-of-kin will be interviewed if the cases are deceased) to obtain work histories and other information pertinent to the origin of this case tumor.

Major Findings: Additional time is necessary to produce a significant report.

Significance to Biomedical Research and the Program of the Institute: This contract will provide data needed to estimate the risk of low-level asbestos exposures in a variety of industries and occupations. The rising incidence of

this tumor and the widespread exposure to asbestos underscores the need to identify new exposure groups so that preventive action may be taken.

Proposed Course: Continue data collection.

Date Contract Initiated: September 30, 1981.

Current Annual Level: $102,140.

TEXAS, UNIVERSITY OF, MEDICAL BRANCH (NO1-CP-91025)

Title: Etiologic Study of Respiratory Cancer in Coastal Texas.

Contractor's Project Director: Patricia A. Buffler, Ph.D.

Project Officers (NCI): Thomas J. Mason, Ph.D and Linda W. Pickle, Ph.D.

Objectives: To examine the respiratory system cancer experience in selected areas of the State of Texas with specific emphasis on quantifying risk factors.

Methods Employed: The methodology is that of a case-control interview study. The contractor interviewed each selected respiratory cancer case and matched controls (or their families when appropriate) to ascertain occupational, environmental, and personal characteristics of the study population. This study was performed in areas which have been found to have exceptionally high mortality rates.

Major Findings: Detailed analysis commenced in July, 1982. Preliminary analyses should be completed by Fall, 1982.

Significance to Biomedical Research and the Program of the Institute: Recent reports of excessive mortality from lung cancer in Texas pointed to the need for a project such as this to assess the relative contribution to this excess from industrial (occupational) as well as common environmental exposures. This type of investigation contributes to studies in the geographic distribution of malignancy in the United States to which the Environmental Epidemiology Branch is committed.

Proposed Course: This contract expired in June 1982. An edited tape for analysis was delivered to the NCI.

Date Contract Initiated: September 30, 1979.

Current Annual Level: $165,000.

TEXAS, UNIVERSITY OF, SYSTEM CANCER CENTER (NO1-CP-01051)

Title: Familial Cancer in Melanoma Patients.

Contractor's Project Director: David Anderson, M.D.

Project Officer (NCI): Mark H Greene, M.D

Objectives: To define the incidence and types of cancer among first-degree relatives of patients with malignant melanoma in order to quantify the risk of melanoma among relatives and to identify other associated neoplasms. Pathologic verification of melanoma cases and other pigmented lesions will be obtained through a subcontract with the University of Pennsylvania. Differences in risk of familial cancer will be assessed by melanoma subtype, and an effort will be made to determine the frequency of the dysplastic nevus syndrome in these melanoma patients.

Methods Employed: Family history of cancer will be obtained from the relatives of 433 consecutive melanoma patients treated at the M.D. Anderson Hospital during 1969-1970. The contractor will locate all relevant pigmented lesion biopsy material and transmit it to the subcontractor for pathology review. Clinical photographs of the melanoma patients will be reviewed to assess the frequency of the dysplastic nevus syndrome in these patients. Risk of melanoma and other cancers in first-degree relatives of cases will be assessed by comparing their frequency to that predicted by general population incidence rates. Analysis will be done by the Project Officer at NCI.

Major Findings: The data collection is now complete. Coded data forms, the major deliverable under this contract, have been submitted to the Project Officer. Data processing at NCI has begun, and the analysis commenced in the summer of 1982.

Significance to Biomedical Research and the Program of the Institute: Having identified what is probably the single most important melanoma precursor to date, the dysplastic nevus syndrome, information on the frequency of this syndrome and melanoma risk among relatives of melanoma patients is now required. This information will permit more effective counseling and management of patients at high-risk of melanoma, and will also be invaluable in planning melanoma prevention and education programs.

Proposed Course: Although originally designed as a one-year contract, a five month extension without additional funds was effected in order to complete patient follow-up. This proved unusually difficult, since ten years had elapsed between the identification of the study group and its current evaluation. All pathologic material has been located and reviewed. This completed the Contractor's responsibilities, and the contract was terminated on February 26, 1982.

Date Contract Initiated: September 25, 1980.

Current Annual Level: $95,852.

VETERANS ADMINISTRATION (YO1-CP-20512)

Title: Mesothelioma and Employment: A Case-control Study Utilizing the Veterans Administration (VA).

Contractor's Project Director: Paul C. LeGolvan, M.D.

Project Officer (NCI): Robert Spirtas, Dr. P.H.

Objectives: To collect information to evaluate the role of occupational exposure in the origin of mesothelioma. Asbestos miners, insulators, and shipyard workers are known to be at high risk for mesothelioma, but information on other industries where exposures to asbestos and other fibrous products are lower is incomplete. This study supplements the study using cases from tumor registries by providing additional cases from the VA.

Methods Employed: The project is a case-control design. Cases of mesothelioma from the VA and their matched controls are being interviewed (next-of-kin will be interviewed if the cases are deceased) to obtain work histories and other information pertinent to the origin of this tumor.

Major Findings: Additional time is necessary to produce a significant report.

Significance to Biomedical Research and the Program of the Institute: This contract will provide data needed to estimate the risk of low-level asbestos exposures in a variety of industries and occupations. The rising incidence of this tumor and the widespread exposure to asbestos underscores the need to identify new exposure groups so that preventive action may be taken.

Proposed Course: Continue data collection.

Date Interagency Agreement Initiated: February 18, 1982.

Current Annual Level: $100,000.

YALE UNIVERSITY (NO1-CP-01029)

Title: Risk of Cancer Following Multiple Chest Fluoroscopies for Tuberculosis in Connecticut.

Contractor's Project Director: Jennifer Kelsey, Ph.D.

Project Officer (NCI): John D. Boice, Jr., Sc.D.

Objectives: To determine the long-term health effects of multiple low-dose radiation exposures in men and women, and to estimate the risk of radiation-induced leukemia, lung cancer, and breast cancer.

Methods Employed: All patients discharged alive from major Connecticut State tuberculosis hospitals between 1930 and 1952 are being studied. Hospital records are being used to determine the extent of the tuberculosis and the number of fluoroscopic examinations performed on each patient. Death certificates will be obtained for those patients who have died. The Connecticut Tumor Registry will be used to determine the incidence of all cancers in this population.

Major Findings: Progress has been made in the following areas: (1) abstraction of patient charts, (2) development of additional capabilities for determining deaths of patients, (3) tracing of patients at the state tuberculosis registry, (4) tracing of patients through city directories, and (5) modification of the Massachusetts mail questionnaire for use in Connecticut. Approximately 4,500 eligible patients have now had medical records abstracted. The total eligible population should be approximately 9,400.

Significance to Biomedical Research and the Program of the Institute: Persons repeatedly exposed to radiation over a period of years will be evaluated. This study will determine whether low-dose fractionated exposures are as effective in producing cancers as single high-dose exposures, and thus has relevance to cancer etiology. This project is also a major component of the Institute's expanded program on the biological effects of ionizing radiation.

Proposed Course: Date collection will proceed for approximately one more year, followed by analysis and publication of study findings.

Date Contract Initiated: September 30, 1980.

Current Annual Level: $345,692.

CARCINOGENESIS EXTRAMURAL PROGRAM

ORGANIZATIONAL OVERVIEW

The Carcinogenesis Extramural Program (CEP): (1) develops, evaluates and administers the Division's program of extramural research in cancer causation and prevention; (2) is responsible for program management, including improved methods and practices; (3) maintains liaison between extramural activities and various organizations and scientists; and (4) assists in allocating resources and evaluating program priorities for these activities. To accomplish its goals, the program makes use of a variety of instruments which include traditional research grants, interagency agreements, and contracts.

The CEP was established to integrate the management and coordination of these diverse activities. Technical review of all research proposals (contract and grant) is now conducted by the Division of Extramural Affairs (DEA) utilizing traditional peer review groups whose members are drawn from the outside scientific community. Steps are being taken to include the technical review of research support and resource contracts within the DEA structure. For contracts, review for relevance, priority and need are still performed by the senior staff of the Division of Cancer Cause and Prevention and all contract activities require concept approval by the Board of Scientific Counselors of the division.

The Carcinogenesis Extramural Program contains three branches: the Biological Carcinogenesis Branch, the Chemical and Physical Carcinogenesis Branch, and the Special Program Branch. It has a current on-board staffing level of 32 full-time permanent positions and has a budget of $131 million in Fiscal Year 1982.

Significant changes for Fiscal Year 1982: We have continued to reduce the level of contract support used in providing resources to the research community in general, and in addition have introduced cost recovery mechanisms into a number of our resource provision activities. Such modifications are expected to further reduce the cost of providing resources in subsequent years as well as resulting in the elimination of some of our resources for which a continuing need has not been demonstrated.

We have continued our efforts to reduce or phase out contract support of investigator initiated research in fields where grants provide adequate coverage. Funds made available in this way are being used to stimulate the development of high priority areas of research which are inadequately covered by grants. A number of such Requests for Grant Applications (RFAs) have been issued during this fiscal year, and others are now being developed for funding in the coming fiscal year. Responses to the RFAs so far released has been excellent.

The overall effects of these modifications continue to be: (1) a gradual transfer of current resources to a cost reimbursement system: (2) increased availability of funding to support the development of new resource activities to meet the changing needs of investigators; (3) the elimination of contract-supported research in areas adequately covered by research grant applications;

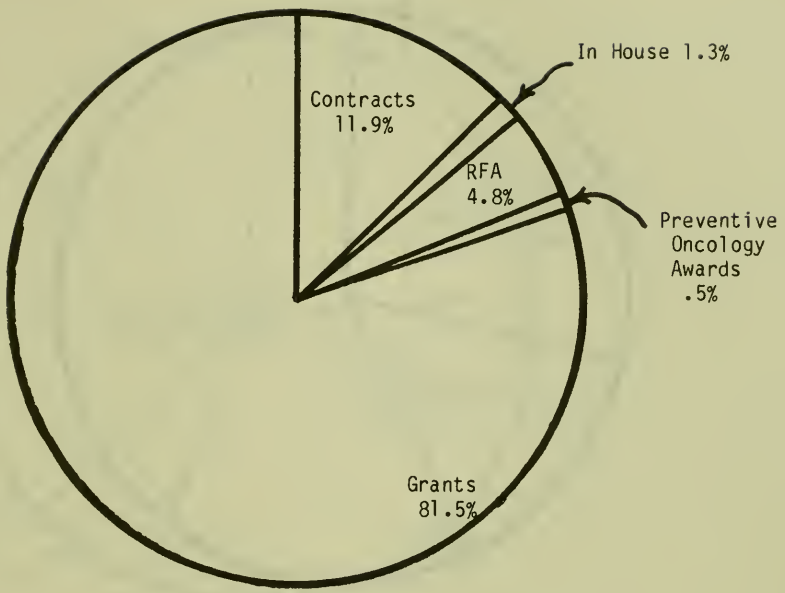
and (4) an increased use of RFAs to stimulate research activity in high priority areas. Considerable progress has been made in the past year toward meeting the cause and prevention related goals of the Carcinogenesis Extramural Program. Such accomplishments are well summarized in the reports of individual research areas.

The Biological Carcinogenesis Branch research program continues to provide valuable insights into the mechanisms of viral carcinogenesis and the means by which the transformation of cells from the normal to the malignant state occurs and might be arrested. Recent evidence from several laboratories suggest that relatively few genes which are present in all human cells may be responsible for transforming normal cells to cancer cells. None of these genes have yet been proven as causes of human neoplasia, but they have caused malignant transformation of cells in culture. Another new development is the recent report that some of the newly discovered human cancer genes are nearly identical to some virus-transmitted animal cancer genes. These discoveries are very important because they point to a common denominator for cancer cause and the possibility of a unified approach to control or reversal of the cancer process.

The Chemical and Physical Carcinogenesis Branch continues to report progress toward an understanding of the metabolism and pharmacokinetics of carcinogenic substances, e.g., polycyclic hydrocarbons, nitrates, arylamines. In the area of chemoprevention, progress continues to be made in the development of model systems for the investigation of the phenomenon, and increased emphasis is being placed on studies designed to elucidate mechanisms of action. New initiatives undertaken (using the research grant mechanism) in the areas of "Mechanisms of Biological and Chemical Prevention of Carcinogenesis" and the "Role of Tumor Promoters, Hormones and Other Cofactors in Human Cancer Causations" have had an excellent response and resulted in a number of awards.

Within the Special Programs Branch, activities focused on mathematical modeling of the carcinogenic process and improved epidemiologic study design and analysis are well underway, as are the development of new or improved techniques for data analysis in a number of contexts. The recently reported findings on the relationship of hepatitis B viral infection (and carrier status) with human hepatocellar carcinoma is of great research interest. It can be anticipated that much additional research in this area will be stimulated and, in the long term, it seems probable that means will be found to reduce the cancer burden related to this tumor in the population. An apparent epidemic of Kaposi's sarcoma and opportunistic injections in homosexual males has come to the attention of the cancer research community in recent months and a considerable effort, on the part of the NCI as a whole, to respond to this situation is underway. The outbreak may present a unique opportunity to gain further understanding of the relationship between immunosuppression and the carcinogenic process.

FY '82



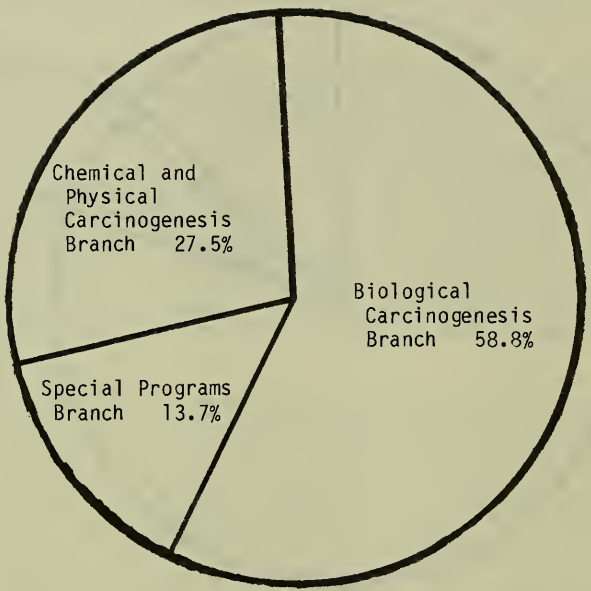
Carcinogenesis Extramural Program

($123.40 Million)

	$	%
Contracts	14.64	11.9
Grants	100.65	81.5
RFAs/CREGs	5.90	4.8
POAA	.61	.5
Subtotal	121.80	98.7
In-House	1.60	1.3
TOTAL	$ 123.40	100%

NOTE: Individual Projects have been adjusted to show analyzed levels of effort and may not correspond to fiscal year budget amounts shown in tables and "pie" charts.

FY '82

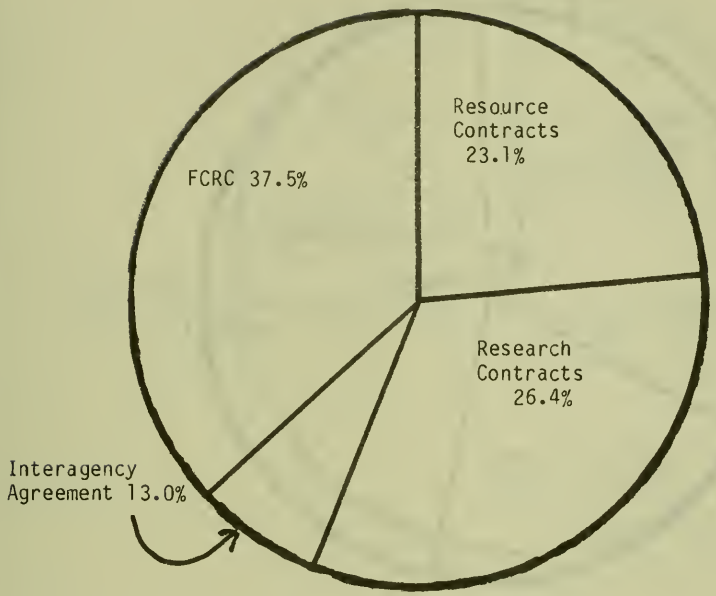


Carcinogenesis Extramural Program Contracts by Branch

($14.64 million)

	$	%
Biological Carcinogensis Branch	8.60	58.8
Chemical and Physical Carcinogenesis Branch	4.03	27.5
Special Programs Branch	2.01	13.7
TOTAL	$ 14.64	100%

FY '82

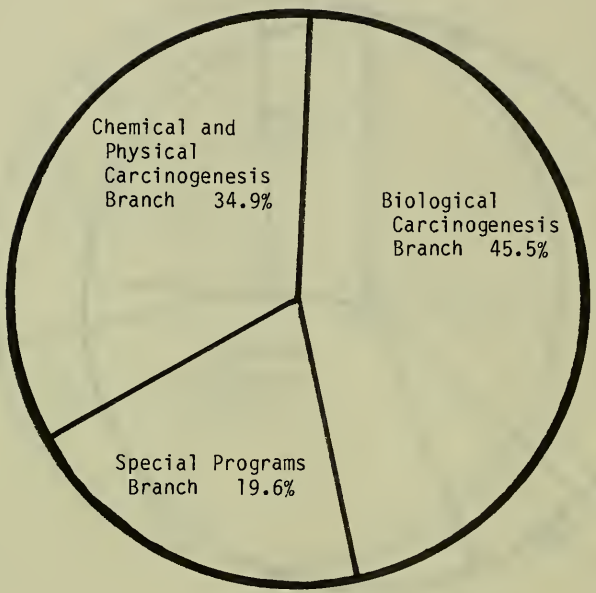


Distribution of Carcinogenesis Extramural Program Contract Funds

($14.64 million)

	$	%
Research Contracts	3.86	26.4
Resource Contracts	3.38	23.1
Interagency Agreements	1.90	13.0
Frederick Cancer Research Center	5.50	37.5
TOTAL	$14.64	100%

FY '82

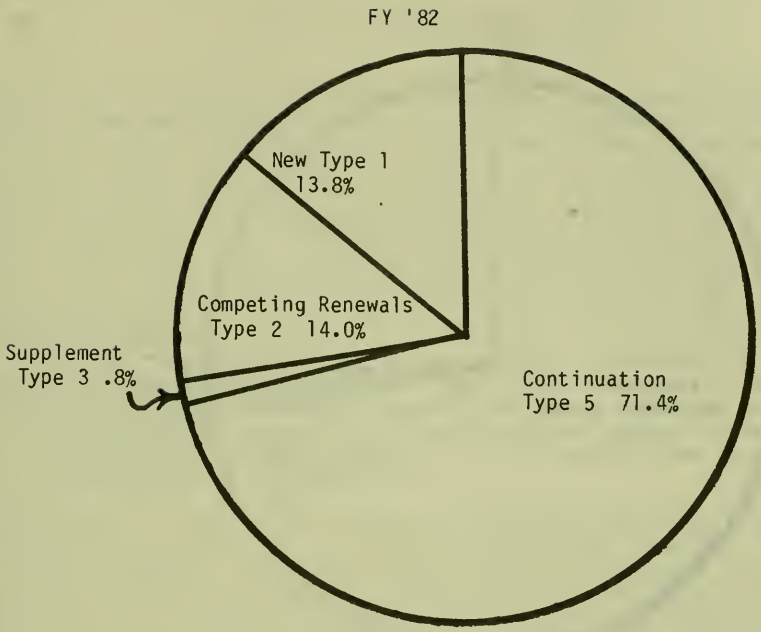


Carcinogenesis Extramural Program Grants by Branch

($107.16 million)

	$	%
Biological Carcinogenesis Branch	48.76	45.5
Chemical and Physical Carcinogenesis Branch	37.40*	34.9
Special Programs Branch	21.00	19.6
TOTAL	$107.16	100%

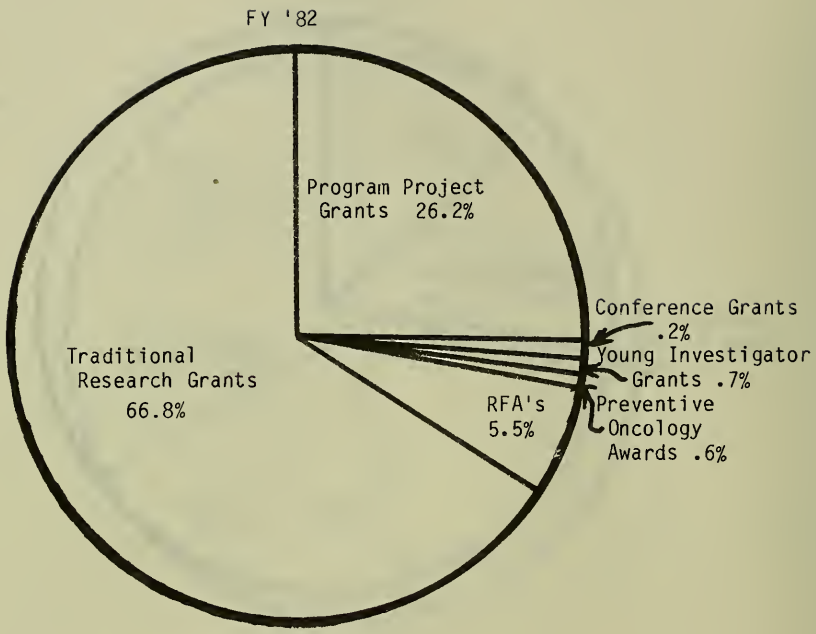
*Includes 1 Cooperative Agreement for $.443 for OD, DCCP.



Carcinogenesis Extramural Program Grants by Type

($107.16 million)

	$	%
New (T1)	14.75	13.8
Competing Renewals (T2)	15.00	14.0
Supplement (T3)	.84	.8
Continuation (T5)	76.57	71.4
TOTAL	$ 107.16	100%



Distribution of Carcinogenesis Extramural Program Grant Funds

($107.16 million)

	$	%
Traditional Research Grants (R01s)	71.56	66.8
Program Project Grants (P01s)	28.08	26.2
Conference Grants (R13s)	.23	.2
Young Investigator Grants	.78	.7
Preventive Oncology Awards (K07s)	.61	.6
RFA's Responses (R01's)	5.90	5.5
TOTAL	$107.16	100%

SUMMARY REPORT

BIOLOGICAL CARCINOGENESIS BRANCH

The Biological Carcinogenesis Branch (BCB) plans, develops, directs and manages a national extramural program of basic and applied research concerned with the role of biological agents as possible etiological factors or co-factors in cancer and on the control of these agents and their diseases; establishes program priorities, and evaluates program effectiveness; provides a broad spectrum of information, advice and consultation to individual scientists and institutional science management officials relative to NIH and NCI funding and scientific review policies and procedures, preparation of grant applications and choice of funding instruments; provides NCI management with recommendations as to funding needs, priorities and strategies for the support of relevant research areas consistent with the current state of development of individual research activities and the promise of new initiatives; plans, develops and manages research resources necessary for the conduct of the coordinated research program; develops and maintains computerized data management systems; and plans, organizes and conducts meetings and workshops to further program objectives, and maintains contact with the relevant scientific community to identify and evaluate new research trends relating to its program responsibilities.

The extramural activities of the Branch are accomplished through contractual agreements with universities, research institutes, and commercial organizations, and through traditional individual research grants and program project grants with universities and research institutes. Currently, the Branch administers over 300 research activities with an annual budget of approximately 57 million dollars. The research projects of the branch divide into three main categories. Research programs on viruses with a DNA core which are known or suspected to be involved in the induction of malignant transformation are included in the DNA Virus Studies. Research dealing with RNA core viruses which are known or suspected of involvement in the malignant transformation of animal and human cells are covered by RNA Virus Studies components. The Branch program component designated RNA Virus Studies I involves research concerning murine, feline, bovine, primate, and hamster viruses. The program component designated RNA Virus Studies II incorporates research involving avian tumor viruses, pox viruses, myxoviruses, picornaviruses, hepatitis B virus, and plant viruses.

To facilitate and support these research activities the research resources component of the Branch is responsible for planning, developing, allocating and maintaining the biological research resources necessary for the extramural research effort. Research resources is assisted by a data management element which is responsible for the automated retrieval and inventories of BCB resources, computer-systems planning, and automated analysis and management support. The automated inventories include the research resources virus inventory, the serum collection, the human tissue collection, and the virus, antisera and cell culture collections of satellite resources systems.

Table I focuses on mechanisms of support of extramural research and related activities in the area of biological carcinogenesis. The total budget in FY 82 is estimated to be five million dollars less than the FY 81 budget. The primary reason for the decreased funding was the programmed termination of approximately 20 contracts and a marked reduction in the funding of the Frederick Cancer Research Facility.

Table II provides an estimate of grant and contract support, respectively, in each of the four Branch components described above. The Branch administers 47 contracts and 282 grants.

In last year's report, it was noted that the DCCP Board of Scientific Counselors had approved the implementation of a resources "payback" system for the division. The payback system is one in which the recipients of resource materials or services reimburse the resource or production contractor directly for the services or materials received, based on a price schedule agreed upon in advance between the NCI and its resource contractor. The contractor in turn credits those funds received from recipients against its production costs and these are shown on monthly vouchers which it submits to the government for payments on the contract. Initiation of this system was the result of a variety of influences: the noticeable shrinking of the budget, an interest in seeing that the resource dollars utilized by grantees, intramural scientists, and contractors were somehow included in a peer-review system and the perception that free distribution of resources did not always result in the most effective utilization of resource funds.

There are two general modes under which the payback system is being implemented in the Branch. The first approach involves the immediate and full implementation of the system. This is most appropriate to contracts in which there are a large number of individual users who are receiving small amounts of material at costs reasonable enough for them to continue to acquire them without financial hardship. In other cases, where past utilization patterns have shown that significant problems would be encountered, it is planned for the payback system to be phased in over a period of time in such a way that investigators would not have to unduly curtail their research activities. In either case, as a general rule, when the resources payback system is fully implemented, all grantees, contractors, and intramural scientists will pay for the resources which they receive. The only exception anticipated to a general implementation will be the resource distributions to investigators that are participating in the special bilateral agreements between the United States and certain foreign countries. These bilateral agreements usually contain specific language relating to the open exchange of resources for cancer research and as such are not appropriate for the payback implementation.

Table III provides a listing of those Branch resource functions which are now in, or are scheduled to enter into, the payback system. As the table indicates, there are seven contracts that are now projected as being included under this system. Four contracts are now in effect (May, 1982), a fifth is being phased in at the present time, and two others are projected to be included in the system in the near future. The dates in the third column indicate when the payback system went in or goes into effect for each contract; in the last column on the right side the FY dollar figures in parentheses are newly projected funding levels for the next contract year for each effort. Three contracts have been in the system for at least a minimum time needed to obtain reasonable information. These are the two at Life Sciences, Inc. and the one at Showa University, all at the top of the table. The one effort that has been under the system for the longest time and for which good information is now available is the contract effort with Life Sciences, Inc. (at the top of the table), for the production of avian myeloblastosis virus and AMV reverse transcriptase enzyme. That payback system has been in effect since May 1981, and we now have a full year's experience with that activity.

The AMV contract will have received about $310,000 in payback funds from recipients by the end of the contract year and has distributed gratis an additional $40,000 worth of materials to foreign investigators under our international agreements. The recovered funds and the remaining undistributed inventory of frozen virus (valued at about $200,000) will make it unnecessary to add any new funds in FY82 to maintain this operation. Since this contract had been projected at its start for an operational budget of $559,564 in FY82, the total cost reduction to the NCI will be about $559,000. The SPF avian materials contract at Life Sciences (second listing) has been experiencing a reduced demand for chicken eggs and chickens since the introduction of the payback method. It is estimated that only about $60,000 worth of gratis and payback materials will be distributed during the contract year. As a result, we expect the level of effort for the contract to be reduced in its next year to about $80,000 to cover only the Japanese quail eggs, which are still in demand with a calculated savings of $160,000 from an earlier earmarked funding level in FY 82 of $240,000. Similar situations prevail at both Showa University and Litton Bionetics, with only modest distributions or use being experienced and a reduction to the level of effort now expected during the next contract year.

With regard to the three payback contracts scheduled for renewal during FY82, the total savings during this fiscal year will be approximately $925,000. It appears that the payback system is doing what was expected of it in budgetary terms, with certain essential efforts paying for themselves and others being reduced in scope when a lack of widespread use is made apparent.

Investigations carried out in the Biological Carcinogenesis research program during the past year have continued to produce valuable insights into the mechanisms of viral carcinogenesis and suggested means by which the transformation of cells from the normal to the malignant state occurs and might be arrested. Recent evidence from several laboratories suggest that relatively few genes which are present in all human cells may be responsible for transforming normal cells to cancer cells. None of these genes have yet been proven as causes of human neoplasia, but they have caused malignant transformation of cells in culture. Another new development is the recent report that some of the newly discovered human cancer genes are nearly identical to some virus-transmitted animal cancer genes. These discoveries are very important because they point to a common denominator for cancer cause and the possibility of a unified approach to control or reversal of the cancer process.

It is now increasingly likely that viral information, present as nucleic acid sequences in the genes of apparently normal human cells and replicating with them, is intimately involved with the development of cancer. Triggered by chemical carcinogens, radiation, hormones, the ageing process, and other influences, these viral sequences may direct the synthesis of the proteins responsible for malignant transformation of the cell. For this reason, increased attention has been given to studies defining the interaction of viruses and cells in both animal and human cancers and to identify minute regions of virus and cell chromosomes which are responsible for malignancy; to understand the molecular pathways of viral replication and to identify virus products which may trigger the transformation of a cell to malignancy; and to understand and enhance immune mechanisms which ultimately may prevent cancer. Most of these basic investigations are discussed in the next summary report which covers the grants activities. The investigations described here represent clinical and applied studies supported by the contract funding mechanism.

A multicenter study is underway to evaluate Epstein-Barr virus (EBV) immunovirological markers as potential clinical tools for the diagnosis and clinical management of American patients with nasopharyngeal carcinoma (NPC) and with occult tumors in the head and neck region. A total of 202 patients with histopathologically confirmed NPC have now been entered into the program. This is an increase of 78 over the last year and included (a) 36 patients previously listed as suspected NPC's and (b) 42 new patients entered into the program from collaborating institutions. Complete clinical records including pathology, clinical staging and treatment are now available on 160 patients which is an increase of 75 over the last reporting period. This reflects the increased effort on the part of all subcontractors to collect all possible clinical information on each patient entered into the study. Follow-up serum samples collected every 3-6 months following diagnosis which are necessary for determining the prognostic value of EBV serology have also been obtained successfully from most of the patients. Three or more follow-up serum samples have now been collected from 84 individual patients which is an increase of 22 over the past reporting period. Thus patient recruitment, collection of clinical information and patient follow-up are still progressing well in this study. Of the 202 patients with confirmed NPC, 45 or 22% of the patients have died over the 3-year period. Serum samples from all patients with suspected NPC and most controls were tested for antibodies to EBV antigens by immunofluorescence tests (IF) at the Mayo Clinic and Children's Hospital of Philadelphia. The tests include IgG antibodies to VCA and EA (D or R) and IgA antibodies to VCA. Antibody-dependent cellular cytotoxicity (ADCC) determinations were performed at the Mayo Clinic only. As usual, there has been excellent agreement in the results on individual serum samples between the two different laboratories. Previous studies established that EBV serology was useful in the diagnosis of the WHO 2 and WHO 3 histopathological types of NPC but not for WHO 1 (well-differentiated squamous cell carcinoma). This conclusion has been strengthened over this past 6-month period. Eighty-eight percent of the sera from 160 patients with WHO 2 or WHO 3 tumor types were positive for IgG anti-EA antibodies, mainly to the D component, and 86% of the same sera were positive for IgA antibodies to VCA. In contrast, only 33% of sera from 42 patients with WHO 1 tumors were positive for antibodies to EA (1/3 anti-R; 2/3 anti-D) while 11% were positive for IgA antibodies to VCA. This is similar to what has been observed in control populations. These results continue to support the conclusions that (1) IgA antibodies are very specific for WHO 2 and WHO 3 histopathological types of NPC and are of clinical value for the diagnosis of this disease including the occult form; and (2) the presence of both IgA antibodies to VCA and IgG antibodies to EA-D in the same serum sample, particularly when present at high levels, is very characteristic of patients with these two histopathological types of NPC.

The relationship of initial antibody titers to stage of disease at diagnosis is currently being evaluated using five different staging procedures: Ho, UICC, AJC, SEER, and Mayo-Scanlon staging system. There should now be sufficient clinical information on enough patients to draw some meaningful conclusions in regard to this question. All patients entered into the study are also being followed to determine the prognostic value of EBV serology. The number of patients who have died or developed recurrent disease over the 3-year period is still too small to draw a meaningful conclusion. However, it has been possible to identify some trends with the IF tests. Generally, in the absence of clinically identifiable recurrent disease, antibody titers measured

by these assays have remained fairly stable. No consistent decreases were noted in titers to any of the antigens in patients presumed to be in clinical remission. This is in contrast to what has been reported in high incidence populations. However, consistent increases, particularly in IgG anti-VCA and anti-EA titers, were noted in patients who developed metastatic disease. These increases, in many cases, preceded the clinical detection of metastatic disease. These observations suggest that increases in antibody titers in these IF tests may indeed signal the presence of active or metastatic disease. This should become more evident over the next contract year as more patients are expected to relapse. The assay that so far appears to be of the greatest prognostic value for this disease is the ADCC assay. Of the 82 patients with confirmed WHO 2 or WHO 3 histopathology who have been on study for at least one year or who have died during this 3-year period, 78% of 54 patients whose ADCC titers were high at diagnosis are in remission and 22% have died or have developed metastasis. In contrast, 36% of the remaining 28 patients whose initial ADCC titers were low are in remission and 64% have died or have recurrent disease. More surprising has been the observation that in some of the patients with high titers at diagnosis who eventually died from the disease, ADCC titers dropped significantly in the last one or two serum samples tested before death. The results to date, therefore, indicate that ADCC titers may indeed be valuable prognostic markers for this disease as previously reported. This conclusion is further supported by results on six patients entered into the study at the time they developed recurrent disease. ADCC titers in these patients were all low ranging from 1:480 to 1:3840.

The obvious utility of a reliable, consistent, and specific marker for carcinoma of the breast has led to numerous studies in which various substances, including enzymes, hormones, proteins, and others as yet undefined, have been investigated as possible candidates. Recent investigations have established that approximately half of human breast carcinomas contain an immunohistochemically detectable antigen which is crossreactive with the 52,000-dalton major glycoprotein (gp52) of the mouse mammary tumor virus (MuMTV). This antigen can be localized in paraffin-embedded sections of routinely-fixed tissues using heterologous antibodies to gp52 or MuMTV. One of the most significant applications of this methodology involves deciding whether a tumor is benign or malignant in borderline cases. A less dramatic, not altogether uncommon situation where such methods could prove useful involves the identification of a clinically occult primary breast carcinoma which presents as a metastatic lesion with an undistinctive histopathological pattern. Preliminary studies have been conducted on patients with metastatic carcinoma in axillary lymph nodes without any clinical evidence of a primary lesion in the breast or elsewhere. The localization of the gp52-related antigen in paraffin-embedded sections of metastatic lesions suggested the presence of primary mammary carcinoma. This suggestion was ultimately confirmed by the finding of primary lesions in which the gp52-related antigen was also found. Further prospective and retrospective studies are planned with the hope that the information gleaned will demonstrate the usefulness of this technique as a prognostic and/or diagnostic tool.

Studies are continuing to increase the sensitivity of a diagnostic test for human breast cancer either by developing a more sensitive assay than the immunoperoxidase test or by improving the reagents utilized in this test. One of the antisera (RII5) raised in New Zealand white rabbits has been thoroughly examined

to determine why the positivity of the peroxidase test went from 46% to 90% when this antiserum was used. After several months of study it was determined that the RII5 antisera recognized a protein, probably of milk origin, which copurified with gp52. Because of the frustrating unpredictability of heterologous xenogeneic antisera in general the research priorities were reoriented in favor of the production of hybridomas and monoclonal antibodies. The laboratory has generated hybridomas producing antibodies against MuMTV gp52 and the particles and particle proteins (p50) of the 47D clones. Seventy-six hybridomas have been produced against the particles of clone 11 of the 47D cell line and 16 against the p50 antigen from the same cell clone. These hybridomas are being cloned and subcloned for subsequent characterization.

AKR leukemia is associated with and can be transferred by an endogenous, vertically transmitted type C retrovirus (AKR virus) which is probably synthesized at birth, reaches detectable levels in the circulation during the perinatal period and attains high levels at 6 weeks of age. AKR mice display a nearly normal immune response to sheep red blood cells and are capable of rejecting tumor transplants. However, as the leukemic phase develops, a severely depressed immune response to sheep erythrocytes is observed. Specific defects in the cellular aspects of the immune system of the AKR mouse, including the possible emergence of specific suppressor cells, has been observed. Such considerations suggest that an important relationship exists between the activities of the virus and those of the host immune system and that these two parameters play a major role in contributing to resistance or susceptibility to leukemia. On this basis, manipulations which would reduce the virus load and swing the balance in favor of the immune defense would be expected to have a suppressive effect on leukemia development. AKR mice were treated with heterologous anti-MuLV gp71 antibodies under various conditions in order to establish the optimal criteria for effective suppression of leukemia development. The strongest effect was observed when mice were administered the antibody at birth, and the data indicate that the effect of continued treatment for 10 days is just as good as the effect produced after 42 days of treatment. A significant observation of these studies concerns the capacity of successfully treated AKR mice to transmit to their offspring characteristics which prevent development of leukemia. The hope here is that it may be possible to generate a nonleukemogenic AKR strain through appropriate mating crosses. The studies should also demonstrate which parameters are important in the mother and the father for transmission of the "protected" characteristics; clearly the results of these long-term studies may point directly to the mechanism of AKR leukemogenesis. The mating studies have resulted in an F6 generation which continues to yield nonviremic, antibody-positive animals that are nonleukemogenic.

Long-term studies continue to examine the levels of infectious ecotropic AKR virus in the tissues of control and gp71-treated animals. The results of these experiments have confirmed preliminary findings that suppression of ecotropic virus activity in the spleen and marrow of treated animals is transient, with levels of infectious virus in these organs eventually reaching those seen in control mice. The major difference between these two groups of animals is the near absolute lack of infectious ecotropic virus in the thymus of treated animals. Since the thymus is the most likely site for genetic recombination between ecotropic and xenotropic viruses, it was felt that this observation may be the key to understanding the effect of antiviral immunotherapy upon leukemogenesis. Studies are now underway to follow viral activity further into the late pre-

leukemic interval. In addition, the susceptibility of the thymus of treated animals to support the replication of eco-, xeno- and amphotropic viruses is currently being examined. These experiments should prove key to our understanding of the therapeutic effect seen with the treatment protocols used in these studies.

The neoplastic progression of the murine mammary gland involves an intermediary stage, the hyperplastic alveolar nodule (HAN), which can be morphologically visualized as lobular alveolar tissue in a nonpregnant, nonlactating host. The HAN can be surgically removed and transplanted into the cleared fat pad of isologous hosts. The resultant growth of the HAN is the hyperplastic outgrowth (HOG), which is delineated by the boundaries of the fat pad, and which has a higher tumor risk than normal mammary epithelium. This transplantation technique allows investigators to experimentally manipulate the HAN and to amplify the number of cells in the HAN for biochemical studies. By serially transplanting the preneoplastic HAN in mammary fat pads seven new HOGs have been established from the low tumor incidence mouse strain, C3H/Sm (formerly designated C3H/StWi). Four HAN lines from virus-free C3H/Sm mice are in transplant generations 2-4; to date no tumors have developed. Three of the HAN lines are from virus-infected C3H/Sm mice; all three lines exhibited a 50% tumor incidence in their second transplant generation. The five BALB/cfC3H "Z series" HOGs are being maintained by serial transplantation. The tumor incidences in these lines have not changed. The high tumor incidence Z4 continued to have a 100% tumor incidence at 6 months post-transplantation while the tumor incidence of the Z3 HPO line is 6% at 18 months. Lines Z4, Z5, Z5cl, and Z5d are now in transplant generation 16.

Restriction endonuclease mapping has been used to detect exogenous provirus and to detect clonal dominant populations. The results indicate that the HOGs are comprised of a heterogeneous population of cells and that some of the different subpopulations can be selected by transplantation. Primary outgrowths are composed of several subpopulations which are selected by the transplantation procedure. Stabilization of the population occurred by the third to fourth transplant generation. Tumors which arise from the HOGs contain the restriction patterns found in their respective outgrowths. Multiple tumors which arose in one HOG have the outgrowth restriction pattern but each tumor has its own unique additional band(s). However, not all tumors contain amplified viral genes. Thus, MuMTV gene amplification may not be required for tumorigenesis. The major role of MuMTV may be in the normal to HAN transformation, and have little or no effect on the HAN to tumor transformation.

On May 3 and 4, 1982, a workshop on "Hepatitis B Virus and Primary Hepatocellular Carcinoma" was held in Bethesda, Maryland. The meeting was co-chaired by Dr. Hilary Koprowski, the Wistar Institute, and Dr. W. Thomas London, Fox Chase Cancer Center. Presentations were made on the state-of-the-art in such areas as the Biology of Hepatitis B Virus (HBV); Epidemiology of HBV and Primary Hepatocellular Carcinoma in Man; Co-Carcinogenesis, HBV and Hepatocellular Carcinoma; Hepatocellular Carcinoma as a Disease in Man and Animals; and Animal Model Systems. Round table discussions were held on biological studies of HBV, and on the role of the virus in PHC in man and other animals. A number of recommendations for areas of increased emphasis were made. These suggestions are now being evaluated for possible new initiatives. Additionally, a workshop entitled "Retroviral Isolates From Human T-Cell Leukemias", was held in August

1982 for the purpose of determining the state of the art for the inter-disciplinary research being conducted with the human T-cell leukemia viruses (HTLV). New initiatives for HTLV research will be based upon input from the workshop.

TABLE I
BIOLOGICAL CARCINOGENESIS BRANCH
(Extramural Activities - FY 1982 - Estimated)

	No. of Contracts/Grants	$ (Millions)
Research Contracts	22	1.16
Research Grants	282	48.77
Traditional Project Grants (257 grants; $34.77 million)		
Conference Grants (1 grants; $0.04 million)		
New Investigator Research Grants (6 grants; $0.30 million)		
Program Project Grants (18 grants; $13.66 million)		
Research Resources Contracts	25	4.42
Frederick Cancer Research Facility	1	3.00
TOTAL	330	57.35

TABLE II
BIOLOGICAL CARCINOGENESIS BRANCH
(Contracts and Grants Active During FY 1982)

	FY 82 (Estimated)			
	CONTRACTS*		GRANTS	
	No. of Contracts	$ (Millions)	No. of Grants	$ (Millions)
DNA Virus Studies	9	0.78	120	22.89
RNA Virus Studies I	13	0.38	80	13.64
RNA Virus Studies II	0	0	82	12.24
Research Resources	25	4.42	0	0
TOTAL	47	5.58	282	48.77

*Does not include FCRF

TABLE III

BIOLOGICAL CARCINOGENESIS BRANCH
Resource Contracts Payback System

Contract	Function	Payback Start Date	Distributions	Cost Reduction
Life Sciences, Inc. NO1-CP1-1013 5/19/81 (start date)	Provides AMV virus and reverse transcriptase	5/19/81	$350,000	$559,564 (FY82-$0)*
Life Sciences, Inc. NO1-CP6-1005 9/01/81	Provides SPF chickens, chicken eggs, and Japanese quail eggs	9/01/81	60,000	$160,000 (FY82-$80,000)
Showa University Research Institute NO1-CP1-1012 8/31/81	Provides Epstein-Barr virus and EBV DNA	1/01/82	110,866	$205,000 (FY82-$110,000)
Litton Bionetics, Inc. NO1-CP9-1022 2/15/82	Holding facility for experimental primates	4/21/82	80,326	$173,379 (FY83-$100,000)
Children's Hospital of Michigan NO1-CP2-1017 4/01/82	Provides cell culture identification service	6/01/82	-	Unknown at present
NO1-CP9-1004 Recompetition 6/16/82	Provides antisera to oncogenic viruses and virus components	7/16/82	-	Unknown at present
NO1-CP7-1014 Recompetition 6/01/82	Breeding and holding of marmosets	8/01/82	-	Unknown at present

*Projected funding levels for the next contract year for each effort

SUMMARY REPORT

GRANTS ACTIVITIES

The Biological Carcinogenesis research program consists of the studies on biological agents as possible etiologic factors or co-factors in cancer and on the control of these agents and their associated diseases. Emphasis is placed on viruses, viral products, and related cellular substances as tumor-inducing agents, and includes biological, biochemical, immunological, and physical investigations of actual, potential or suspected, oncogenic viruses, and their interactions with, and effects on their hosts at all levels of biological organization. In general, the emphasis of the program is on the dynamic relationship of biological agents to the oncogenic and oncologic process and applications arising from these considerations. The program may also include research on infectious processes in a host organism induced by oncogenic and possibly oncogenic viruses, since viral latency and persistence may impact on the development of malignancy. Research on gene expression and other cell regulatory functions, utilizing biological agents as tools, is also appropriate for the program, provided the relationship and significance to neoplasia is clearly indicated and documented. While the greatest interest resides in animal viruses and animal systems, including human, projects which involve other forms of life may also be considered if these offer unique advantages and their pertinence to neoplasia is documented. Within the Branch, the grants research activities are grouped into three components comprising DNA virus studies, RNA virus studies I, and RNA virus studies II.

In the DNA Virus Studies component there are approximately 120 grants. Of these, the major research emphasis lies in mechanisms of transformation which includes genome structure, function, and expression (49%); and, virus-cell interactions (37%). In terms of the viruses being studied, 33% concern the herpesviruses (herpes simplex virus, 16%; Epstein-Barr virus, 12%), and 67% concern the better known smaller DNA viruses, the adenoviruses and papovaviruses.

The genome of herpes simplex virus type 1 consists of two covalently linked components, L and S, that invert relative to each other. The L and S components consist of unique DNA sequences bracketed by inverted repeats. The inverted repeats of the L component are designated ab and b'a' and those of the S component are designated a'c' and ca. The number of a sequences at the termini and at the L-S component junction varies from one to several copies. Insertion into the middle of the L component of a DNA fragment consisting of 156 base pairs (bp) of the b sequence, an entire α sequence of 501 bp, and 618 bp of the c sequence created a new site through which additional inversions in the genome occurred. Comparison of the nucleotide sequences of DNA fragments containing one and two α sequences defined the domain of the α sequence. The single α sequence consists of two 20-bp direct repeats (designated as DR1) bracketing a region that contains 19 tandem direct repeats of a 12-bp sequence (DR2) adjacent to three direct repeats of a 37-bp sequence (DR4), in addition to short stretches of unique sequences. The fragment with two tandem α sequences contained three copies of DR1; i.e., the intervening DR1 was shared by the two α sequences. Furthermore, one α sequence contained 22 copies of DR2 and two copies of DR4 whereas the second α sequence contained 19 copies of DR2 and two copies of DR4. These observations suggest that (i) amplification of the number of terminal and internal α sequences is the consequence of intramolecular or intermolecular recombination

through DR1, (ii) the number of copies of DR2 and DR4 within the α sequence is not fixed and may vary as a consequence of unequal crossing over or slippage, and (iii) inversion results from intramolecular recombination between terminal and inverted α sequences. (Mocarski & Roizman, 1981)

Affinity chromatography and immunoprecipitation experiments were performed to determine whether cells infected with herpes simplex virus type 2 (HSV-2) expressed a glycoprotein that was functionally and antigenically related to the HSV-1 Fc-binding glycoprotein designated gE. A protein from extracts of HSV-2-infected HEp-2 cells was found to bind specifically to an Fc affinity column and that the electrophoretic mobility of this protein in sodium dodecyl sulfate-acrylamide gels was slightly less than the mobility of HSV-1 gE. Immunoprecipitation experiments performed with an antiserum prepared against HSV-1 gE revealed that (i) extracts from HSV-2-infected cells contained a glycoprotein that was antigenically related to HSV-1 gE; (ii) the electrophoretic mobility of the HSV-2 gE was indistinguishable from the mobility of the HSV-2 Fc-binding protein; (iii) the antiserum reacted with both newly synthesized transient forms and stable fully processed forms of both HSV-1 gE and HSV-2 gE; and (iv) the transient and stable forms of HSV-2 gE all had lower electrophoretic mobilities than their HSV-1 counterparts. Electrophoretic analyses of gE precipitated from extracts of HEp-2 cells infected with two sets of HSV-1 X HSV-2 intertypic recombinant viruses suggested that the gene for gE is located at the right end of the HSV genome (0.85 to 0.97 map units) in the unique portion of the S component. (Para et al, 1982)

Defective genomes present in serially passaged herpes simplex virus (HSV) stocks have been shown to consist of tandemly arranged repeat units containing limited sets of the standard virus DNA sequences. Invariably, the HSV defective genomes terminate with the right (S component) terminus of HSV DNA. Because the oligomeric forms can arise from a single repeat unit, it has been concluded that the defective genomes arise by a rolling circle mechanism of replication. Current studies of defective genomes packaged in viral capsids accumulating in the nuclei and in mature virions (enveloped capsids) translocated into the cytoplasm of cells infected with serially passaged virus reveal that, upon electrophoresis in agarose gels, the defective genomes prepared from cytoplasmic virions comigrated with nondefective standard virus DNA. In contrast, DNA prepared from capsids accumulating in nuclei consisted of both full-length defective virus DNA molecules and smaller DNA molecules of discrete sizes, ranging in M_r from 5.5 to 100 x 10^6. These smaller DNA species were shown to consist of different integral numbers (from 1 to approximately 18) of defective genome repeat units and to terminate with sequences corresponding to the right terminal sequences of HSV DNA. It was concluded on the basis of these studies that (i) sequences from the right end of standard virus DNA contain a recognition signal for the cleavage and packaging of concatemeric viral DNA, (ii) the sequence-specific cleavage is either a prerequisite for or occurs during the entry of viral DNA into capsid structures, and (iii) DNA molecules significantly shorter than full-length standard viral DNA can become encapsidated within nuclear capsids provided they contain the cleavage/packaging signal. However, capsids containing DNA molecules significantly shorter than standard virus DNA are not translocated into the cytoplasm. Finally, the results of these studies might be of relevance to the previous observations that infections of cells with certain virus stocks containing defective HSV genomes, as well as virus stocks containing defective genomes of other herpesviruses, result in significant reduction in virus particle production, in reduced and delayed cleavage of helper

virus DNA concatemers, and, in some cases, in increased production of incomplete forms of virus capsids. Thus, the defective virus genomes containing multiple copies of packaging and cleavage signals could efficiently compete with helper virus DNA molecules on limiting cleavage machinery or on limiting capsid constituents produced in the serially passaged virus-infected cells. If so, the obligatory presence within defective virus genomes of the ac recognition sites specifying cleavage/packaging of virus DNA could constitute a specific pathway of defective genome interference with homologous standard virus replication. (Vlazny, Kwong, and Frenkel, 1982)

Experimental trigeminal ganglion and corneal infection in mice was studied with three thymidine kinase-positive (TK+) strains of herpes simplex virus type 1 (HSV-1) and eight TKHSV-1 mutants. Viruses were extensively tested in cell culture to determine whether any were temperature sensitive (ts) for virus replication or for TK activity. TKmutants were no more ts than were TK+ viruses. By arabinosylthymine testing and measurement of thymidine phosphorylation, it was apparent that some TK mutants were, in fact, intermediate for TK activity (TK+). After corneal inoculation of individual viruses it was observed with one exception that TK, TK+, and TK+ viruses replicated in ocular tissues. However, TK mutants were rarely isolated from trigeminal ganglia, whereas TK+ and to a lesser degree TK+ viruses were isolated frequently. In vivo complementation studies were performed by corneal inoculation of (TK^+-TK^-) and (TK^--TK^-) virus mixtures. TK+ HSV complemented TK virus since significant amounts of TK+ HSV were isolated from trigeminal ganglia. In addition, after inoculation of certain (TK^--TK^-) pairs, complementation in ganglia was observed. These studies support the hypothesis that HSV-1 TK expression is necessary for sensory ganglion (neuron) infection in three ways: HSV-1 TK mutants that replicated in ocular tissues and were not ts mutants did not replicate in vivo in trigeminal ganglia; (ii) there was a correlation between level of viral TK activity and trigeminal ganglion virus titer; and (iii) when complemented by TK+ or TK HSV, TK virus replicated in trigeminal ganglia. (Tenser, Ressel, and Dunstan, 1981)

Squamous-cell carcinoma in situ of the vulva is appearing with increasing frequency. It is also appearing more commonly in women under the age of 40. It has been suggested that common pathogenic factors, possibly viral, may be associated with carcinogenesis of the vulva, cervix, and vagina. In particular, a temporal relation between this disease and genital herpesvirus (HSV-2) infection has been noted. The relation between HSV-2 infections and cervical carcinoma has been well documented. This study concerns attempts to identify HSV-2 DNA-binding proteins in biopsy samples from patients with carcinoma in situ of the vulva. Antigens induced by herpes simplex virus Type 2 (HSV-2) were found to be associated with squamous-cell carcinoma in situ of the vulva in nine of 10 patients. The HSV-2 induced antigens are DNA-binding proteins that are normally present in the nuclei of infected cells, but in the cells of the carcinomas in situ they were found in the cytoplasm. Whole-virion structural antigens were not present, although there was serologic evidence of previous HSV-2 infection in patients tested for the presence of antibodies. These observations and the recent parallel rise in the prevalence of both HSV-2 infections and vulvar carcinoma in situ, particularly in women under 40 years of age, suggest an association of HSV-2 infection with this type of neoplasia, the nature of which remains to be determined. (Kaufman et al, 1981)

Cellular proteins with subunits about 53 kilodaltons (kDal) have been described in several transformed cell systems and in other cells in active growth. A

protein of this size occurs in cells transformed by simian virus 40 (SV40), associated with the large tumor (T) antigen, and in cells transformed by other papovaviruses. A 53-kDal protein has also been detected in Epstein-Barr virus-transformed cells, where it may be complexed with the Epstein-Barr virus-determined nuclear antigen (EBNA), and in cells transformed with Abelson murine leukemia virus. In nonviral systems, proteins of this size have been reported from methylcholanthrene-induced sarcomas, concanavalin A-stimulated cells, embryonic cells (including embryonal carcinoma cells), and normal thymus. A 53-kDal protein is also a common feature in several human tumor cell lines. Thus, this class of proteins appears ubiquitous in rapidly proliferating cell populations and are coded for by cellular DNA. The fact that they may be associated with T antigen and are found in a variety of different tumor systems and embryonic tissues suggest that 53-kDal proteins may be of general importance in transformation or growth regulation. It also appears possible that such a protein may form a link between viral, chemical, and other mechanisms of cellular transformation. Immunological studies suggest that some 53-kDal proteins from several sources are similar, regardless of the etiological agent involved. These findings are also consistent with peptide mapping experiments although peptide differences have also been noticed. As yet, there is no direct proof that all these studies concern the same protein; even the molecular mass values reported have been slightly different, and various forms of 53-kDal protein have been found to occur in a single cell. To date, direct chemical characterization has been lacking and no sequence data have been reported for any of the 53-kDal proteins. A heat-stable DNA-binding protein with subunits of about 53 kDal was purified from two virally transformed human cell lines (Epstein-Barr virus-positive Raji and Namalwa) and two mouse tumor cell lines (methylcholanthrene-induced Meth A sarcoma and TA3 mammary carcinoma). All four 53-kDal proteins showed closely related total amino acid compositions, similar peptide maps, and identical NH_2-terminal amino acid sequences for 20 residues. These 53-kDal proteins are therefore evolutionarily highly conserved, independent of whether they originate from virally or chemically transformed cells. The NH_2 terminal sequence and the protein chain as a whole are not hydrophobic; however, some unexpected residue distributions were observed. Comparisons with other proteins reveal no clear sequence similarity with known tumor antigen structures, homologous immunoglobulins, or some other proteins of known sequence. Epstein-Barr virus-determined nuclear antigen also appears to have a different NH_2-terminal sequence. Thus, the results show that the 53-kDal proteins represent a unique protein type with little species variation; this finding suggests that these proteins must perform an important common function in different transformation systems. (Jornvall et al 1982)

Most strains of Epstein-Barr virus (EBV) cause transformation of normal human lymphocytes with very high efficiency. The usual target cells for EBV infection in vitro are B lymphocytes which bear complement receptors and which are in a resting state at the time of inoculation with virus. The mechanism by which EBV causes resting lymphocytes to proliferate continuously is not known but some of the early events in the process have been placed in order. Viral attachment and penetration are completed within 2 hr. The EB virus-determined nuclear antigen (EBNA) is synthesized within 12-24 hr after inoculation and before the onset of blastogenesis and DNA synthesis. In early studies virus-induced DNA synthesis measured by (^{3}H) thymidine incorporation could not be detected before three days following inoculation. However, with assay systems employing longer pulses with (^{3}H)thymidine or populations enriched for susceptible cells, virus-induced DNA synthesis has been detected within 24 to 36 hr after infection. Nearly all the

DNA synthesized appears to be cellular although the possibility that a small fraction may represent viral DNA replication cannot be excluded. Since multiple copies of the EBV genome are found in transformed cells, some viral DNA synthesis probably occurs after infection; but whether it is involved in a critical event leading to transformation, or whether it takes place gradually over several cell cycles after the transformation event is not known. Although the timing of DNA synthesis in B lymphocytes following exposure to virus has been well documented, the onset of cellular proliferation in infected cells has not been studied. In this investigation the physical association of EBNA with metaphase chromosomes was used as a marker for dividing infected cells in order to determine the time course of cellular proliferation in relationship to EBNA expression and DNA synthesis. EBNA was first detectable 6-8 hr after infection in 1-10% of the cells. Even though cells were exposed to virus under conditions which would maximize the chances of synchronous infection, the proportion of EBNA (+) cells increased until 32-36 hr when a maximum level was reached. DNA synthesis in infected cultures increased above control levels between 20 and 36 hr after exposure to virus and thereafter increased progressively. The first cell divisions observed in EBNA (+) cells occurred between 36 and 48 hr after infection and the mitotic index of EBNA (+) cells increased with time reaching 25% of the EBNA (+) cells by 96 hr. However, the total cell number and the percentage of EBNA (+) cells remained unchanged after 36 hr suggesting that the rate of cell death in EBNA (+) cells was equivalent to the rate of proliferation. Adenine arabinoside (Ara A) and cytosine arabinoside (Ara C), at concentrations which inhibit VCA expression in EBV producer cell lines, did not effect EBNA expression during the first 24 hr after infection. These data together with the very early appearance of EBNA after inoculation show that EBNA synthesis is independent of viral or cellular DNA synthesis and support the hypothesis that synthesis of EBNA is one of the crucial earliest events in lymphocyte transformation by EBV. (Robinson and Smith, 1981)

Evidence that cytomegalovirus (CMV) may be associated with epithelial tumors of the bowel is two-fold. First, cytological and cultural evidence for the presence of CMV in bowel wall has been reported in several series of patients with ulcerative colitis, a disease which after 10 years' duration is associated with a markedly increased incidence of colonic cancer. Secondly, two recent studies utilizing sensitive biochemical techniques for detecting viral nucleic acid sequences in human tissues have reported CMV DNA to be present in diseased bowel of patients with ulcerative colitis, familial polyposis, and carcinoma of the colon. Patients with Crohn's disease, an illness with little or no increase in cancer risk, were uniformly negative for CMV DNA. These two reports, however, involved small numbers of patients, did not employ cultural or other techniques for confirmation, and used methods of only modest sensitivity for detecting CMV DNA, in the range of ~2 genome-equivalents per diploid cell. Therefore, a study was conducted to test the strength and specificity of the association between epithelial tumors of the colon and CMV in a consecutive series of individuals undergoing bowel surgery. The specificity and strength were tested by analysis for: CMV-DNA by a DNA-DNA reassociation kinetic (hybridization) procedure; latent virus by co-cultivation of fresh tissue with indicator fibroblasts; and CMV viral antigens by immunofluorescence. Ten of 13 cancer patients whose colonic tissue was able to be examined by all techniques showed some evidence of active or prior CMV infection. Four cancer specimens were CMV-DNA (hybridization)- positive; an additional specimen from a cancerous colon was culture-positive. In six instances, CMV DNA was detected in mucosal cells adjacent to colon adenocarcinoma. In tissue from one of three patients with ulcerative colitis and two of seven patients with

other non-neoplastic colonic disease, CMV DNA was also detected. No fresh colonic tissues were demonstrated to have CMV surface or nuclear antigens when examined by immunofluorescence. Culture of peripheral lymphocytes was positive for CMV in three of 14 cancer patients. A CMV-specific defect in humoral immunity could not be documented in that most cancer patients, as well as cancer-free patients, exhibited circulating specific antibody to CMV and had a normal capacity for CMV-specific antibody-dependent cellular cytotoxicity. It was concluded that CMV, probably in a latent form, is readily detectable in colonic cells of man, including those derived from malignant, pre-malignant and non-malignant tissues. Neither preferential replication in damaged tissue nor carriage of CMV by peripheral lymphocytes homing to gut appear to explain the presence of CMV in colon cells. (Roche et al, 1981)

Cytomegalovirus (CMV) can cause a mononucleosis syndrome either spontaneously (community-acquired) or in patients who have received multiple blood transfusions. This syndrome is characterized by malaise, protracted fever, and peripheral blood lymphocytosis with atypical lymphocytes. It has been shown that patients with CMV-mononucleosis develop a hyporesponsiveness to certain mitogens and herpes virus antigens. However, the mechanisms of CMV-induced hyporesponsiveness remain unclear. Recent reports have emphasized the immunoregulatory role of T lymphocyte subpopulations. Abnormalities in these T cell subpopulations have been associated with a number of human diseases, including the closely related Epstein-Barr virus (EBV) induced mononucleosis. In this study, peripheral blood mononuclear cells from patients with CMV-mononucleosis were characterized by means of T lymphocyte-specific monoclonal antibodies. During acute CMV-mononucleosis, a reversal in the normal ratio of helper to suppressor T lymphocytes accompanies a diminished lymphocyte responsiveness to Con A. During convalescence, helper T lymphocytes increase, suppressor T lymphocytes decrease, and mitogen responses return to normal. It has not yet been possible to separate T suppressor cells from T cytotoxic cells. If these cells are indeed identical, suppression of mitogen responsiveness may be a necessary correlate of effective antiviral cell mediated immunity. However, if these cells are separable, then it may eventually be possible to selectively eliminate suppressor cells or to augment helper cells, thereby reversing the immunosuppression induced by this virus. (Carney et al, 1981)

The existence of adenovirus tumor-specific transplantation antigen(s) (TSTA) in rodent cells transformed with different adenovirus serotypes has been well documented. Immunization of mice with adenovirus type 12 (Ad12) or Ad12-transformed cells produces specific isograft immunity. Cellular cytotoxicity was further demonstrated by in vitro colony-inhibition assays. Sera of tumor-bearing animals are cytotoxic to transformed cells in vitro and exert blocking effects in cellular cytotoxicity assays. The nature of adenovirus TSTA, however, has not been closely studied and it has, as yet, not been firmly associated with any specific portion of adenovirus genome. Analysis of the immune response of tumor-bearing inbred rats to syngeneic Ad12-transformed cells indicated, however, that the early gene block E_1 of Ad12 codes for a membrane-associated antigen active in both cellular and humoral immunity. In agreement with this observation it has also been reported that products of the E_1 gene block of Ad12 in the transformed cells give rise to transplantation immunity in hamsters. In Ad2-transformed rat cells, a 19 K glycoprotein, an "early" product of the right end of Ad2 genome forms a ternary complex with the major histocompatibility antigen subunits on the cellular membrane and may thus be a component of the adenovirus TSTA. The cytotoxic immune response to three well-characterized Ad2 transformed cell lines

it is the src gene and pp60^{src} product. For both viruses, a second gene (ts-a for polyoma and pol for Rous sarcoma virus) is needed to bring about a stable association of the viral genome with the cell. Although the two viruses are profoundly different in their structures, life cycles, and natural histories, the pattern of cellular changes caused by the two "oncogenes" is strikingly similar. Dramatic morphological alterations occur; these are accompanied by decreased cell-substratum adhesion and by structural changes at the cell surface (lectin agglutinability) and in the cytoplasm (stress fibers). Growth control is relaxed, leading to decreased regulation by cell density, by serum concentration, or by the availability of a solid substrate. Protein modification reactions controlled directly or indirectly by the products of these viral genes have been implicated in the biochemical mechanisms underlying their pleiotropic action in cells. Expression of the hr-t gene leads to hyperacetylation of histones H3 and H4 encapsidated in virions, a finding consistent with a pleiotropic regulatory model of hr-t gene action proposed on the basis of the host range and transformation properties of hr-t mutants. Immune precipitates containing pp60^{src}, the product of the src gene, show a kinase activity leading to phosphorylation of pp60^{src} and of the heavy chain of immunoglobulins. The activity is absent or reduced when mutants defective in the src gene are used. Analogous experiments with polyoma virus T antigens show a similar activity, with the 56,000-dalton (56K) middle T antigen being the major phosphate acceptor; hr-t mutants are uniformly negative in this reaction. In mutant NG-59, an alteration from Asp to Ile-Asn at a specific site within the sequence shared by the middle and small T proteins is sufficient to abolish the in vitro kinase activity. Specificity for tyrosine as the phosphate acceptor is one feature shared by the kinases associated with the transforming proteins of polyoma virus, Rous, Fujinami, and Y73 sarcoma viruses, and Abelson leukemia virus. Another common feature is the association of the viral gene products with plasma membranes. To understand the mechanism of action of these viral oncogenes required further detailed study of the protein modification reactions under their control. A new species of polyoma virus middle T antigen was found which differed from the previously recognized 56K species in having a slightly higher apparent molecular weight (58K) and a different pattern of phosphorylation in vivo. Two species of polyoma virus middle T antigen were detected in both lytically infected and transformed cells by in vitro kinase assay of immunoprecipitates. A minor species with an apparent molecular weight of 58,000 (58K) represented less than 10% of the total middle T protein. This species was roughly 10 times more active as a phosphate acceptor than was the predominant 56K form. Partial proteolytic mapping experiments showed that the same site was phosphorylated in both species. Mapping of the middle T antigens from a series of deletion mutants suggested that the major site of phosphorylation is tyrosine residue 315. Phosphorylation occurred on both middle T species in vivo, involving sites predominantly other than the tyrosine labeled in vitro. The 56K and 58K middle T forms differed from each other in their in vivo phosphorylation patterns. Some phosphate was incorporated into the 58K species in a region of the molecule to which at least part of the apparent molecular weight difference could be mapped. Mutant NG-59, which codes for a slightly altered middle T, produced only a single species (56K) which was inactive in the in vitro kinase reaction. Moreover, no 58K species appeared in vivo with this hr-t mutant. Therefore hr-t mutants are defective in both aspects of phosphorylation. Phenotypically normal revertant cells of a polyoma transformed line failed to express any middle T antigens or associated kinase activity. A fundamental question concerns whether either of the middle T forms is indeed a catalytic protein, as opposed to being simply a substrate for one or more cellular kinases or perhaps regulatory subunits thereof. Because the

Asp to Ile-Asn substitution in hr-t mutant NG59 would appear unlikely to alter middle T antigen radically as a substrate, it was earlier argued that the middle T antigen may itself be a kinase. However, the present results showing that NG59 fails to induce a 58K form detectable by either ^{35}S or ^{32}P labeling in vivo suggest that this hr-t mutation may lead to a defective substrate for some in vivo phosphorylation reactions. Futhermore, efforts to demonstrate a catalytic role for middle T antigen by direct labeling with ATP affinity reagents have proven negative thus far. (Schaffhausen and Benjamin, 1981)

SV40-transformed mouse cells and murine embryonal carcinoma cells contain a 54,000 MW cellular tumor antigen which was detected with antibodies from animals bearing SV40-induced tumors. An antigen with a similar molecular weight has been detected in a variety of transformed mouse cell lines employing an antisera raised against a chemically induced murine sarcoma. To examine the structural relationships between these cellular tumor antigens, the 54,000 MW proteins from a variety of murine transformed cell lines have been analyzed by chromatography of the methionine-containing tryptic peptides derived from these proteins. The results of this analysis demonstrate: (1) SV40 tumor sera and a monoclonal antibody, directed against a cellular tumor antigen of a chemically transformed cell line, each immuno-precipitated the same 54,000 MW protein from SV40-transformed cells as shown by the fact that each protein had an identical peptide map. (2) The 54,000 MW proteins obtained from (a) murine embryonal carcinoma cells, (b) 3T12 cells, (c) chemically transformed cell lines, and (d) an in vitro translation of m-RNA from SV40-transformed cells, all had similar or identical peptide maps. The 54,000 MW proteins from all these sources had eight methionine-containing peptides in common with the 54,000 MW protein obtained after in vivo labeling of SV40-transformed cells in cell culture. However, the 54,000 MW protein derived from SV40-transformed cells labeled in vivo produced a variable number (3-5) of additional methionine-containing tryptic peptides not detected in the other cell lines or with the 54,000 MW protein translated in vitro. These additional peptides may be the result of a post-translational modification of the 54,000 MW protein that is specific to SV40-transformed cells. The results of these experiments demonstrate the structural similarity or identity of the cellular 54,000 MW tumor antigens derived from a variety of murine cell lines transformed by diverse agents. (Maltzman, Oren, and Levine, 1981)

The ability of simian virus 40 (SV40) to malignantly transform cells depends upon the early half of the genome. Via partially overlapping spliced mRNA's, this early region codes for two known proteins, a 94,000-dalton (94K) large T antigen, which is required for lytic growth, and a 17K small t antigen. Both proteins influence viral transformation. Analysis of deletion mutants suggests that the small t antigen is required for the efficient initiation of transformation when growth is restricted soon after infection but plays a minimal role in the subsequent maintenance of the transformed phenotype either in vitro or in vivo. Large T antigen, on the other hand, may play a continuingly active role in transformation. It is required for the induction of stable transformants by SV40 virions and has often appeared to be continuously required for the maintenance of transformation, since clones of rat, hamster, rabbit, or mouse cells transformed by mutants with temperature-sensitive lesions in large T may lose various transformed characteristics when grown at the nonpermissive temperature. Mutant tsA58, maps by marker rescue with the bulk of a tsA mutants studied to date between 32.4 and 37.3 map units in the middle of the coding region for large T antigen. Mutant tsA1499 is a temperature-sensitive deletion mutant which lacks 81 base pairs at 21 map units lying within the carboxy-terminal portion of the

T-antigen-coding region. Both mutants fall into complementation group A for lytic growth; both can be marker rescued by fragments falling within the known coding region of the large T antigen; both direct the synthesis of a thermolabile large T antigen in permissive and nonpermissive cells; and both are severely heat sensitive for lytic growth. Despite these similarities, when these two mutants are assayed for transformation under identical conditions on the same cell line, opposite results are obtained. Mutant tsA58 conforms to its lytic phenotype, being defective in generating and maintaining transformants at the high temperature where lytic growth is inhibited. Conversely, tsA1499 generates and maintains transformants efficiently at the temperature where lytic growth is inhibited and is severely defective for transformation at the lytically permissive low temperature. This dissociation of the lytic and transforming phenotypes as expressed by mutant tsA1499 reveals that the transforming and lytic activities of the A region of SV40 virus can function independently. (Pintel, Bouch, and DiMayorca, 1981)

The function of 53K is unknown, but several interesting properties have been attributed to this protein in addition to its association with large T antigen. Transformed cells seem to express significantly higher levels of 53K than normal cells. Similarly, SV40-infected mouse or monkey cells synthesize 53K more rapidly than uninfected cells. Furthermore, lymphocytes freshly prepared from the spleens of young mice express detectable levels of 53K only after mitogenic stimulation with an agent such as concanavalin A. Another interesting observation is that some mice bearing SV40-induced tumors raise autoantibodies against 53K. Because 53K synthesized by SV40-transformed or infected cells becomes complexed with large T it was of interest to determine how these proteins interact and whether this interaction relates to the role of large T in promoting DNA synthesis, regulating early mRNA synthesis and in cellular transformation. A significant fraction of the T antigen extracted from cell lysates is tightly bound to a cellular phosphoprotein of molecular weight 53,000 (53K). The direct interaction of highly purified T antigen (D2T antigen or SV80 T antigen) with 53K in lysates from a variety of mouse cell lines was demonstrated in vitro. All the 53K detected in these lysates was able to bind to added T antigen, and it was the only cellular protein with which T antigen formed a stable complex. It may be concluded that 53K binds directly to a specific site on T antigen and that binding occurs without previous modification and without involvement of other virus-encoded or induced proteins. (McCormick et al, 1981)

In vitro transformation of rodent cells by papovaviruses has been well established. For SV40 and polyoma, it has been shown that the early regions of these viruses are essential for transformation. The early region of SV40 codes for large and small T-antigens. Both of these antigens appear to have some role in cell transformation. The role of these antigens in cell transformation has been investigated by the use of mutants in the genes coding for these antigens. The human papovavirus, BKV, also transforms cell in vitro. The genome organization of this virus resembles that of SV40. The early region of this virus also codes for large and small T-antigens and these antigens are expressed early during infection and also in transformed cells. The role of these antigens in cell transformation has not, however, been directly studied due to the unavailability of mutants of this virus. The genome of RFV, a closely related variant of BKV, consists of two complementary defective molecules, one, R1, with a deletion corresponding to 20-30% of the coding region for capsid proteins VP2 and VP3 and the other, R2, with a deletion corresponding approximately to 50% of the coding region for large T-antigen of BKV. Blot-hybridization of cellular DNA from hamster, rat, and guinea pig cells transformed by RFV reveals that all three

transformed cell types contain viral DNA integrated into high-molecular-weight DNA. Moreover, all the cell lines contain the viral DNA species which has the intact early region. None of the cell lines contains both DNA species. RFV is propagated very effectively as two defective viral DNA species without parental helper, probably due to the presence of repeated origins of replication. Therefore, it will be of great significance to see if the DNA species with an intact early region and a tandem repeat of the origin of replication has a greater efficiency of transformation in lytic human cells and nonlytic rodent systems. Since nonlytic systems are generally more readily transformable, if a human cell is infected with the DNA species containing only the functional transforming genes, the DNA and tumor antigens will be readily produced and the absence of late functions will preclude cell death before transformation can occur. If higher transformation efficiency is observed, especially in human cells, greater attention will have to be paid to the oncogenic potential of the BKV variants which have more than one DNA species as their genome. This is an important consideration since this type of genome organization appears to be a common but unique feature of human papovaviruses. (Pater et al, 1981)

Recent colposcopic and cytopathologic studies have focused attention on the high prevalence of condylomatous lesions of the cervix in sexually active women. According to present experience, condylomatous lesions on the cervix are at least twice as prevalent as cervical intraepithelial neoplasia (CIN). This recent recognition of an increased prevalence of cervical condylomata is a result not of an "epidemic outbreak" of new cases, but of an increasing use of colposcopy and a reinterpretation of cytologic, pathologic, and colposcopic findings. A large proportion of cervical lesions classified in the past as CIN grade 1 or 2 (mild to moderate dysplasia) are considered today to be the flat variant of the relatively rare, papillary, condyloma acuminatum. Unlike the papillary lesion, flat condyloma cannot be readily recognized by naked eye examination. The papillomavirus etiology of the flat condylomata has been supported by the detection of human papilloma virus (HPV) particles and HPV antigen in a significant proportion of these lesions. Cervical condylomata as well as CIN are venereally transmitted lesions. The most studied agent suspected to be etiologically related to CIN is herpes simplex virus type 2. However, the frequent association of cervical condylomata with and occasional progression to CIN are circumstantial evidence that HPV may also have an etiologic role in cervical carcinogenesis. The ultrastructural and immunohistochemical study reported confirms previous postulates that cervical lesions considered both cytologically and histologically condylomata are indeed associated with HPV. Consistent with previous ultrastructural studies of condylomatous lesions of the cervix, it was found that HPV chiefly resides within koilocytotic cells. This cellular distribution of HPV provides for a direct correlation between presence of HPV and extent of koilocytotic cells in condylomatous lesions. The general submicroscopic alterations of cells with koilocytotic changes including marked cytoplasmic cavitation and degenerated, pyknotic nuclei are consistent with irreversible cell injury. The latter may be a result of productive viral infection. This contention seems to be supported by the considerably larger number of HPV particles in koilocytotic cells than in those without such alterations. On the other hand, there were many koilocytotic cells in which neither particles nor antigen was demonstrable. HPV was not identified by either transmission electron microscopy (TEM) or immunoperoxidase technique (IPT) in nonkoilocytotic cells of the basal and parabasal layers of condylomatous epithelium. Using TEM, only 25% of the 97 condylomata contained HPV. The limitations of TEM are eliminated by using IPT by which comparatively larger tissue areas are examined. This aspect

is especially important since HPV has often an irregular distribution within condylomatous epithelium alternating with HPV-negative cellular zones. Also, IPT appears to be more sensitive than TEM. In the present study, IPT yielded twice as many HPV-positive cases (48%) as TEM (25%). In 52% of cervical condylomata, a series of 97 condylomata, were devoid of HPV as evidenced by negative IPT. Morpohologically, no significant differences were noted in comparison of HPV-negative and HPV-positive condylomata. However, HPV-negative lesions contained comparatively fewer instances with levels 2 and 3 koilocytosis than their HPV-positive counterparts. Extent of levels of koilocytosis of condylomata was positively correlated with HPV detection rates, explaining at least partly, the HPV-free nature of a certain proportion of condylomatous lesions. Moreover, recent studies have shown that genital warts which do not yield viral particles may still contain many copies of free viral DNA and that HPV type 6, a newly characterized papillomavirus serotype, may be responsible for a large proportion of the papillomavirus infection of the genital tract. The immunoperoxidase technique is likely to be of a value in defining the clinical and pathological spectrum of these infections and in evaluating their role in producing genital tract cancer. (Ferenczy et al, 1981; Krzyzek et al, 1980)

It has been known for some time that papillomaviruses cause benign warty tumors in a number of animal species, including humans. Moreover, in several animal systems papillomaviruses, or components thereof, have been identified associated with malignant tumors occupying similar anatomical sites and suspected of arising from papillomas. Although papillomaviruses clearly cause benign wart disease in man, their involvement or even association with human malignant disease is presently unknown. Certain chronic wart disease syndromes have been known to exhibit a propensity towards malignancy and there has been considerable interest in determining whether human papillomaviruses are, at the very least, associated with, and quite possibly involved in the development of, malignant tumors in these patients. In an effort to resolve this issue studies were initiated on a group of patients exhibiting the wart disease syndrome, epidermodysplasia verruciformis (EV), which is a rare, familial disease characterized by a life-long progression of cutaneous, wart-like lesions to the malignant phenotype. This specific wart disease syndrome is of particular interest because of the high incidence (ca. 25%) of patients displaying transition from benign papillomas to frank, multiple cutaneous carcinomas. Although human papillomavirus (HPV) has been demonstrated to be associated with benign papillomas from patients with EV, no virus has been detected in carcinomas suspected to have arisen from wart tissue in these patients. Recent studies have in fact demonstrated that benign lesions from EV patients contain two types of HPV, type 3 and type 5. Whereas HPV 3 appears to be present exclusively in those EV patients that do not exhibit malignant conversion, HPV-5 is associated with warts from EV patients that invariably develop carcinomas. In addition to a specific type of HPV, other factors appear involved in the development of carcinomas in these patients. For instance, because carcinomas develop on sun-exposed areas of the skin, it has been postulated that ultraviolet light is in part responsible for the transition from papillomas to carcinomas. Moreover, many patients exhibit defective cell-mediated immunity, which may play a role in their disposition to viral infection and susceptibility to carcinoma. Because of the lack of apparent virus in carcinomas from patients with EV studies were initiated to determine whether HPV-specific DNA can be demonstrated in carcinoma tissue from these patients exhibiting this particular wart disease syndrome. DNA extracted from squamous cell carcinomas from patients with the chronic wart disease syndrome, EV, was analyzed for the presence of HPV-specific DNA sequences by Southern blot

hybridization analysis. Employing an HPV probe obtained by molecular cloning of viral DNA purified from benign warts from these patients, HPV-specific nucleotide sequences were unequivocally identified in squamous cell carcinomas from these patients. Restriction endonuclease mapping indicated that the DNA present in the carcinomas was of the same type (type 5) as that found in the benign tumors from these patients and was present as unintegrated, free viral DNA. Moreover, the presence of HPV-5 DNA was demonstrated in a subcutaneous metastatic tumor from one of these patients. This latter observation essentially eliminates the possibility that the HPV-5 DNA present in the malignant tumors in these patients resulted from cross-contamination from an adjacent benign warty lesion. In addition to wildtype HPV-5 DNA, both the primary and metastatic carcinomas analyzed also contained an HPV-5 DNA species lacking approximately 20% of the HPV-5 DNA genome. These subgenomic forms of HPV-5 DNA could not be detected in benign papillomas from these patients. The exact significance of these subgenomic forms and their involvement in transformation is presently unclear. (Ostrow et al, 1982)

In the RNA virus studies I component there are approximately 80 grants utilizing the murine (70), feline (8), primate (1), and hamster (1) model systems. Of these, 33% are involved with studies of gene organization, control and expression; 36% are devoted to studies of virus-cell interaction; 19% are involved in the studies on the characterization and biological activity of retroviruses; 2% support studies on detection in human material of activities or components characteristic of RNA viruses; 4% support research on cocarcinogenesis, 5% involve studies on the inhibition of viral replication and cell transformation; and 1% support conference grants.

Retroviruses first attracted widespread attention as oncogenic agents that amplify their RNA genomes through DNA intermediates. Vigorous study of these viruses during the past decade has illuminated them from several other fascinating perspectives: as agents with varied pathological potential, dispersed through many species and transmitted by vertical as well as horizontal routes; as parasites well-adapted to host functions, thereby facilitating orderly integration and expression of viral genomes; as intermediates themselves in the relocation of DNA proviruses, which are structural homologues of the transposable elements of other organisms; as mutagens equipped to interrupt or activate cellular genes; and as vectors able to transduce cellular genes and potentially act as agents of evolutionary change. No other class of animal viruses exhibits such profound intimacy with the genomes of their hosts. Information gathered concerning this relationship seems likely to elucidate our understanding of the transformation process.

All retroviruses whose genomes have been closely examined share a common feature; the presence of a nucleotide sequence which encodes a protein unnecessary for viral replication but required for the induction and maintenance of the transformed phenotype. Such sequences have been found to be closely related to a sequence that occurs in the uninfected host cell yet is distinctive from the genome of any endogenous viruses which might be present. The virus transformation-specific sequences have been generally referred to as onc genes. Presently there are at least 15 distinct onc genes which have been identified in about 20 isolates of transforming retroviruses. The importance of these findings is that they may lead to the formulation of general principles of the biogenesis of neoplasia and provide useful reagents for diagnosis, prevention or treatment of cancer.

Recently developed molecular cloning techniques have made possible the isolation of genes that transform a healthy cell into a cancer cell. A mouse cell line has been transfected with the DNAs from a human bladder carcinoma, a human colon carcinoma and a human promyelocytic leukemia. The genes in the transfected human DNA were easily identified because they were associated with a highly repeated class of human DNA which is not crossreactive with any sequences in the mouse background. The data indicates that there were three distinctly different human oncogenes operative in these three different types of malignancy. The proteins induced by transforming genes are also under study. Preliminary evidence indicates that a phosphoprotein, which is structurally similar to and crossreactive with a phosphoprotein present in mouse cells transfected by the DNA from a rat neuroblastoma cell line, is formed after transfection with the DNA from cells of human neuroblastoma cell line. Studies are underway to probe the relationship between the human oncogene and the induced phosphoprotein, since indirect evidence suggests that this transforming gene encodes the structure of the protein. These findings raised the question concerning the relationship of the transforming genes found in the human cancer cells to retroviral onc genes. Of all the known viral onc genes, two related sarcoma genes have been found to resemble oncogenes from the human cancer cells. These similarities have been detected by using DNA copies of the RNA onc genes to look for corresponding DNA segments in mouse cells transformed with human cancer cell DNA. Researchers found that cells transformed by DNA from the EJ line of human bladder carcinoma cells contain a DNA fragment that reacted with a probe for the ras oncogene of the Harvey strain of murine sarcoma virus; therefore, the two are related. Other investigators studying the bas gene, which is present in a sarcoma virus that causes cancer in BALB/c mice and is nearly identical to the ras oncogene, found the transforming gene of the T24 line of human bladder carcinoma cells to be closely related to the bas gene. Earlier work has already suggested that the EJ and T24 transforming genes might be identical. Other relationships between retroviral onc genes and the human onc genes are presently under study.

Analysis of the functions of the different parts of the Abelson murine leukemia virus (A-MuLV) genome requires an assay for the biological activity of the cloned DNAs. Experiments by Goff et al., 1982, demonstrated that a cloned, permuted DNA copy of the A-MuLV genome was capable of eliciting the morphological transformation of NIH/3T3 fibroblasts when applied to cells in a calcium phosphate precipitate. The efficiency of the process was extremely low, yielding approximately one transformant per microgram of DNA under conditions which give 10^4 transfectants per microgram of other DNAs (e.g., Moloney sarcoma virus proviral DNA). The DNA was able to induce foci, even though the 3' end of the genome was not present. The transforming gene was thus localized to the 5' portion of the genome. The transformed cells all produced viral RNA and the virus-specific P90 protein. Transmissible virus could be rescued from these cells at very low frequencies by superinfection with helper virus; the rescued A-MuLV virus had variable 3' ends apparently derived by recombination with the helper. Dimerization of the permuted A-MuLV cloned genome to reconstruct a complete provirus did not improve transformation efficiency. Virus could be rescued from these transformants, however, at a high efficiency. Cotransfection of the permuted A-MuLV DNA with proviral Moloney murine leukemia virus (M-MuLV) DNA yielded a significant increase in the efficiency of transformation, and cotransfection of dimeric A-MuLV and proviral M-MuLV resulted in a high-efficiency transformation yielding several thousand more transformants per microgram than A-MuLV DNA alone. It is proposed that helper virus efficiently rescues A-MuLV from transiently transfected cells which would not otherwise have grown into

foci. It was hypothesized that multiple copies of A-MuLV DNA introduced into cells by transfection are toxic to cells. It is likely that the toxic effect is mediated by expression of the P90 protein and is not a direct effect of the A-MuLV DNA because inactive clones and fragmented clones did not show the effect. In support of this hypothesis, it was shown that A-MuLV DNA sequences inhibited the stable transformation of cells by other selectable DNAs.

During the life cycle of retroviruses, the viral genomic RNA is transcribed to DNA, some of which is integrated into the host chromosome. This crucial function carried out by viral encoded reverse transcriptase is obligatory for establishment of infection. Although the mechanism of reverse transcription is quite complex and not fully understood, several forms of double-stranded viral DNA including linear, circular, and supercoiled DNAs have been reported in infected cells. Both the in vivo- and in vitro-synthesized viral DNAs have two types of genome-length molecules: (i) those that contain 5'-end genomic sequences (U_5) repeated at their 3' end (U_3), forming a structure 5' . . . $U_3U_5$3' and (ii) those that, in addition to having 5' genomic RNA sequences repeated at their 3' end, also contain 3' genomic RNA sequences repeated at their 5' end, forming a structure 5'-U_3U_5 . . . U_3U_5-3'. However, analysis of the integrated viral DNA shows only structures containing U_3U_5 . . . U_3U_5 sequences. The U_3U_5 unit is referred to as the long terminal repeat (LTR). The biological function of the LTR is not clearly understood, but its structure warrants several speculations. The LTR contains control elements for both the promotion and termination of viral RNA transcripts and may mediate or facilitate the integration of viral DNA into the host chromosomal DNA. Nucleotide sequence analysis of LTRs from several proviral DNAs has shown: (i) direct duplication of sequences at the termini of viral DNA, i.e., LTRs; (ii) inverted repeats at the termini of each LTR; and (iii) direct repeat of adjacent cellular sequences at both termini of proviral DNA. These structural attributes of LTRs are reminiscent of structures associated with bacterial transposons, TY1 elements of yeasts, and the copia element in Drosophila. In recent studies (Van Beveren et al., 1982), the nucleotide sequence of the long terminal repeat (LTR) of three murine retroviral DNAs has been determined. The data indicate that the U_5 region (sequences originating from the 5' end of the genome) of various LTRs is more conserved than the U_3 region (sequences from the 3' end of the genome). The location and sequence of the control elements such as the 5' cap, "TATA-like" sequences, "CCAAT-box," and presumptive polyadenylic acid addition signal AATAAA in the various LTRs are nearly identical. Some murine retroviral DNAs contain a duplication of sequences within the LTR ranging in size from 58 to 100 base pairs. A variant of molecularly cloned Moloney murine sarcoma virus DNA in which one of the two LTRs integrated into the viral DNA was also analyzed. A 4-base-pair duplication was generated at the site of integration of LTR in the viral DNA. The host-viral junction of two molecularly cloned AKR-murine leukemia virus DNAs (clones 623 and 614) was determined. In the case of AKR-623 DNA, a 3- or 4-base-pair direct repeat of cellular sequences flanking the viral DNA was observed. However, AKR-614 DNA contained a 5-base-pair repeat of cellular sequences. The nucleotide sequence of the preintegration site of AKR-623 DNA revealed that the cellular sequences duplicated during integration are present only once. Finally, a striking homology between the sequences flanking the preintegration site and viral LTRs was observed.

The retrovirus expression of eight independent lymphoid cell lines derived from spontaneous thymomas of AKR mice was investigated (Pedersen et al., 1982). The RNase T_1 fingerprints of viral 70S RNA produced by these cell lines were compared

with genome structure of the nonleukemogenic Akv virus and with two types of cloned leukemogenic viruses derived from one of the thymoma cell lines. Viral RNAs from three cell lines, SL3, 4, and 7, were indistinguishable from one another. The fingerprint patterns indicated that these cell lines produce equal amounts of two prototype, leukemogenic SL viruses that were previously isolated from the SL3 cell line. Viral RNA produced by the SL1 and SL2 cell lines contained similar components, but at a different ratio. Two other cell lines (SL5 and SL11) produced viral RNAs that resemble those of AKR mink cell focus-forming viruses. One additional line, SL9, produced viral RNA of a novel structure. The complex pattern of viral RNA expression observed for these lymphoid cell lines can be interpreted in terms of the recombination between RNA products of three types of proviral loci: the locus that codes for the Akv virus, proviral loci related to inducible xenotropic viruses, and a locus that codes for at least the gag-pol and 3' region of the SL virus that is here designated the spontaneous leukemia virus locus. Further studies of the structure and expression of such endogenous viral loci may contribute to our understanding of the etiology of spontaneous AKR lymphoma.

The sequence of 2,191 nucleotides encoding the env gene of murine retrovirus Akv was determined by using a molecular clone of the Akv provirus (Lenz et al., 1982). Deduction of the encoded amino acid sequence showed that a single open reading frame encodes a 638-amino acid precursor to gp70 and p15E. In addition, there is a typical leader sequence preceding the amino terminus of gp70. The locations of potential glycosylation sites and other structural features indicated that the entire gp70 molecule and most of p15E are located on the outer side of the membrane. Internal cleavage of the env precursor to generate gp70 and p15E occurs immediately adjacent to several basic amino acids at the carboxyl terminus of gp70. This cleavage generates a region of 42 uncharged, relatively hydrophobic amino acids at the amino terminus of p15E, which is located in a position analogous to the hydrophobic membrane fusion sequence of influenza virus hemagglutinin. The mature polypeptides are predicted to associate with the membrane via a region of 30 uncharged, mostly hydrophobic amino acids located near the carboxyl terminus of p15E. Distal to this membrane association region is a sequence of 35 amino acids at the carboxyl terminus of the env precursor, which is predicted to be located on the inner side of the membrane. By analogy to Moloney murine leukemia virus, a proteolytic cleavage in this region removes the terminal 19 amino acids, thus generating the carboxyl terminus of p15E. This leaves 15 amino acids at the carboxyl terminus of p15E on the inner side of the membrane in a position to interact with virion cores during budding. The precise location and order of the large RNase T_1-resistant oligonucleotides in the env region were determined and compared with those from several leukemogenic viruses of AKR origin. These comparisons showed that the only region altered in all of them relative to Akv is the region extending from approximately the carboxyl terminus of p15E into the U_3 portion of the LTR sequence. A corresponding region has been implicated in the leukemogenic potential of avian leukosis virus. In addition to the coding function, this region plays a role in the origin of second-strand DNA synthesis during reverse transcription and possibly in the control of RNA transcription. The role of these alterations in the leukemogenic potential of these viruses remains to be determined.

The proviruses of the AKv strain of ecotropic murine leukemia viruses, also referred to as type 1 MLVs, are genetically transmitted among many laboratory strains of the mouse. These genetically transmitted proviruses segregate in

a Mendelian fashion and have, in some cases, been mapped to specific mouse chromosomes. The gel electrophoresis-hybridization technique of Southern can be used to detect and resolve AKv proviruses integrated at different sites in the mouse genome. In previous work, this technique was used to associate restriction endonuclease-generated DNA fragments of AKR mouse DNA with the endogenous AKv proviruses Akv-1 and Akv-2. One surprising result of these studies was that three sublines of AKR mice, the AKR/J, AKR/N, and AKR/Cum sublines, differed in the pattern of AKv-containing restriction endonuclease fragments. Specifically, a restriction fragment shown to carry the Akv-2 provirus was found in the DNA of the AKR/N subline, but not in the AKR/J or AKR/Cum sublines. The AKv proviruses of sublines of AKR mice, of C3H mice, and of a series of AKXL recombinant inbred strains have been analyzed by the gel electrophoresis-hybridization technique of Southern (Steffen, Taylor and Weinberg, 1982). The restriction endonuclease EcoRI fragments containing two previously unidentified, genetically transmitted AKv proviruses of AKR mice were identified. Comparison of different sublines of AKR mice revealed considerable heterogeneity in their complement of germ line proviruses. This heterogeneity provides evidence that the provirus complement of AKR mice is not stable. Rather, the number of genetically transmitted proviruses increases during inbreeding. Examination of a series of sublines of the C3H strain indicated that this amplification is dependent on viremia. It was estimated that, in viremic strains of mice, one new provirus becomes fixed in the germ line every 15 to 30 years. Since integration of a provirus into the mouse genome is mutagenic, the accumulation of proviruses integrated into novel sites in the mouse genome and the resulting polymorphism of provirus integration sites might be an important source of genetic instability and polymorphism among mice.

Crosses of mice of the high-lymphoma mouse strains, AKR and C58, with mice of the low-lymphoma strains, C57L, DBA/1, and DBA/2, produce F_1 generations with low and late incidences of the spontaneous disease. Backcross populations, obtained from high-lymphoma parental strain X low-lymphoma F_1 matings, showed lymphoma incidences of ≅75%, indicating that the low lymphoma incidence in F_1 mice of these crosses was due to the simultaneous presence of at least two independently segregating dominant genes inherited from the low-lymphoma parental strain. In none of the three crosses was lymphoma resistance linked to the Fv-1 locus, and in only one (AKR X C57L) was H-2 type a significant determinant of the disease. This finding indicates that previously undiscovered genes possess the capacity to suppress spontaneous murine lymphoma. Studies of the expression of endogenous ecotropic and xenotropic murine leukemia viruses in mice of these crosses suggest that one of the new genes involved in lymphoma suppression may act by delaying the expression of xenotropic virus. It is not excluded that an allele at the Akvr-1 locus might be involved in the findings reported here. A dominant allele, identified so far only in wild mice, at this presently unmapped locus efficiently suppresses lymphomagenesis in crosses with AKR mice, but this same allele also causes profound suppression of ecotropic virus expression in the AKR X wild mice crosses. If the Akvr-1 locus is involved in lymphoma and MuLV suppression in the present crosses, then the allele carried by the low-lymphoma inbred strains must be a third one, different from that of either AKR or wild mice.

The Rauscher virus complex (RV) is characterized by its ability to induce erythroblastosis, myeloblastosis, and thymic lymphoma in mice. It was shown that RV consists of at least four separate viruses, three replication-competent viruses and one replication-defective virus. The question arises whether different viral components are responsible for the induction of different diseases, or whether

some components can induce more than one disease. The viral component in the complex causing a rapid erythroblastosis both in newborn and in adult mice is the replication-defective component. Another viral component of the RV which induces an erythroblastosis is the dualtropic mink cell focus-inducing virus (R-MCF). This virus induces a slow erythroblastosis when injected into newborn mice, but causes no apparent disease when injected into adult mice. The pathogenicity of two ecotropic viruses cloned from an RV, that had been passaged in the mouse, has been studied by M. Vogt, 1982. One of the viruses, R-MuLV, gives rise to syncytia in the XC test (XC-positive), the other, R-XC$^-$ virus, does not (XC-negative). Both cloned viruses induce erythroblastosis and thymic lymphoma after inoculation into newborn NIH Swiss mice. Another clonal isolate of R-MuLV, from a stock which has been propagated in tissue culture, similarly induces both diseases when injected into newborn NIH Swiss mice. All R-MuLV and R-XC$^-$ induced splenic and thymic tumors produced, in addition to the injected virus, mink cell focus-inducing virus. This is consistent with the idea that MCF viruses are the actual (proximal) viruses causing the proliferative processes in the hematopoietic cells which lead to the different diseases. The finding that an R-MCF virus, which in most cases induces erythroblastosis, can also induce thymic lymphoma at a low frequency suggests that both diseases could be induced by the same MCF virus.

To explore the role of endogenous retroviruses in radiation-induced leukemogenesis in the mouse, virus-encoded proteins in nine BALB/c leukemias have been examined by pulse-chase labeling procedures and serological typing with monospecific and monoclonal antibodies (Tress et al., 1982). The major gag precursor protein, $Pr65^{gag}$, was observed in all cases, but only three leukemias expressed detectable amounts of the glycosylated gag species, $gP95^{gag}$, or its precursor, $Pr75^{gag}$. No evidence was found for synthesis of gag-host fusion proteins. None of the leukemias released infectious xenotropic or dualtropic virus, but all nine expressed at least one env protein with xenotropic properties. In two instances a monoclonal antibody, 35/56, which is specific for the MuLV G_{IX} antigen, displayed a distinctive reactivity with this class of env protein, although this antibody is unreactive with replicating xenotropic viruses. An ecotropic/xenotropic recombinant env protein with the same 35/56 phenotype was observed in a leukemia induced by a strongly leukemogenic virus isolated from a BALB/c radiation leukemia. Because of the frequent association of env recombinant murine leukemia viruses (MuLVs) with virus-induced leukemias of AKR and other mouse strains, there has been considerable interest in the hypothesis that recombinant env proteins might play some essential role in the leukemogenic process. This might not necessitate production of infectious virus with such an env phenotype; expression of an env protein related to xenotropic MuLV might occur as a viral gene product only in the leukemia cell itself. This could result from genetic deletions in replication-competent virus, or reflect activation of replication-defective endogenous MuLV genes.

Recent studies by Levenson et al., 1982, have shown that the level of cytoplasmic calcium ions appears to be important in the control of murine erythroleukemia (MEL) cell differentiation. Interest in this study focuses on the relationship between the regulation of calcium concentration and differentiation. The fluorescent membrane probe $DiOC_6$ was used to examine the relationship between MEL cell mitochondria and changes in cytoplasmic calcium levels occurring at the initiation of commitment. Fluorescence microscopy reveals the selective association of $DiOC_6$ with MEL cell mitochondria, where an enhanced fluorescence is observed. Treatment of cells with dimethylsulfoxide (DMSO) or other inducers causes a decrease in mitochondria-associated fluorescence levels that occurs

with the initiation of commitment. A decrease in $DiOC_6$ fluorescence is caused by agents that reduce mitochondrial membrane potential, but is only slightly affected by agents that alter plasma membrane potential. Amiloride and EGTA, agents that prevent commitment and inhibit calcium uptake, also prevent the decrease in $DiOC_6$ uptake caused by DMSO. The effect of DMSO on MEL cell mitochondria is mimicked by FCCP, a proton ionophore that dissipates mitochondrial membrane potential. FCCP also caused MEL cell mitochondria to release calcium into the cytoplasm. When MEL cells are treated with DMSO plus FCCP, commitment is initiated without the lag period observed when cells are treated with DMSO alone. These results are consistent with the hypothesis that mitochondrial transmembrane potential is important in the regulation of cytoplasmic calcium levels at the time of commitment of MEL cells to terminal differentiation.

Mouse mammary tumor virus (MuMTV) is a retrovirus which causes mammary carcinomas in mice. MuMTV also infects certain heterologous cells in culture, but does not appear to alter their growth or morphological characteristics. The infected heterologous cells and virus-producing cells derived from mammary tumors have been used extensively to examine the synthesis and structure of viral DNA, RNA, and proteins and the hormonal response of MuMTV to glucocorticoid hormones. By taking advantage of the rapid hormone induction of MuMTV RNA and its large augmentation, the kinetics of dexamethasone induction of MuMTV RNAs and proteins in virus-infected rat XC cells and GR mouse mammary tumor cells have been studied by Robertson and Varmus, 1982. A detectable increase in viral RNA in infected XC cells was present within 10 min after hormone addition, and half-maximal induction was achieved in less than 2 hr. The increase in viral RNA concentration was apparent first in nuclear RNA and later in the cytoplasm. Within the first 15 min of induction, only genome-sized RNA (35S, 7.8 kilobases) was present in augmented amounts, whereas the major subgenomic RNA (24S, 3.8 kilobases) did not appear until at least 30 to 60 min postinduction. The sequential appearance of these RNAs, the probable mRNA's for the gag and env proteins, paralleled the order of appearance of the gag and env proteins, respectively, after hormone treatment. An additional species of viral RNA (20S, 2.5 kilobases) was detected during these induction experiments, but the role of this RNA is not known. Both subgenomic RNAs contain sequences derived from both the 5' and 3' termini of genomic RNA and are presumably spliced. After dexamethasone induction of infected XC cells, two smaller env-related proteins which were not found in full hormone induction were detected. The functional role of these smaller proteins is not known. A previously reported smaller species of RNA (13S, 1.0 kilobase) did not appear to be induced and was shown to be cellular rather than viral in origin. In the fully induced infected XC and GR mammary tumor cells, the only viral RNAs present were the 35S and 24S RNAs. In addition, mammary tumors contained only these two viral RNAs. Thus, tumor cells appear to contain only the viral RNAs which direct the synthesis of the gag, pol, and env proteins of the virion.

The sequencing of the long terminal repeats (LTRs) of two horizontally transmitted MuMTVs from the C3H and RIII strains of mice have been completed. The sequences confirm the presence of a long open reading frame capable of allowing the translation of a 36,000 dalton protein; they have been compared to LTR sequences adduced by other investigators. The 5' and 3' ends of the envelope gene have been determined and attempts have been made to correlate the sequence with available information about the structure of envelope proteins. An unusual cloned provirus representing a reverse transcript of an envelope messenger RNA was used to determine the splice junction in envelope messenger RNA; also the region of the splice donor site and splice acceptor site from wild type DNA have been sequenced. The

splice signals appear to be similar to those used for most eukaryotic messenger RNAs. The splice acceptor site is one nucleotide upstream from the beginning of the longest open reading frame for the envelope gene. The sequencing of three integration sites for MuMTV proviruses has also been completed. All the results to date suggest that six nucleotides of host DNA are duplicated during the insertion of MuMTV DNA.

MuMTV proviruses have been examined in over 35 mammary carcinomas and the cellular DNA which flanks a single exogenous provirus in one of those tumors has been cloned. A probe prepared from unique sequence DNA from the flanking region was then used to probe integration sites in other tumors, and a total of 5 tumors were found to harbor proviruses in the same 6 kilobase region of the host genome. Thirty kilobases of surrounding DNA were retrieved from a bacteriophage library of mouse DNA and investigated for known onc genes and for transcriptional units. To date, no transcriptional units have been identified in this area, and no known onc genes have been assigned to this domain. The site has been mapped to chromosome 4 or 19. Experiments in process include the cloning of flanking DNA from other unique proviruses in additional tumors, looking for additional insertions further along the chromosome from the first site, and screening large numbers of tumors in transplantable tumor lines for insertions in the original and other domains.

MuMTV-induced mammary tumors in mice are characterized by increased levels of MuMTV proviral DNA, RNA, and proteins. Recently, it has been shown that MuMTV(C3H)-induced mammary tumors in BALB/c mice contain undermethylated copies of MuMTV(C3H) proviral DNA. This finding is significant in view of recently proposed mechanisms concerning the regulation of eukaryotic genes. In particular, the suggestion that active genes are undermethylated, as has been found with the globin and ovalbumin genes and certain eukaryotic viruses, may also apply to active MuMTV proviruses. Various normal, premalignant, and malignant tissues of GR/A mice have been analyzed to determine whether a correlation exists between MuMTV undermethylation and amplification (Fanning, Vassos, Cardiff, 1982). The proviral methylation pattern was examined with the restriction enzyme HhaI, which fails to cleave methylated DNA. MuMTV proviral DNA from liver, kidney, and heart was highly methylated. Proviral DNA was somewhat undermethylated in mammary gland cells from virgin and lactating mice and extensively undermethylated in cells from premalignant outgrowths, pregnancy-dependent tumors, and pregnancy-independent tumors. The restriction enzyme SacI was used to detect additional proviruses in the same cells. No additional proviral copies of MuMTV were detected in liver, kidney, or heart cells or in mammary gland cells from virgin mice. Some mammary gland cells from lactating mice appeared to contain additional copies of the endogenous, highly oncogenic GR-MTV-2 provirus. Premalignant outgrowth, pregnancy-dependent tumor, and pregnancy-independent tumor cells contained an average of two to three additional copies per cell of the GR-MTV-2 provirus. Thus, neoplasia in GR/A mice was directly associated with quantized increases in MuMTV proviral DNA undermethylation and GR-MTV-2 proviral DNA amplification. Restriction enzyme analysis suggested that premalignant outgrowths and pregnancy-dependent tumors both consisted largely of heterogenous cell populations, although some evidence of clonal dominance was detected.

The feline sarcoma virus (FeSV) has been found to be etiologically associated with naturally occurring multicentric fibrosarcomas in young domestic cats. Three independent isolates of the virus have been biologically and biochemically analyzed. These have been designated as the Snyder-Theilen (ST), Gardner-Arnstein (GA), and

McDonough (SM) strains. Each has been shown to transform fibroblasts in tissue culture and to require a replication-competent helper virus for transmission. The replication defectiveness is one consequence of the derivation of these viruses by a recombination event involving replication-competent feline leukemia virus (FeLV) and cat cellular genetic information. The ST and GA strains appear to have been derived from the same or closely related cat cellular sequences while the SM-FeSV-specific sequences appear to have been derived from a different subset of host sequences. Translation products of the genomes of the ST and GA FeSVs have been shown by Snyder, 1982, to possess associated protein kinase activities. These activities are similar to those associated with a number of rapidly transforming retroviruses in that they appear to mediate transfer of radiolabel from [γ-^{32}P]ATP to selected tyrosine residues in substrates and to be independent of the effects of cAMP and cGMP in vitro. The FeSV activities, when detected in specific immune complexes bound to Staphylococcus aureus, result predominantly in autophosphorylation of FeSV polyproteins. Partially purified polyprotein also induces phosphorylation of an exogenous acceptor (casein) in a soluble protein kinase assay system. The FeSV enzymes display marked preference for Mn^{+2} and Co^{+2} over Mg^{+2} while Ca^{+2} fails to initiate activity in vitro. The optimal pH for the reactions is near 7.0. There is a strict requirement for ATP as phosphoryl donor. High concentrations of ADP and dADP are inhibitory to the reaction but low concentrations of dADP are stimulatory. A comparison of these properties with those of other retroviral-associated protein kinases suggests that these activities fall into three, and possibly four, classes. One class, exemplified by RSV pp60^{src}, uses Mn^{+2} and Mg^{+2} with equal efficiency and has broad specificity for phosphate donors and nucleoside effector molecules. A second class, exemplified by Abelson murine leukemia virus kinase, prefers Mn^{+2} and Co^{+2} to Mg^{+2} and can utilize GTP as a phosphoryl donor, although at much lower efficiency than ATP. A third class, exemplified by the FeSV and Fujinami avian sarcoma virus kinases, also prefers Mn^{+2} but appears to have rather strict specificity for ATP as phosphate donor and for adenosine nucleotides as effectors. The transforming proteins of this third class have also been shown recently to be immunologically related. The recent observation that the translation product of Y73 avian sarcoma virus is structurally unrelated to the proteins of the above three classes of virus leaves open the possibility that a fourth class of retrovirus-associated protein kinase activity may yet be defined. It is not clear whether these distinctions are significant in terms of any unifying hypothesis concerning protein kinase-mediated transformation. As representatives of each of the three defined classes of viral protein have been shown to have a normal cellular analog, these differences may simply reflect properties of enzymes existing in the host cells from which the viruses originated. On the other hand, the classes of protein kinases may represent enzymes with quite different primary substrate specificities in vivo. Further studies of the biochemical properties of the viral enzymes, especially after rigorous purification, will be crucial to the understanding of how these kinases compare with normal cellular kinases and how they transform cells.

Early experiments have shown that gag-fes proteins which reacted with antisera to the feline oncornavirus-associated cell membrane antigen (FOCMA) were present in nonproducer cells of nonfeline origin that were transformed by FeSV in vitro. More recently it was found that the same gag-fes proteins were also present in cells that produce FeLV as well as nonproducer cells. Cat cells transformed by chemical carcinogens such as dimethylbenzanthracene did not contain gag-fes. Also, FOCMA-positive feline lymphoma cells, both FeLV-positive and FeLV-negative, lack this protein.

To determine if cats could mount an antibody response to the fes portion of the gag-fes molecule, animals were inoculated with their own biopsied cells following transformation of the cells in vitro with FeSV. The resulting antisera, confirmed to be negative for reactivity with all virion structural proteins, still gave the typical FOCMA-type membrane immunofluorescence reaction on both transformed fibroblasts and lymphoma cells. Antisera prepared in this manner would also successfully immunoprecipitate the gag-fes protein, obviously on the basis of reactivity with the fes determinant. Perhaps of even greater interest, high-titered anti-FOCMA sera taken from cats that had only been exposed to FeLV would also react specifically with fes. This suggested that FeLV must somehow activate fes-related sequences (c-fes) in some of the cells of infected cats, at least those cats that mount high anti-FOCMA responses. In those cats examined, the anti-FOCMA response coincided with the anti-fes response.

In another series of experiments, tumor cells taken from cats with FeSV-induced tumors were examined to determine if they expressed the same gag-fes polyproteins as cells transformed with FeSV in vitro. Cells from progressively growing fibrosarcomas were biopsied, prepared as single-cell suspensions, and tagged with ^{35}S-methionine. Although some were examined at in vitro culture intervals of 1-2 days or less, others were examined after 20 or more subcultivations to determine if the gag-fes proteins were expressed under all such conditions. In every instance tumor cells from animals with FeSV-induced tumors expressed the characteristic polyprotein. The gag-fes polyprotein found in the tumor cells was compared to the analagous protein found in cells transformed in vitro by FeSV using two-dimensional tryptic peptide mapping. No differences could be detected. Additionally, the gag-fes proteins found in tumor cells had the same protein kinase activity found for the protein detected in cells transformed in vitro. Tumor cells taken from virus-negative cats with various types of spontaneous tumors lacked the characteristic gag-fes polyproteins.

Finally, since different "gag-onc" polyproteins identified in association with the avian leukemia viruses have been postulated to mimick differentiation proteins that might function in a given restricted lineage of hematopoietic cells, it was decided to check tumor cells taken from FeSV-induced tumors originating from different embryonic germ layers. Melanomas, which reportedly arise from neuroectodermal cells (whereas both fibrosarcomas and lymphomas arise from mesodermal cells) were induced with FeSV and similarly examined. FeSV-induced melanomas were found to have the same gag-fes polyproteins as cells from fibrosarcomas.

Characterization of that sequence of human DNA that is homologous to the FeSV sarc regions of the ST strain of FeSV has been initiated. Three recombinant phages have been isolated which contain portions of the human chromosomal locus that are strongly homologous to the FeSV sequence. The restriction endonuclease cleavage map of this region has been determined, and the results demonstrate that the portion of the human genome homologous to the FeSV sarc regions contains several regions of strong homology to the FeSV sequence. This region is not a continuous coding region and appears to be interrupted by introns.

Introduction of purified eukaryotic genes into heterologous host cells provides a powerful means for studying the expression of specific genes outside their usual cellular environment. Specific genes have been introduced into mammalian cells in tissue culture by cotransfer with a marker gene for which there is dominant selection. The herpes simplex virus thymidine kinase gene has been used as a selectable marker for introduction of the genes for rabbit β-globin,

chicken ovalbumin, and adenine phosphoribosyltransferase into cells. Stable expression of each gene was achieved but in a nonregulated fashion. Recently, it has been shown that cloned genes transfected into heterologous cells can be expressed and induced by glucocorticoids.

It was of interest to identify the mechanisms and sites involved in the expression and regulation of eukaryotic genes. The rat growth hormone (rGH) gene provides a model for these studies because its expression can be studied in vivo, in primary pituitary cell cultures, and in vitro, in a stable clonal cell line. The use of cultured cell lines permits direct analysis of hormonal regulation of genes not possible in vivo or using organ cultures.

The use of DNA-mediated gene transfer to assess the functional significance of sequences involved in the expression and regulation of this gene has been reported (Doehmer et al., 1982). The rGH gene was introduced into mouse 3T3 cells, using a plasmid vector that contains the entire genome of Moloney mouse sarcoma virus (Mo-MSV). Mo-MSV is able to transform fibroblasts in vitro and thus provides a selection criterion. Then a 7.6-kilobase pair (kb) BamIII fragment of rat genomic DNA was introduced into NIH/3T3 mouse fibroblasts by inserting it into Mo-MSV DNA. The ability of the viral DNA to induce foci in the recipient cells was used as a dominant selection marker. Several copies of rat growth hormone DNA were integrated in the mouse cells. The transformed mouse cells expressed rat growth hormone-specific mRNA and secreted mature rat growth hormone. In rat cells, the expression of this gene is regulated by glucocorticoids. This study demonstrated that hormone-dependent regulation transfers with the clone and thus appears to be an intrinsic property of the gene or its RNA products.

In the RNA Virus Studies II component, there are 82 research grants. Of these, approximately 90% are predominantly involved with studies of avian tumor virus. The remaining 10% touch on a variety of subjects which are more distantly related to human disease. Many of the studies funded by the RNA Virus Studies II area are involved with, or seek to explain, the molecular nature of the transformation process, the definition and discovery of new oncogenes and possible explanation(s) of how viruses without definitive oncogenes can be involved in oncogenesis. A few examples of the types of studies accomplished in the last year will follow: From studies of these viruses, two patterns of viral oncogenesis have emerged. Some viruses possess genetic loci or oncogenes whose actions initiate and maintain the neoplastic phenotype of the infected cell. Other viruses are devoid of specific oncogenes and induce tumors by more subtle means whose particulars are now beginning to be understood. Both forms of viral oncogenesis are united by the persistance of at least a portion of the viral genome in the host cell, either as an integral part of the host chromosome or as an independently replicating unit.

The replicative unit in all retrovirus genomes is composed of three genes: gag (structural proteins of the virion); pol (reverse transcriptase); and env (glycoproteins of the viral envelope). An oncogene may be inserted into this unit in at least four distinctive ways. First, as an independently expressed gene that does not impose on either the structure or function of the replicative genes and is expressed from a subgenomic mRNA. The v-src of the Rous sarcoma virus is a well known example of this class. Secondly, as an independently expressed gene that replaces part or all of a replicative gene, env for example, and is expressed from a subgenomic mRNA. The v-myb of avian myeloblastosis virus is an example. Thirdly, as a fusion between v-onc and a portion of gag that is accompanied by

deletions in one or more of the replicative genes, usually pol and portions of gag and env and is expressed as a polyprotein produced from a genomic-length mRNA, an example is v-myc from avian myelocytomatosis virus. And fourth, as two separately expressed v-onc domains, one fused with a portion of gag, the other expressed independently and the two together replacing portions of replicatative genes. In this instance the gag-onc protein is produced from a genomic length mRNA. The second onc protein from a subgenomic mRNA e.g. erb-A and erb-B of avain erythroblastosis virus. With the exception of v-src, the insertion of oncogenes into retrovirus genomes creates genetic defects that preclude the production of virus unless the defective function is provided by a second helper virus.

A large number of retrovirus genes and genomes have now been cloned, including the entire genomes of Rous sarcoma virus, avian erythroblastosis virus, mylocytomatosis virus and Rous assessory viruses one and two. Numerous subclones of these viruses that define functional and structural domains of the genome as well as the oncogenes of AMV and PRCII avian sarcoma virus have also been cloned. Transfection has been used to demonstrate that each of the cloned genomes is infectious.

Attention has been focused on the long terminal redundancy (LTR) of retrovirus genomes. The mechanism by which this structure is generated during reverse transcription has now been largely elucidated both in vitro and in vivo. Although no laboratory has yet demonstrated directly a role for the LTR in the integration of viral DNA, two other functions have been discovered. First, it directs the initiation of transcription from viral DNA and can also be used as a fairly efficient artificial promoter when fused into other genetic units. Second, it can enhance the frequency with which viral or other DNAs are assimilated and expressed by mammalian cells. The precise mechanism of this enhancement is not yet clear but the phenomenon has practical value in designing vectors for the delivery of foreign genes to cells.

The structure of integrated retrovirus DNA has all of the hallmarks of transposable elements found in bacteria, yeast and Drosophila. This statement is supported by extensive sequencing data, however, there is no evidence to date that retrovirial DNA can transpose directly from one position to another in cellular DNA.

The genesis of retrovirus messenger RNAs has been further elucidated. The splice donor and acceptor sites have been mapped precisely on the genome and messenger RNAs of RSV and AEV. The patterns of mRNA produced from various categories of endogenous retrovirus proviruses in chicken cells have been described. The results indicate that lesions in the proviruses can cause anomalies in either the processing or the nuclear-cytoplasmic transport of viral RNAs.

Several functional domains within the protein encoded by src (pp60 src) have been eluicidated. A portion, no longer than 10 kilodaltons at the amino-terminus of the protein holds the molecule to the plasma membrane. The carboxyterminal half of the protein is fully active in phosphotransfer to tyrosine; a serine residue in position 17 has been found to be phosphorylated and the sole residue of phosphotyrosine in the protein is located at position 419. Identification of these domains opens the way to an exploration of structure-function relationships by site directed mutagenesis.

Within the last year it has been demonstrated that pp60 src is synthesized on soluble polyribrosomes and that it moves within ten minutes directly to the cytoplasmic

surface of the plasma membrane without cleavage of a signal sequence. It has also been found that the mechanism of this transit may be novel. All of the pp60 src while soluble is complexed with two cellular proteins: an 89 kilodalton protein which is also a heat shock protein of the cell; and a 50 kilodalton protein which is phosphorylated on tyrosine, at least in avian cells, and it may therefore be a target for the kinase activity of pp60 src. Once bound to the membrane, pp60 src is no longer associated with the two cellular proteins. The complex of these three proteins may be designed to keep this very hydrophobic pp60 src protein in solution, and to guarantee its specific arrival at the plasma membrane rather than at other membranous surfaces within the cell.

Only a few cellular proteins phosphorylated by pp60 src have been identified to date. Of these, a protein with molecular weight of about 36,000 is perhaps the best studied. This protein has been purified and monospecific antibodies have been prepared and have been used as reagents to demonstrate that the protein is associated with the plasma membrane of both normal cells and cells transformed by pp60 src. Approximately half of the total 36 kilodalton protein in the cell is bound to the plasma membrane and can be released only by treatment with nonionic detergent. The remainder of the protein is more loosely bound to all of the membranous fractions obtained from disrupted cells.

Efforts in other laboratories devoted to identifying intercellular targets for the viral encoded tyrosine protein kinases have resulted in discovery of their obvious relevance to seven putative substrates of pp60 src, the transforming protein of Rous sarcoma virus in transformed chick cells. Two of these proteins, of molecular weights 46,000 and 39,000 respectively have been characterized. The 39K protein may be closely related to the 36K protein discussed above. The unphosphorylated counterparts of these two phosphoproteins have been purified from normal chick cells by conventional means in order to raise monospecific antisera. In combination with two dimensional gel analysis, these sera have been used to screen cells for the presence of both the phosphorylated and unphosphorylated forms of these proteins and to determine their subcellular locations. The 46K protein is a monomeric, soluble protein which by immunofluorescence appears to be uniformly distributed throughout the cytoplasm. There seems to be a related protein present in mammalian cells. So far, no function has been defined for this protein. The 39K protein is phosphorylated both in cells transformed by viruses and in A 431 human tumor cells treated with epidermal growth factor. The 39K protein is a highly conserved material, found in many different cell types of species as diverse as chickens and humans. Interestingly, the 39K protein is lacking from certain lymphoid cell lines. The protein is a dimer in detergent lysates of cells. It has unusual properties when subject to conventional cell fractionation procedures. Upon detergent lysis of adherent cells the 39K protein and its phosphorylated counterpart are found associated with the cytoskeleton. They are released into the soluble fraction if the cells are suspended by trypsinization before lysis. Upon hypotonic lysis in the presence of EDTA, both species of the 39K protein are soluble, but in the presence of divalent magnesium a considerable fraction is particulate. Immunofluorescence studies on briefly permeablized cells show that 39K protein distributed throughout the cytoplasm in a lattice-like structure which is not a familiar organization for any of the known cytoskeletal proteins. It is suspected that this protein may have a structural role in the cell.

PRC II virus, another virus of the type which encodes tyrosine protein kinases, although of a different class than RSV, has the same set of seven tyrosine

phosphorylated proteins found in RSV transformed chick cells. The transforming protein of this virus has been studied and the primary translation product is a 105 kilodalton protein. Within 5 minutes of the synthesis of this material, some fraction is converted by post-translational modification to a 110 kilodalton protein. The p105 material is primarily found in the soluble fraction of transformed cells while the p110 is mostly associated with the cytoskeleton. Both the p110 and the p105 contain phosphoserine and phosphotyrosine but the p110 has considerably more phosphotyrosine than p105.

Sequencing of the myb oncogene of AMV has been completed. Both the initiator codon and the terminator codon for the gene are located outside the domain of the gene that represents nucleotide sequences acquired by recombination with the genome of the host. Initially it has been found that avian erythroblastosis virus apparently contains two separate oncogenes, erb-A and erb-B. Molecular cloning has been employed to isolate and characterize vertibrate genetic loci relating to the oncogenes src, myc, erb-A, erb-B, myb, and fps. Cellular versions of src have been isolated from both chicken and human DNA. In both instances a locus was found which matches the topography of viral src well except that the cellular gene is interrupted repeatedly by introns. It is now suspected that both chicken and human DNA contain a second version of the src locus which may be incomplete or otherwise anomalous but these loci have not been well characterized. The splice donor site and the cellular mRNA for src has been mapped and it is identical to that found in the mRNA for viral src. The topography of myc and myb isolated from chicken DNA has been studied in detail. Both contain one or more introns, both are expressed by transcription into large precursor RNAs that are then processed through several recognizable intermediates to the mature mRNA. Nucleotide sequencing of cellular and viral myb indicates exceptional similarity between the two genes.

The structure and expression of the cellular erb genes are complex. The erb-A and erb-B genes are represented by separate, possibly unlinked genes in chicken DNA. Both cellular loci contain multiple introns and cover large expanses of DNA. Both are transcribed into multiple mRNAs. The transcription may be specific to certain tissues with the two loci being active in different tissues, a further testimony to their apparent independence. Patterns of transcription from cellular src, myc, erb, and myb genes in different avian tissues have been examined. Myb is expressed preferentially in hematopoetic tissues, but the other genes are expressed in a wide variety of tissues with no apparent preference for particular embryological lineages. Levels of expression detected, however, were all low and it remains possible that each of the cellular genes is vigorously expressed in specific developmental compartments that are not well represented in materials studied to the present. It is of some interest that avian leukosis viruses, which contains no oncogene, induced bursal lymphoma with a high frequency in chickens and related birds. In these tumors, viral DNA is almost inevitably found integrated within a few kilobase pairs of a cellular myc gene. As a seeming consequence of the integration, the myc gene is transcribed at levels far above those found in normal bursal tissue and in other normal cells. In many instances, the augmented transcription for myc is due to the fusion of the viral LTR into the cellular genetic unit. The LTR directly drives transcription from the cellular gene.

A few important exceptions to this rule have been found. In some instances, the LTR is integrated upstream from myc but in the opposite orientation from that required to derive transcription from myc. And in one instance, viral DNA was

integrated downstream from myc. These exceptions still display high levels of transcription from myc, demonstrating that the effects of the LTR, and perhaps of other domains in viral DNA on transcription, extend beyond the direct provision of a promoter.

Other labs have demonstrated that expression of the endogenous retroviral genomes resident in normal chick embyro cells correlates inversely with the degree of methylation at CpG residues. Thus the viral locus EV-3, which is constitutively expressed in a wide variety of cell types, is hypomethylated while EV-1, which is not expressed, is hypermethylated. In a single embryo which spontaneously expresses EV-1, undermethylation has been detected at sites which are normally methylated in cells which do not express EV-1. Furthermore, transient treatment of EV-1 cells with 5 azacytidine, a nonmethylatable analog of cytosine, results in expression of EV-1, production of a novel defective virus particle, induction of DNAase I sensitivity and hypersensitive sites in both the 5' prime and 3' prime LTR regions.

Recent studies on the control of another endogenous virus locus, EV-2, which encodes RAV-0, an infectious retrovirus, have produced other interesting findings. EV-2 has been postulated to be controlled by a cis-acting repressor which accounts for low levels of virus expression and lack of transfectibility of EV-2 DNA. Results of studies on this locus demonstrate that treatment with 5 azocytodine results in an enormous increase in the level of EV-2 expression and demethylation of the genome. In collaborative experiments, EV-2 DNA from azacytidine treated cells, but not from nontreated cells, is infectious in a transfection assay. These data are consistent with the model that a methyl transferase binding site which is itself functional only when methylated is located in the vicinity of the EV-2 genome.

The putative transforming protein of avian myelocytomatosis virus (MC29) is a 110 kilodalton phosphlorated polyprotein consisting of sequences derived from both the gag region p19 and p27 and the myc region. Immunoprecipitation with anti-gag serum of lysates of q8 cells, a nonproducer line of MC29 transformed quail embryo fiberblasts, demonstrates the presence of p110 and another 100 kilodalton phosphorprotein also related to MC29. From cellular fractionation and immunofluorescence analysis of subcellular fractions of MC29 transformed cells, and by immunoprecipitation the majority of the p110 and the p100 is found in a nuclear fraction of nonproducer q8 cells. Additionally, indirect immunofluorescence experiments, utilizing rabbit anti-p27 gag serum, which has been preabsorbed to normal chick cell lysates, showed specific intense immunofluorescence in the nuclei of fixed q8 cells. This fluorescence could be eliminated by preadsorbing the anti-p27-gag serum with either purified RSV or purified p27 gag. These two independent lines of evidence suggests that significant levels of the putative MC29 transforming protein are present in the nuclei of MC29 transformed cells. Up to this time no retrovirus proteins had been shown to be localized in the cell nucleus. The detection of MC29 proteins in the nuclei of transformed cells and tumors may be linked to the fact that the phenotype of cells transformed by this virus is different from cells transformed by other retroviruses. The growth rate, for example, is increased in MC29 transformants, but not in AEV or ASV transformants.

The action of viral oncogenes may provide useful analogs for the enzymatic mechanisms that give rise to and sustain the malignant phenotype. It appears that viruses bearing oncogenes are not usually responsible for tumorigenesis in human beings. Studies of the cell itself are necessary if the common origins

of malignancy are to be discerned. In particular, studies to identify the events that spark the onset of oncogenesis and to determine whether a particular cellular gene or set of genes always mediates progression to and maintenance of the malignant phenotype are needed.

The mechanism(s) of oncogenesis initiation are unknown. The matter has elicited great controversy, with some investigators arguing for mutation, others for chromosomal rearrangements, transpositions of DNA or even reversible epigenetic events. By contrast, the discovery of c oncs may have brought to view genes whose actions can mediate oncogenesis once the cell has sustained an initiating lesion. There are diverse reasons to suspect the existance of such cancer genes. One, a number of malignancies have appeared as heritable traits in human pedigrees and it has even been suggested that each of the roughly 100 types of malignancies will eventually be attributable to abnormalities affecting a specific genetic locus. Two, several efforts have been made to enumerate the genetic loci that might mediate neoplastic transformation by chemical carcinogens. In most instances the results indicate no more than a few dozen, or a few hundred genes as potential mediators. Three, DNA extracted from some lines of chemically transformed cells and from certain tumors induces neoplastic transformation when transferred into cells and cultures. Of course the efficiency of transformation is generally low, the transformation occurs reliably in only a few established cell lines, particularly the mouse NIH 3T3 line, and only a limited number of transformed cell lines or tumors have so far yielded DNA capable of inducing transformation. But the data do suggest that the DNA from at least some forms of neoplastic cells contain stable and heritable changes that are responsible for the malignant phenotype. If first sheared to molecular weights of about .3 to 3 X 10^6, even DNA from normal cells can transform NIH 3T3 cells at a very low frequency, and the transformed mouse cells, in turn, yield DNA that can induce transformation at much higher efficiencies. It is as if the original shearing of normal DNA unleased a potentially oncogenic gene whose action is now stably established in the transformed cell. Activation of the gene has been attributed to disruption of linkage between the oncogenic gene and a cis-active regulator. Are c oncs, then, among the cancer genes of normal cells. Is the induction of their activity responsible for at least some form of oncogenesis? Answers to these questions may come eventually from surveys of c onc expression in naturally occuring tumors. The study of viral agents far removed from human cancers appear to have established the elusive connection between tumor virology and human oncology. The issue is not whether viruses might cause human tumors (as perhaps they may) but rather how much viral oncology can shed light on the mechanisms by which human tumors arise.

Workshops and conferences supported by grants in FY 82 included the following: Structure of DNA, June 2-9, 1982, under the auspices of Cold Spring Harbor Symposium on Quantitative Biology (R13 CA 02809).

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SUMMARY REPORT

DNA VIRUS STUDIES

DNA Virus Studies supported by the contract mechanism concern research to explore the possible etiological relationship of DNA viruses to human malignancy and applied research to develop methods of diagnosis, prognosis, and intervention for neoplastic diseases associated with DNA viruses. The major effort during FY82 has centered on the Epstein-Barr virus (EBV) and nasopharyngeal carcinoma (NPC).

A multicenter study is underway to evaluate Epstein-Barr virus (EBV) immunovirological markers as potential clinical tools for the diagnosis and clinical management of American patients with NPC and with occult tumors in the head and neck region. A total of 202 patients with histopathologically confirmed NPC have now been entered into the program. This is an increase of 78 over the last year and included (a) 36 patients previously listed as suspected NPCs and (b) 42 new patients entered into the program from collaborating institutions. Complete clinical records including pathology, clinical staging and treatment are now available on 160 patients which is an increase of 75 over the last reporting period. This reflects the increased effort on the part of all subcontractors to collect all possible clinical information on each patient entered into the study. Follow-up serum samples collected every 3-6 months following diagnosis for determining the prognostic value of EBV serology have also been obtained successfully from most of the patients. Three or more follow-up serum samples have now been collected from 84 individual patients which is an increase of 22 over the past reporting period. Thus patient recruitment, collection of clinical information and patient follow-up are still progressing well in this study. Of the 202 patients with confirmed NPC, 45 or 22% of the patients have died over the 3-year period. Serum samples from all patients with suspected NPC and most controls were tested for antibodies to EBV antigens by immunofluorescence tests (IF) at the Mayo Clinic and Children's Hospital of Philadelphia. The tests include IgG antibodies to VCA and EA (D or R) and IgA antibodies to VCA. Antibody-dependent cellular cytotoxicity (ADCC) determinations were performed at the Mayo Clinic only. As usual, there has been excellent agreement in the results on individual serum samples between the two different laboratories. Previous studies established that EBV serology was useful in the diagnosis of the WHO 2 and WHO 3 histopathological types of NPC but not for WHO 1 (well-differentiated squamous cell carcinoma). This conclusion has been strengthened over this past 6-month period. Eighty-eight percent of the sera from 160 patients with WHO 2 or WHO 3 tumor types were positive for IgG anti-EA antibodies, mainly to the D component, and 86% of the same sera were positive for IgA antibodies to VCA. In contrast, only 33% of sera from 42 patients with WHO 1 tumors were positive for antibodies to EA (1/3 anti-R; 2/3 anti-D) while 11% were positive for IgA antibodies to VCA. This is similar to what has been observed in control populations. These results continue to support the conclusions that (1) IgA antibodies are very specific for WHO 2 and WHO 3 histopathological types of NPC and are of clinical value for the diagnosis of this disease including the occult form; and (2) the presence of both IgA antibodies to VCA and IgG antibodies to EA-D in the same serum sample, particularly when present at high levels, is very characteristic of patients with these two histopathological types of NPC.

The relationship of initial antibody titers to stage of disease at diagnosis is currently being evaluated using five different staging procedures: Ho, UICC, AJC, SEER, and Mayo-Scanlon staging system. There should now be sufficient clinical information on enough patients to draw some meaningful conclusions in regard to this question. All patients entered into the study are also being followed to determine the prognostic value of EBV serology. The number of patients who have died or developed recurrent disease over the 3-year period is still too small to draw a meaningful conclusion. However, it has been possible to identify some trends with the IF tests. Generally, in the absence of clinically identifiable recurrent disease, antibody titers measured by these assays have remained fairly stable. No consistent decreases were noted in titers to any of the antigens in patients presumed to be in clinical remission. This is in contrast to what has been reported in high incidence populations. However, consistent increases, particularly in IgG anti-VCA and anti-EA titers, were noted in patients who developed metastatic disease. These increases, in many cases, preceded the clinical detection of metastatic disease. These observations suggest that increases in antibody titers in these IF tests may indeed signal the presence of active or metastatic disease. This should become more evident over the next contract year as more patients are expected to relapse. The assay that so far appears to be of the greatest prognostic value for this disease is the ADCC assay. Of the 82 patients with confirmed WHO 2 or WHO 3 histopathology who have been on study for at least one year or who have died during this 3-year period, 78% of 54 patients whose ADCC titers were high at diagnosis are in remission and 22% have died or have developed metastasis. In contrast, 36% of the remaining 28 patients whose initial ADCC titers were low are in remission and 64% have died or have recurrent disease. More surprising has been the observation that in some of the patients with high titers at diagnosis who eventually died from the disease, ADCC titers dropped significantly in the last one or two serum samples tested before death. The results to date, therefore, indicate that ADCC titers may indeed be valuable prognostic markers for this disease as previously reported. This conclusion is further supported by results on six patients entered into the study at the time they developed recurrent disease. ADCC titers in these patients were all low ranging from 1:480 to 1:3840. In addition to the above studies, experiments were initiated to evaluate NK and T-cell cytotoxicity functions in patients with NPC. These experiments have only been performed on Mayo Clinic patients. Lymphocytes from 10 patients were evaluated in these assays. NK cytotoxicity was determined against K562 cells and specific T-cell killing against Daudi cells. Some of the lymphocyte donors had not yet undergone treatment, others were in remission and one had metastatic disease. All of these patients including the patient with metastatic disease whose lymphocytes showed good NK activity against K562 cells and were also cytotoxic to Daudi cells had lymphocyte functions comparable to those noted in different control populations. Therefore, in this limited number of patients, there is no indication that these two lymphocyte functions are impaired in patients with NPC. Lymphocytes from a larger number of patients with NPC will have to be monitored for NK and T-cell functions, however, before drawing any definitive conclusions on this point.

DNA VIRUS STUDIES

CONTRACT INDEX

CONTRACT REPORTS

DNA VIRUS STUDIES

Dr. Maurice L. Guss

ARMED FORCES INSTITUTE of PATHOLOGY (YO1-CP9-0500)

Title: Application of Epstein-Barr Virus Markers to Diagnosis and Prognosis of Nasopharyngeal Carcinoma and Occult Tumors of the Nasopharynx Area in U.S.A.

Contractor's Project Director: Dr. Vincent Hyams

Contractor's Project Officer (NCI): Dr. Maurice L. Guss

Objectives: The contractor provides support to the Mayo Foundation, NO1-CP9-1006, by supplying clinical information and materials and histopathology consultation.

Major Findings: The contractor has enrolled 34 nasopharyngeal carcinoma patients in the collaborative study.

Significance to Biomedical Research and the Program of the Institute: This project provides support for a contract to demonstrate that Epstein-Barr virus assays can be used for improved diagnosis and management of American patients with head and neck tumors. It is anticipated that the knowledge gained could be transferred to cancer centers for wider application in cancer control. The study will further permit assessment of the specificity of the viral markers in the identification of high risk populations.

Proposed Course: This project will continue without change.

Date Contract Initiated: November 28, 1978

Current Annual Level: $1,500

CALIFORNIA, UNIVERSITY OF, LOS ANGELES (NO1-CPO-1022)

Title: Studies of Interrelationship of Viruses, Genetics and Immunity in the Etiology of Human Cancer.

Contractor's Project Director: Dr. Mitsuo Takasugi

Project Officer (NCI): Dr. Maurice L. Guss

Objectives: (1) To detect cellular and humoral immunity in cancer patients and to determine the specificity of these reactions; (2) to understand the cellular components of the immune response to cancer and its interaction with antibodies in terms of resistance to susceptibility to cancer; and (3) to study the immune response of cancer patients and normals to different viruses and virus-induced antigens.

Major Findings: Patients with Burkitt's lymphoma (BL) and nasopharyngeal carcinoma (NPC) were tested for specific antibody dependent cellular cytotoxicity (ADCC) activity against Epstein-Barr virus (EBV)-associated antigens on EBV-infected target cells. Results obtained with serum from patients with BL showed a significant correlation between specific ADCC and survival. Patients with strong ADCC to EBV antigens survive longer than patients with weak ADCC. If ADCC is related to stage of disease as suggested, it then remains to be determined if specific ADCC to EBV is greater among long term than short term survivors in the same stage. Should this be the case, ADCC test results may be used to predict prognosis.

In the collaborative studies with the Mayo Clinic (NO1-CP9-1006), 56 more patients have been entered, and early follow-up ADCC data show a weak trend for better survival of strong ADCC reactors. No difference in survival was observed for groups according to age, sex, or race.

Significance to Biomedical Research and the Program of the Institute: Studies in progress may help to determine whether certain groups of people have specific responses to EBV or other suspected tumor viruses. The studies are likely to provide useful techniques for studying antigenic expression and modulation following virus infection or transformation, and in the diagnosis, prognosis and treatment of various forms of cancer.

Proposed Course: This contract terminated on July 31, 1982.

Date Contract Initiated: July 12, 1971

Current Annual Level: No funding in FY82

CENTRE NATIONAL de la RECHERCHE SCIENTIFIQUE (NO1-CP9-1035)

Title: Comparison and Evaluation of IgA Antibody Levels to Epstein-Barr Virus-VCA in Nasopharyngeal Carcinoma Patients from High, Intermediate, and Low Risk Populations.

Contractor's Project Director: Dr. Guy de Thé

Project Officer (NCI): Dr. Maurice L. Guss

Objectives: The objective is the determination of the usefulness of IgA antibody to Epstein-Barr virus (EBV) in the diagnosis and prognosis of nasopharyngeal carcinoma (NPC) in low, intermediate, and high risk populations. The study of three regions of differing disease frequency will provide information on the relationship of environmental factors to disease and on the significance of low VCA titers in healthy family members.

Major Findings: Progress was made in evaluating the EBV IgA reactivities for the management of NPC patients in Hong Kong, Tunisia and France. The number of patients who have entered the study now total 124 in France, 99 in Hong Kong, and 59 in Tunisia. The serological testing for both the IgG and IgA class antibodies to VCA, EA and to EBNA has been carried out in Lyon and simultaneously in Hong Kong and Tunis for the respective sera. The original plan requested a follow-up of patients at 6 months interval for 3 years to compare the clinical and serological evaluation in the three ethnic groups involved. The number of patients at the one year follow-up is too small to allow adequate statistical analysis.

The use of the IgA-VCA serological test was confirmed as a unique tool for early detection of NPC in high risk areas. The results from Dr. Ho in Hong Kong and from Dr. Zeng in the People's Republic of China suggest that individuals at immediate risk for this tumor may be identified by this test.

Following the study of 56 IgA positive normal Chinese, among whom were 14 individuals with EBV DNA sequences in their biopsied nasopharyngeal mucosa, the presence of EBV DNA sequences in exfoliated cells of the nasopharynx was investigated in two sub-population groups with and without IgA VCA antibodies. Unexpectedly, a similar proportion of EBV DNA positive individuals were found in each group. The nature of the cells (epithelial or lymphoid) containing these EBV DNA sequences needs to be determined.

Significance to Biomedical Research and the Program of the Institute: This project offers the opportunity to demonstrate that EBV assays can be used for improved diagnosis and management of patients with head and neck tumors. It is anticipated that the knowledge gained could be transferred to cancer centers for wider application in cancer control. The study will further permit assessment of the specificity of the viral markers in the identification of high risk populations.

Proposed Course: This project will continue without change.

Date Contract Initiated: August 16, 1979

Current Annual Level: $180,880

CHILDREN'S HOSPITAL OF PHILADELPHIA (NO1-CP3-3272)

Title: The Propagation and Sero-epidemiology of Epstein-Barr Virus.

Contractor's Project Director: Dr. Gertrude Henle

Project Officer (NCI): Dr. Maurice L. Guss

Objectives: To investigate the relationship between Epstein-Barr virus (EBV) and human cancer, primarily, the determination of frequencies and titers of antibodies to various EBV-determined antigens in EBV-associated diseases providing prognostic information and support for a causal relation of EBV to given human malignancies.

Major Findings: Numerous sera from patients with Burkitt's lymphoma (BL), nasopharyngeal carcinoma (NPC), and other malignancies have been collected at the Kenyatta National Hospital in Nairobi because this source of material will soon no longer be available. These sera have been tested for their spectrum and titers of EBV-related antibodies to serve for future pertinent studies.

Three further cases of BL have been detected in pre-bled children in the West Nile District. The pre-sera again revealed high anti-VCA titers indicative of a high, persistent viral load.

Of 7 juvenile patients with NPC studied at St. Jude Children's Research Hospital 3 have been in remission for 42-53 months and their EBV-specific antibody spectra returned to close to normal. The other 4 patients are moribund or have died and their antibody titers remained high or increased substantially.

Studies on NPC in Alaskan natives have yielded results comparable in all aspects to those obtained in Southern China or East Africa. A prospective study has been initiated, screening all individuals over 30 years of age in several communities for IgA antibodies to VCA. Positive sera are then titrated for the whole spectrum of EBV-specific antibodies and the donors are examined and further followed for evidence of NPC.

In regard to primary and persistent EBV infections in immunologically compromised individuals, further studies have been pursued in collaboration with various investigators in this country and abroad which include patients with ataxia telangiectasia and other inheritable immune defects, with renal transplants, Hodgkin's disease and other malignant lymphomas and now also acute lymphocytic leukemia. Abnormal patterns of EBV-specific antibodies are thought to reflect deficiencies or dysfunctions of given leukocyte subpopulations. There seem to be certain correlations emerging but much more work is required before a clear-cut picture can be presented. It appears though that the EBV-specific serology may serve as a parameter in assessing cellular immunity.

The contractor is providing tests for the presence or absence of EBV in newly established cell lines derived from lymphoproliferative malignancies, such as Hodgkin's disease, histiocytic lymphomas B cell lymphomas, etc. The contractor is also providing sera with certified antibody spectra for the monitoring of various EBV-specific antigens during their purification, their identification in EBV DNA-transfected cells, etc.

Significance to Biomedical Research and the Program of the Institute: The primary purpose of these studies is to aid in the determination of the etiologic relationships of EBV to certain human malignancies. Fingerprints of EBV have been found in nearly all BL and NPC biopsies. EBV-related serology may serve to detect advancing disease, to provide prognostic information, and to monitor the effectiveness of therapy.

Proposed Course: This contract will terminate on May 31, 1982. An RFP for continuation of these studies has been issued and proposals submitted are now undergoing review.

Date Contract Initiated: March 1, 1973. This is a continuation of the Contract PH 43-66-477 initiated February 2, 1966.

Current Annual Level: $186,620

INDIAN HEALTH SERVICE (YO1-CP9-0501)

Title: Application of Epstein-Barr Virus Markers to Diagnosis and Prognosis of Nasopharyngeal Carcinoma and Occult tumors of the Nasopharynx Area in U.S.A.

Contractor's Project Director: Dr. Anne Lanier

Contractor's Project Officer (NCI): Dr. Maurice L. Guss

Objectives: This contractor will provide support to the Mayo Foundation, NO1-CP9-1006, by supplying clinical information and materials.

Major Findings: The Indian Health Service has enrolled 24 nasopharyngeal carcinoma patients in the collaborative study.

Significance to Biomedical Research and the Program of the Institute: This project provides support for a contract to demonstrate that Epstein-Barr virus assays can be used for improved diagnosis and management of American patients with head and neck tumors. It is anticipated that the knowledge gained could be transferred to cancer centers for wider application in cancer control. The study will further permit assessment of the specificity of the viral markers in the identification of high risk populations.

Proposed Course: This project will continue without change.

Date Contract Initiated: November 30, 1978

Current Annual Level: $21,420

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER (NO1-CP9-1009; successor to NO1-CP4-3296)

Title: Sero-Epidemiologic and Laboratory Studies on Nasopharyngeal Carcinoma and Burkitt's Lymphoma.

Contractor's Project Director: Dr. John Higginson

Project Officer (NCI): Dr. Maurice L. Guss

Objectives: To conduct epidemiological studies of virus associated cancers.

Major Findings: In the malaria suppression study, the community wide chloroquine distribution in North Mara continued during the year but failed to have any appreciable effect on the level of malaria parasitaemia in the area. The malaria suppression study was therefor terminated in North Mara at the end of 1981.

Sufficient newly diagnosed cases of NPC in Singapore have been typed for HLA locus A and locus B antigens to give a definitive description of the associated risk for NPC. Three antigens A2, BW17 and BW46 (formerly SIN2) are each associated with an increase in risk of 50 to 100%. In addition it appears that the presence of A2 in the absence of BW17 and BW46 is associated with better survival.

Significance to Biomedical Research and the Program of the Institute: EBV is a naturally-occurring virus strongly suspected of an etiologic role in human cancer. The research under this contract should help elucidate the role of EBV in the Burkitt's lymphoma. Studies of blood genetic types, together with the sero-epidemiological results, may provide the means for detecting NPC high risk groups among a normal population.

Proposed Course: This contract terminated on January 14, 1982.

Date Contract Initiated: April 1, 1979

Current Annual Level: $8,165

MASSACHUSETTS GENERAL HOSPITAL (NO1-CP4-3222)

Title: Activation of Oncogenic Viruses and Induction of Cancer by Immunologic and Nonimmunologic Methods.

Contractor's Project Directors: Dr. Martin S. Hirsch

Project Officer (NCI): Dr. Maurice L. Guss

Objectives: To determine the effects of human interferon in the prophylaxis of virus infections in immunosuppressed kidney transplant patients.

Major Findings: Virus infections and their complications are major limiting factors in the success of human renal transplantation. Cytomegalovirus (CMV) is the principal recognized viral pathogen in this population, although infections with other viruses, e.g. herpes simplex, Epstein-Barr, and members of the Papova group are well-recognized. Renal transplant recipients also have an extraordinarily increased incidence of lymphoma, Kaposi sarcoma, and other neoplasms that may be virus-induced.

The contractor is conducting double-blind, placebo-controlled clinical trials of human interferon alpha (IFN) in renal transplant recipients. The initial studies demonstrated that 3.0×10^6 units of IFN administered twice weekly for six weeks delayed the onset of CMV and EBV excretion and reduced CMV viremia. Effects on other virus infections were observed, but numbers were too small to reach statistical significance. Patients enrolled in this study continue to be followed for late sequellae of infection or treatment, particularly neoplasia. No tumors have yet developed, and graft survival is equivalent in interferon and placebo recipients.

Two current studies are expanding information on interferon, viruses, and cancer in the high risk renal transplant population. One involves patients at risk for primary CMV infection (seronegative recipients of kidneys from seropositive donors), and is a multicenter collaborative trial. The second involves patients susceptible to reactivation CMV infection (seropositive recipients). In both trials, treatment courses have been extended to 14 weeks and total IFN doses have been increased from 36 million to 102 million units. The mechanisms of IFN-induced protection in these subjects is being studied by performing pharmacokinetic and immunological function studies on individual recipients. To date, 55 patients have been enrolled, 39 in the reactivation study and 16 in the primary infection study.

Significance to Biomedical Research and the Program of the Institute: The studies on the inhibition of virus infection in immunosuppressed patients should provide valuable data on interferon prophylaxis, which may later be more directly applicable to cancer prophylaxis and therapy.

Proposed Course: The current clinical studies will be continued according to the protocols already established, with appropriate modifications as these seem clinically indicated.

Date Contract Initiated: September 15, 1971

Current Annual Funding: $207,140

MAYO FOUNDATION (NO1-CP9-1006)

Title: Application of Epstein-Barr Virus Markers to Diagnosis and Prognosis of Nasopharyngeal Carcinoma and Occult Tumors of the Nasopharynx Area in U.S.A.

Contractor's Project Director: Dr. Gary Pearson

Project Officer (NCI): Dr. Maurice L. Guss

Objectives: The objective of the project is the determination of the usefulness of Epstein-Barr virus (EBV) markers in established serological tests for diagnosis and prognosis of nasopharyngeal carcinoma (NPC) and primary tumors of the head and neck in the United States.

Major Findings: Over the 3-year period this contract has been active, 202 patients with histopathologically confirmed NPC have been entered into the program including 78 patients over this reporting period. This includes 36 patients, previously listed as suspected NPC's whose diagnosis has now been confirmed and 42 new patients entered into the program. Complete clinical records including pathology, clinical staging and treatment are now available on 160 patients. Three or more follow-up serum samples collected at 3-6 month intervals have been obtained from 84 of these patients. Of the 202 patients with confirmed NPC, 45 or 22% have died during this 3-year period. The serological findings continue to indicate that the EBV serology is useful for the diagnosis of NPC including the occult form. Eighty-eight percent of the sera from 160 patients with WHO 2 or WHO 3 histopathological types of NPC were positive for IgG

anti-EA antibodies and 86% of the same sera were positive for IgA antibodies to VCA. In contrast, only 33% of the sera from patients with WHO 1 tumors were positive for IgG anti-EA antibodies and 11% were positive for IgA antibodies to VCA. This is similar to the frequencies in different control populations. The relationship of EBV titers to stage of disease as determined by five different staging procedures is currently being evaluated. In regard to the prognostic value of EBV serology, increases in IgG antibodies to VCA and IgG antibodies to EA have been noted consistently in patients who developed metastatic disease. These increases, in some cases, were noted before the clinical detection of metastatic disease. ADCC titers at diagnosis also appear to be of prognostic value. Seventy-eight percent of the patients with high ADCC titers at diagnosis have now been in remission for one year or longer as opposed to 36% of those whose ADCC titers were low at diagnosis. Preliminary investigations on NK and T-cell functions on a limited number of patients have not yet revealed any apparent defects in these lymphocyte populations.

Significance to Biomedical Research and the Program of the Institute: This project offers the opportunity to demonstrate that EBV assays can be used for improved diagnosis and management of American patients with head and neck tumors. It is anticipated that the knowledge gained could be transferred to cancer centers for wider application in cancer control. The study will further permit assessment of the specificity of the viral markers in the identification of high risk populations.

Proposed Course: This project will continue without change.

Date Contract Initiated: October 23, 1978

Current Annual Level: $290,000

OHIO STATE UNIVERSITY (NO1-CP8-1021)

Title: Studies on the Epstein-Barr Virus and its Association with Nasopharyngeal Carcinoma.

Contractor's Project Director: Dr. Ronald Glaser

Project Officer (NCI): Dr. Maurice L. Guss

Objectives: Determination of the role of Epstein-Barr virus (EBV) in nasopharyngeal carcinoma (NPC) by conducting studies (1) on infection by EBV of normal human epithelial cells, particularly nasopharyngeal cells; (2) on cellular hybridization with an HR-1 cell line containing one EBV genome per cell; (3) on transformation of normal human primary epithelial cells with EBV by a nuclear exchange procedure; and (4) on in vivo cell fusion of EBV genome positive lymphoid cells and nasopharyngeal cells.

Major Findings: Both the owl monkeys and the common marmosets have been maintained and examined periodically for disease symptoms related to the inoculum that they originally received, i.e., EBV alone, Plasmodium falciparum or P. knowlesi alone, or both EBV and P. falciparum or P. knowlesi. None of the animals have shown any sign of disease. The data obtained in this study are equivocal because of the fact that it has not been possible to establish a chronic malaria infection in either the owl monkeys or the common marmosets, since the animals developed antibodies against the malaria organisms. However, a cell-mediated response related to natural killer (NK) activity in these animals, as well as EBV serology was shown. It may be concluded that at least in owl monkeys and in common marmosets, two primate species which are not particularly susceptible to EBV infection, co-infection of these animals under the conditions used did not result in a major interaction which produced disease, either malignant or acute.

Significance to Biomedical Research and the Program of the Institute: Sero-epidemiological surveys have demonstrated a relationship between EBV and cancer. These studies will provide information as to whether EBV plays a role in NPC and if the association of the EBV genome in the epithelial cells of the tumor is important for the induction of the tumor.

Proposed Course: The basic research portion of the contract terminated on March 31, 1981. The nonhuman primates under test were maintained and observed for 12 months to termination of the contract on March 31, 1982.

Date Contract Initiated: March 29, 1978

Current Annual Level: No funding in FY82

SUMMARY REPORT

RNA VIRUS STUDIES (I)

The studies in the RNA Virus (I) component supported by the contract mechanism are concerned with research on viruses of mouse, hamster, cat, bovine or primate origin containing a ribonucleic acid core which are known or suspected to be involved in the induction of malignant transformation of animal and human cells. The approach used for most of the investigations has been to study several model systems for evidence of viral carcinogenesis. Such animal studies are necessary for developing reagents, techniques and new approaches that might be applicable to studies concerning the etiology and control of human cancer. These studies can be divided into three major categories: (1) detection of retroviral information in human tumors, (2) studies related to cancer prophylaxis, and (3) the role of type B and type C RNA viruses in differentiation, transformation and carcinogenesis.

Detection of Retroviral Information in Human Tissues: The obvious utility of a reliable, consistent, and specific marker for carcinoma of the breast has led to numerous studies in which various substances, including enzymes, hormones, proteins, and others as yet undefined, have been investigated as possible candidates. Recent investigations have established that approximately half of human breast carcinomas contain an immunohistochemically detectable antigen which is crossreactive with the 52,000-dalton major glycoprotein (gp52) of the mouse mammary tumor virus (MuMTV). This antigen can be localized in paraffin-embedded sections of routinely-fixed tissues using heterologous antibodies to gp52 or MuMTV.

One of the most significant applications of this methodology involves deciding whether a tumor is benign or malignant in borderline cases. A less dramatic, not altogether uncommon situation where such methods could prove useful involves the identification of a clinically occult primary breast carcinoma which presents as a metastatic lesion with an undistinctive histopathological pattern. Preliminary studies have been conducted on patients with metastatic carcinoma in axillary lymph nodes without any clinical evidence of a primary lesion in the breast or elsewhere. The localization of the gp52-related antigen in paraffin-embedded sections of metastatic lesions suggested the presence of primary mammary carcinoma. This suggestion was ultimately confirmed by the finding of primary lesions in which the gp52-related antigen was also found. Further prospective and retrospective studies are planned with the hope that the information gleaned will demonstrate the usefulness of this technique as a prognostic and/or diagnostic tool.

Studies are continuing to increase the sensitivity of a diagnostic test for human breast cancer either by developing a more sensitive assay than the immunoperoxidase test or by improving the reagents utilized in this test. One of the antisera (RII5) raised in New Zealand white rabbits has been thoroughly examined to determine why the positivity of the peroxidase test went from 46% to 90% when this antiserum was used. After several months of study it was determined that the RII5 antisera recognized a protein, probably of milk origin, which copurified with gp52. Because of the frustrating unpredictability of heterologous xenogeneic antisera in general the contractor's priorities were reoriented in favor of the production of hybridomas and monoclonal antibodies. The contractor has generated hybridomas producing antibodies against MuMTV gp52

and the particles and particle proteins (p50) of the 47D clones. Seventy-six hybridomas have been produced against the particles of clone 11 of the 47D cell line and 16 against the p50 antigen from the same cell clone. These hybridomas are being cloned and subcloned for subsequent characterization.

Studies Related to Cancer Prophylaxis: AKR leukemia is associated with and can be transferred by an endogenous, vertically transmitted type C retrovirus (AKR virus) which is probably synthesized at birth, reaches detectable levels in the circulation during the perinatal period and attains high levels at 6 weeks of age. AKR mice display a nearly normal immune response to sheep red blood cells and are capable of rejecting tumor transplants. However, as the leukemic phase develops, a severely depressed immune response to sheep erythrocytes is observed. Specific defects in the cellular aspects of the immune system of the AKR mouse, including the possible emergence of specific suppressor cells, has been observed. Such considerations suggest that an important relationship exists between the activities of the virus and those of the host immune system and that these two parameters play a major role in contributing to resistance or susceptibility to leukemia. On this basis, manipulations which would reduce the virus load and swing the balance in favor of the immune defense would be expected to have a suppressive effect on leukemia development. AKR mice were treated with heterologous anti-MuLV gp71 antibodies under various conditions in order to establish the optimal criteria for effective suppression of leukemia development. The strongest effect was observed when mice were administered the antibody at birth, and the data indicate that continued treatment for 10 days is just as good as the effect produced after 42 days of treatment. A significant observation of these studies concerns the capacity of successfully treated AKR mice to transmit to their offspring characteristics which prevent development of leukemia. The hope here is that it may be possible to generate a nonleukemogenic AKR strain through appropriate mating crosses. The studies should also demonstrate which parameters are important in the mother and the father for transmission of the "protected" characteristics; clearly the results of these long-term studies may point directly to the mechanism of AKR leukemogenesis. The mating studies have resulted in an F6 generation which continues to yield nonviremic, antibody-positive animals that are nonleukemogenic.

Long-term studies continue to examine the levels of infectious ecotropic virus in the tissues of control and treated animals. The results of these experiments have confirmed preliminary findings that suppression of ecotropic virus activity in the spleen and marrow of treated animals is transient, with levels of infectious virus in these organs eventually reaching those seen in control mice. The major difference between these two groups of animals is the near absolute lack of infectious ecotropic virus in the thymus of treated animals. Since the thymus is the most likely site for genetic recombination between ecotropic and xenotropic viruses, it was felt that this observation may be the key to understanding the effect of antiviral immunotherapy upon leukemogenesis.. Studies are now underway to follow viral activity further into the late preleukemic interval. In addition, the susceptibility of the thymus of treated animals to support the replication of eco-, xeno- and amphotropic viruses is currently being examined. These experiments should prove key to our understanding of the therapeutic effect seen with the treatment protocols used in these studies.

A series of preliminary studies assessing the effect of antiviral therapy upon cell surface expression of gp71 antigen has been completed. For this purpose fluorescence by flow cytofluorimetry was quantitated using a fluorescence-activated

cell sorter. These studies revealed that expression of gp71 antigen is never completely shut down either during or after the administration of immune IgG. There are insignificant differences in the percentage of fluorescing cells from tissues of treated and control mice. However, the overall intensity of fluorescence, especially in the thymus, is greatly reduced in the immune IgG treatment group. The appearance of intensely staining cells roughly correlates with the appearance of infectious ecotropic virus.

Attempts have been made to produce monoclonal antibodies against AKR viral antigens for the specific purpose of passive therapy. It is hoped that such efforts will result in several monoclonals of differing isotype and epitope specificity. C57B1/6 mice were hyperimmunized with AKR virus producing cells, and the spleens from these animals were fused with drug-sensitive myeloma cells. Three separate fusions have thus far been performed, resulting in approximately 10 hybridomas producing antiviral antibodies. The specificity of these antibodies is currently under study.

Intrathymic injection of SMX-1, a dualtropic MuLV originally derived from MSV stock protected AKR mice from developing MuLV-accelerated and spontaneous leukemia. The thymus of SMX-1 injected mice showed no change in weight, morphology or thymocyte size and quantitative expression of differentiation antigens. Injection of SMX-1 does not prevent X-ray induced leukemias in B6 mice, or MCA induced leukemias in RF mice.

Role of Retroviral Expression in Differentiation, Transformation and Carcinogenesis:
The neoplastic progression of the murine mammary gland involves an intermediary stage, the hyperplastic alveolar nodule (HAN), which can be morphologically visualized as lobular alveolar tissue in a nonpregnant, nonlactating host. The HAN can be surgically removed and transplanted into the cleared fat pad of isologous hosts. The resultant growth of the HAN is the hyperplastic outgrowth (HOG), which is delineated by the boundaries of the fat pad, and which has a higher tumor risk than normal mammary epithelium. This transplantation technique allows investigators to experimentally manipulate the HAN and to amplify the number of cells in the HAN for biochemical studies. By serially transplanting the preneoplastic HAN in mammary fat pads seven new HOGs have been established from the low tumor incidence mouse strain, C3H/Sm (formerly designated C3H/StWi). Four HAN lines from virus-free C3H/Sm mice are in transplant generations 2-4; to date no tumors have developed. Three of the HAN lines are from virus-infected C3H/Sm mice; all three lines exhibited a 50% tumor incidence in their second transplant generation. The five BALB/cfC3H "Z series" HOGs are being maintained by serial transplantation. The tumor incidences in these lines have not changed. The high tumor incidence Z4 continued to have a 100% tumor incidence at 6 months post-transplantation while the tumor incidence of the Z3 HPO line is 6% at 18 months. Lines Z4, Z5, Z5cl, and Z5d are now in transplant generation 16.

Restriction endonuclease mapping has been used to detect exogenous provirus and to detect clonal dominant populations. The results indicate that the HOGs are comprised of a heterogeneous population of cells and that some of the different subpopulations can be selected by transplantation. Primary outgrowths are composed of several subpopulations which are selected by the transplantation procedure. Stabilization of the population occurred by the third to fourth transplant generation.

Tumors which arise from the HOGs contain the restriction patterns found in their respective outgrowths. Multiple tumors which arose in one HOG have the outgrowth restriction pattern but each tumor has its own unique additional band(s). However, not all tumors contain amplified viral genes. Thus, MuMTV gene amplification may not be required for tumorigenesis. The major role of MuMTV may be in the normal to HAN transformation, and have little or no effect on the HAN to tumor transformation.

In order to identify single copy DNA sequences and to work with very small quantities of DNA, refinement of their restriction mapping technology was required. The development of a new method of producing specific MuMTV probes using the M13 phage system should allow the contractor to work with much smaller quantities of DNA.

A cellular protein of 53,000 daltons, suggested to be a correlate with transformation in other mammalian cell systems, has been detected in mouse mammary tumor cells by immunoprecipitation. In a separate study, malignant cells of mouse transformed by a variety of different agents have been found to express high levels of a 55,000 dalton phosphoprotein (p53) with phosphotransferase activity. Little or no p53 can be detected in normal cells with the exception of thymocytes. In view of the fact that several different oncogenic viruses have kinase activity, the association of this activity in p53 is important with regard to the possibility of a common pathway of transformation by diverse agents.

It has been determined that the envelope protein of the virion is the virus receptor molecule; specific antibody to this protein may prevent development of cancer. However, the cooperating receptor molecule has not been identified. Information relevant to the initial virus-cell interaction is basic to determining the complete course of events leading to malignant transformation. Toward this end, the identity of BCL_1 lymphoma derived immunoglobulins and associated proteins have been examined for the molecules bearing idiotypic determinants that are detected by rat antibodies designed to see idiotypic markers. By preparing hybridomas from the spleen of the rat producing anti-idiotypic antisera, several clones of cells producing monoclonal antibodies directed against BCL_1 surface determinants were obtained. Several antibodies detected idiotypic determinants on immunoglobulin, while one that had appeared to be idiotypic-like detected a type specific determinant in the BCL_1 murine leukemia virus envelope, a not surprising finding given that the BCL_1 immunoglobulin was postulated to bind to these envelope gene products. True anti-idiotypic antibodies block binding of virus both to the isolated immunoglobulin, and to BCL_1 lymphoma cells. Other B cell lymphomas with crossreactive-idiotypic determinants also had diminished binding of retroviruses to their surface receptors by pretreatment with these anti-idiotypic antibodies. Two of the entire set of monoclonal idiotypic antibodies detected surface markers on a minor subset of selected T lymphomas, and such anti-idiotypic antibodies block cognate retrovirus binding to these cells also. While screening several T cell and T lymphoma lines for the possibility that they have rearrangement of their genomic immunoglobulin genes, an apparent rearrangement involving kappa light chain genomic sequences was discovered in the Lyt-2,3 T cell lymphoma, RadLV induced VL3. By northern mRNA analysis, kappa related sequences are expressed in these cells although they do not bear surface antigenic determinants of the constant region of the kappa light chain. The cDNA library from these cells revealed a clone bearing sequences cross-hybridizing with both RadLV and two variable regions of the kappa chain. Of specific interest is the possibility that the immunoglobulin related

sequence in this particular clone may represent downstream readings of the RadLV promoter which was inserted during the development of the RadLV lymphoma. This gains great interest when one considers the similarity to the independent promoter-insertion and receptor-mediated leukemogenesis hypothesis.

CONTRACT INDEX

BAYLOR COLLEGE OF MEDICINE (NO1-CP9-1020)

Title: Induction and Control of MuMTV Expression in Mouse Mammary Preneoplastic Tissues

Contractor's Project Director: Dr. Janet S. Butel

Project Officer (NCI): Dr. Garrett V. Keefer

Objectives: To investigate the induction and control of mouse mammary tumor virus (MuMTV) expression in mouse mammary preneoplastic tissues as a model system for developing concepts, techniques and reagents which can be applied to precancerous human mammary tissues.

Major Findings: Seven new outgrowth lines have been established from the low tumor incidence mouse strain, C3H/Sm (formerly designated C3H/StWi). Four HAN lines from virus-free C3H/Sm mice are in transplant generations 2-4; to date no tumors have developed. Three of the HAN lines are from virus-infected C3H/Sm mice; all three lines exhibited a 50% tumor incidence in their second transplant generation.

Antisera were prepared in rabbits against affinity-purified MuMTV polypeptides. These antisera, against MuMTV glycoproteins (gp52/gp36) and against p14 were shown to react specifically with the appropriate viral polypeptides in immunoprecipitation tests. The antisera also recognize env and gag precursor polyproteins in mammary tumor cells.

A cellular protein of 53,000 daltons, suggested to be a correlate with transformation in other mammalian cell systems, has been detected in mouse mammary tumor cells by immunoprecipitation.

Significance to Biomedical Research and the Program of the Institute: Recent reports indicate that small atypical lesions, similar to the hyperplastic nodules in murine mammary cancer, exist in the dysplastic and carcinoma-in-situ cells of the human breast and that hyperplastic regions of the human mammary gland appear to contain an antigen that demonstrates crossreactivity with the glycoprotein (gp52) of MuMTV; therefore, it would be worthwhile to investigate the induction and control of MuMTV expression in mouse mammary preneoplastic tissues as a model system which can then be applied to an understanding of precancerous human mammary tissues.

Proposed Course: This contract terminates November 30, 1982. This contract was funded for fifteen months in FY 81.

Date Contract Initiated: September 1, 1979

Current Annual Level: No funding in FY 82

CALIFORNIA, UNIVERSITY OF, DAVIS (NO1-CPO-1008)

Title: Induction and Control of MuMTV Expression in Mouse Mammary Preneoplastic Tissues

Contractor's Project Director: Dr. Robert D. Cardiff

Project Officer (NCI): Dr. Garrett V. Keefer

Objectives: To investigate the induction and control of MuMTV expression in mouse mammary preneoplastic tissues as a model system for developing concepts, techniques and reagents which can be applied to precancerous human mammary tissues.

Major Findings: The five BALB/cfC3H "Z series" hyperplastic outgrowths (HPO) are being maintained by serial transplantation. The tumor incidences in these lines have not changed. The high tumor incidence Z4 continued to have a 100% tumor incidence at 6 months post-transplantation while the tumor incidence of the Z3 HPO line is 6% at 18 months. Lines Z4, Z5, Z5cl, and Z5d are now in transplant generation 16. The Z3 HPO line was nearly lost at transplant generation 13. However, a recent transplant was successful and Z3 HPO is now in transplant generation 15.

Restriction endonuclease mapping has been used to detect exogenous provirus and to detect clonal dominant populations. The results indicate that the HPO are comprised of a heterogeneous population of cells and that some of the different subpopulations can be selected by transplantation. Primary outgrowths are composed of several subpopulations which are selected by the transplantation procedure. Stabilization of the population occurred by the third to fourth transplant generation.

Tumors which arise from the HPOs contain the restriction patterns found in their respective outgrowths. Multiple tumors which arose in one HPO have the outgrowth restriction pattern but each tumor has its own unique additional band(s). However, not all tumors contain amplified viral genes. Thus, MuMTV gene amplification may not be required for tumorigenesis. The major role of MuMTV may be in the normal to hyperplastic alveolar nodule (HAN) transformation, and have little or no effect on the HAN to tumor transformation.

In order to identify single copy DNA sequences and to work with very small quantities of DNA, refinement of their restriction mapping technology was required. The development of a new method of producing specific MuMTV probes using the M13 phage system should allow the contractor to work with much smaller quantities of DNA.

Significance to Biomedical Research and the Program of the Institute:
It has been reported that small atypical lesions, similar to the hyperplastic nodules in murine mammary cancer, exist in the dysplastic and carcinoma-in-situ cells of the human breast and that hyperplastic regions of the human mammary gland appear to contain an antigen that demonstrates crossreactivity with the glycoprotein (gp52) of MuMTV. Therefore, it would be worthwhile to investigate the induction and control of MuMTV expression in mouse mammary preneoplastic tissues as a model system which can then be applied to a better understanding of precancerous human mammary tissues.

Proposed Course: This contract terminated September 30, 1982.

Date Contract Initiated: October 1, 1979

Current Annual Level: $143,850

CALIFORNIA, UNIVERSITY OF, LOS ANGELES (NO1-CP9-1010)

Title: Identification of Cell Surface Receptor for Oncornavirus gp70 on Murine Fibroblasts

Contractor's Project Director: Dr. C. Fred Fox

Project Officer (NCI): Dr. Garrett V. Keefer

Objectives: To obtain sufficient quantities of purified gp70 receptor for biochemical characterization and production of specific antiserum.

Major Findings: This contract was active for one and one-half months in FY 82. During this time the Principal Investigator was in the process of bringing all experiments to a timely end and developing the appropriate reports.

Significance to Biomedical Research and the Program of the Institute: Infection with oncornaviruses may lead to malignant transformation in many animal species; prevention of infection can thus prevent the development of malignancy. It has been determined that the gp69/71 of the virion is the virus receptor molecule; specific antibody to this protein may prevent development of cancer. However, the cooperating cellular receptor molecule has not been definitively identified. Information relevant to the virus-cell interaction is basic to determining the complete course of events leading to malignant transformation.

Proposed Course: This contract terminated November 14, 1981.

Date Contract Initiated: November 15, 1978

Current Annual Funding: No funding in FY 82

COLUMBIA UNIVERSITY (NO1-CP7-1016)

Title: The Diagnostic and Clinical Implications of Viral-Related Proteins in Human Cancer

Contractor's Project Director: Dr. Sol Spiegelman

Project Officer (NCI): Dr. Garrett V. Keefer

Objectives: To define an antigen in human mammary neoplasia which is immunologically-related to the major envelope glycoprotein (gp52) of murine mammary tumor virus and determine the significance of this crossreactivity.

Major Findings: The contractor has thoroughly examined one of the antisera (RII5) raised in New Zealand white rabbits to determine why the positivity of the peroxidase test went from 46% to 90% when this antiserum was used. After several months of study it was determined that the RII5 antisera recognized a protein, probably of milk origin, which copurified with gp52. Because of the frustrating unpredictability of heterologous xenogeneic antisera in general the contractor's priorities were reoriented in favor of the production of hybridomas and monoclonal antibodies. The contractor has generated hybridomas producing antibodies against MuMTV gp52 and the particles and particle proteins (p50) of the 47D clones. Seventy-six hybridomas have been produced against the particles of clone 11 of the 47D cell line and 16 against the p50 antigen from the same cell clone. These hybridomas are being cloned and subcloned and their characterization was in progress at the contract's termination. A grant has been submitted to continue this work.

Significance to Biomedical Research and the Program of the Institute: A systematic molecular biological study has demonstrated the presence in human cancer of particulate materials possessing characteristics unique to the known animal RNA tumor viruses. Whether the cause or the consequence of human malignancy, the presence of the tumor related particles and their uniqueness provided a novel opportunity to generate information of potentially practical importance for the diagnosis and management of breast cancer in humans. An example is the observation that an antigen found in human breast cancer crossreacts with antibody developed to the MuMTV gp52. Definite data as to its usefulness as a practical clinical tool for the diagnosis or prognosis of human breast cancer should be obtained from this contract effort.

Proposed Course: This contract terminated June 30, 1982.

Date Contract Initiated: October 29, 1969

Current Annual Level: No funding in FY 82

CORNELL UNIVERSITY (NO1-CP9-1007)

Title: Immunoprevention of Cancer in Cats

Contractor's Project Director: Dr. Fernando de Noronha

Project Officer (NCI): Dr. Garrett V. Keefer

Objectives: To determine the nature of autogenous immunity to feline leukemia virus (FeLV) and feline oncornavirus associated antigen (FOCMA) and correlate findings with natural disease occurrence; conduct detailed studies of immune responsiveness of specific pathogen-free (SPF) cats during vaccination with FL74 cell membrane fractions and/or purified FOCMA; conduct immunosurveillance of humans exposed to FeLV; and attempt to transform feline fibroblast cells with nonviral agents.

Major Findings: This contract was active for one month in FY 82. During this time the Principal Investigator was in the process of bringing all experiments to a timely end and developing the appropriate reports.

Significance to Biomedical Research and the Program of the Institute: In the cat, tumors induced by FeLV and FeSV express a cell surface antigen designated "feline oncornavirus-associated cell membrane antigen" (FOCMA). Analysis of anti-FOCMA titers in sera of virus-exposed cats has suggested that development of antibody directed against FOCMA may constitute an immunosurveillance defense against tumor development. Recently, FOCMA has been shown to be distinct from all known FeLV-coded structural proteins and has been demonstrated in spontaneous lymphomas of cats even in the absence of detectable levels of FeLV structural proteins. Moreover, FOCMA has been shown to be expressed in FeSV-transformed cells in the form of a precursor containing two amino terminal FeLV structural-proteins. These findings demonstrate FOCMA to represent a transformation-specific FeSV-coded protein, and suggest that activation of cellular gene coding for a protein analogous to FOCMA may represent a general mechanism for tumor induction in the cat.

Proposed Course: This contract terminated October 31, 1981.

Date Contract Initiated: November 1, 1978

Current Annual Funding: No funding in FY 82

DUKE UNIVERSITY (NO1-CP3-3308)

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. Garrett V. Keefer

Objectives: (1) To study in detail the properties of structural components of RNA tumor virus particles, particularly those of mammalian RNA tumor viruses. (2) To utilize these materials for preparation of highly specific antisera which can be applied to the analysis of cells for the presence of similar virus gene products. (3) To develop appropriate antisera which can be employed for detection and identification of tumor virus activities in human malignant cells. (4) To use purified structural viral proteins and the corresponding antisera for possible immunological control of viral disease.

Major Findings: AKR mice were treated with heterologous anti-MuLV gp71 antibodies under various conditions in order to establish the optimal criteria for effective suppression of leukemia development. The strongest effect was observed when mice were administered the antibody at birth, and the data indicate that continued treatment for 10 days is just as good as the effect produced after 42 days of treatment. A significant observation of these studies concerns the capacity of successfully treated AKR mice to transmit to their offspring characteristics which prevent development of leukemia. The hope here is that it may be possible to generate a nonleukemogenic AKR strain through appropriate mating crosses. The studies should also demonstrate which parameters are important in the mother and the father for transmission of the "protected" characteristics; clearly the results of these long-term studies may point directly to the mechanism of AKR leukemogenesis. The mating studies have resulted in an F6 generation which continues to yield nonviremic, antibody-positive animals that are nonleukemogenic.

Long-term studies continue to examine the levels of infectious ecotropic virus in the tissues of control and treated animals. The results of these experiments have confirmed preliminary findings that suppression of ecotropic virus activity in the spleen and marrow of treated animals is transient, with levels of infectious virus in these organs eventually reaching those seen in control mice. The major difference between these two groups of animals is the near absolute lack of infectious ecotropic virus in the thymus of treated animals. Since the thymus is the most likely site for genetic recombination between ecotropic and xenotropic viruses, it was felt that this observation may be the key to understanding the effect of antiviral immunotherapy upon leukemogenesis. Studies are now underway to follow viral activity further into the late preleukemic interval. In addition, the susceptibility of the thymus of treated animals to support the replication of eco-, xeno- and amphotropic viruses is currently being examined. These experiments should prove key to our understanding of the therapeutic effect seen with the treatment protocols used in these studies.

Expression of Viral Antigen in Tissues of Control and Treated Mice: A series of preliminary studies assessing the effect of antiviral therapy upon cell surface expression of gp71 antigen has been completed. For this purpose fluorescence by flow cytofluorimetry was quantitated using a fluorescence-activated cell sorter. These studies revealed that expression of gp71 antigen is never completely shut down either during or after the administration of immune IgG. There are insignificant differences in the percentage of fluorescing cells from tissues of treated and control mice. However, the overall intensity of fluorescence, especially in the thymus, is greatly reduced in the immune IgG treatment group. The appearance of intensely staining cells roughly correlates with the appearance of infectious ecotropic virus.

Attempts have been made to produce monoclonal antibodies against AKR viral antigens for the specific purpose of passive therapy. It is hoped that such efforts will result in several monoclonals of differing isotype and epitope specificity. C57B1/6 mice were hyperimmunized with AKR virus producing cells, and the spleens from these animals were fused with drug-sensitive myeloma cells. Three separate fusions have thus far been performed, resulting in approximately 10 hybridomas producing antiviral antibodies. The specificity of these antibodies is currently under study.

Significance to Biomedical Research and the Program of the Institute: Although human leukemias or other tumors are not known to be associated with replicating RNA tumor viruses, one cannot exclude the possibility that virus genes exist in human cancer cells and are expressed as discrete antigens on the cell surface in a fashion similar to that in animal cells. This study has shown that, if indeed this does occur, unequivocable detection of the viral antigens on human cells may be an exceedingly difficult task to accomplish. Even so, there is considerable evidence that many other aspects, particularly the immunological consequences of animal and human leukemias, are distinctly related. Therefore, an understanding of the immunological mechanisms in the animal models which are of key importance for host defense, coupled with protocols to artifically stimulate those leading to effective prevention and control of the disease, is of value to reach a better understanding of related events in human cancer.

Proposed Course: This contractor will continue to study effective serum therapy against AKR leukemia.

Date Contract Initiated: March 1, 1973

Current Annual Funding: $189,000

ENERGY, DEPARTMENT OF (YO1-CP9-0503)

Title: Retroviral Genetic Expression in Human Cancers: Analysis by Primer tRNA Binding Approach

Contractor's Project Director: Dr. Wen K. Yang

Project Officer (NCI): Dr. Garrett V. Keefer

Objectives: (1) Isolation of poly(A) RNAs from various human normal and cancer cells; quantitative and qualitative comparison of their capacity to bind selective tRNA species; assessment of the primer property of the bound tRNAs by reverse transcription reaction; and determination of whether different types of human cancers contain poly(A) RNA of different tRNA binding specificity. (2) Structure analysis of tRNA poly(A) RNA binding nucleotide sequences, as well as reversely transcribable 5' end sequences of the tRNA-binding site in the poly(A)RNA, by employing nucleotide sequence determination and molecular hybridization. (3) Purification of the specific poly(A)RNAs by using the unique "primer tRNA" binding specificity. (4) Characterization of the specific poly(A)RNAs, including messenger RNA activity by protein synthesis assay and possible changes of level during neoplastic process.

Major Findings: The examination of various human cancer cell lines is continuing for the presence of poly(A) RNA molecules which contain the tRNA binding activity. This included human lung, colon and bladder carcinoma cell lines. All carcinoma cell lines examined gave negative results, except in the case of some human breast cancer cell lines (ALAB, T47D, and MCF-7 lines). These human breast cancer cells contain a 22S size RNA which can bind tRNA and, with bound tRNA, can serve as template for reverse transcription. Two attempts were made without success to clone the small size cDNA from reverse transcription of the 22S size RNA of ALAB breast cancer cells. Continued efforts in this regard are being applied.

Since all retrovirus proviral DNAs contain long terminal repeats similar to prokaryotic transposons, it is important to know whether or not such DNA molecules are present in human cancer cells. A method for the detection of such DNA molecules in the cell has been devised, by using mouse tumor cells as an experimental model.

Significance to Biomedical Research and the Program of the Institute: One of the objectives of the Program is detection of tumor viral information in human cancer. To date, partial expression of retrovirus-like information, but rarely complete virus, has been detected in human leukemias, breast carcinomas, and melanomas, as well as normal fibroblasts and placental tissues; however, only partial homology to retroviruses of other species has been described. This project offers a new approach to the detection of partial viral information, as well as to study restriction mechanisms operative in human cells.

Proposed Course: This contract terminated May 31, 1982.

Date Contract Initiated: February 1, 1979

Current Annual Funding: $46,530 (4 months funding in FY 82)

SCRIPPS CLINIC AND RESEARCH FOUNDATION (NO1-CP9-1012)

Title: Genetic Analysis of Immune Response of Mice to Recombinant gp70 Oncornavirus

Contractor's Project Director: Dr. Richard A. Lerner

Project Officer (NCI): Dr. Garrett V. Keefer

Objectives: Correlate genetic control of immune responsiveness of inbred mouse strains to different murine oncornavirus gp70 determinants; determine relative contributions of humoral immunity and cell-mediated immunity in affording protection against tumor antigens and tumor growth; induce tumors in appropriate parental strains with cloned characterized N- and B-tropic oncornaviruses, then test the tumors for growth in F_1 hybrids; identify distinct members of the gp70 family to which Ir gene controlled responses are directed; test F_1 hybrid mouse strains rejecting parental N-tropic virus-induced tumor for ability to reject parental tumor induced by an N-tropic recombinant virus; and determine the molecular basis for immune responses directed to molecules other than gp70.

Major Findings: This contract was active for one month in FY 82. During that time the Principal Investigator was in the process of bringing all experiments to a timely end and developing the appropriate reports.

Significance to Biomedical Research and the Program of the Institute: One of the principal areas of interest in viral oncology is the identity and function of the viral coded transforming proteins. Detailed structural analysis of murine oncornavirus virion proteins has enabled production of highly specific immunological probes for these proteins. The functional role these proteins play in virogenesis and oncogenesis and cellular control of the synthesis and functioning of these proteins can now be probed as model systems.

Proposed Course: This contract terminated October 31, 1981.

Date Contract Initiated: November 1, 1978

Current Annual Funding: No funding in FY 82

SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH (NO1-CP8-1054)

Title: Immunogenetic and Virological Study of Leukemogenesis in the AKR Mouse

Contractor's Project Director: Dr. Lloyd J. Old

Project Officer (NCI): Dr. Garrett V. Keefer

Objectives: (1) Extend present serological typing systems to identify new cell surface antigen systems specified by various classes of endogenous MuLV. (2) Characterize new antigens biochemically and determine relationship to viral structural components. (3) Conduct detailed immunogenetic and virological analyses of preleukemic phase of AKR mice. (4) Use genetic approach to analyze age-dependent amplification of MuLV antigenic expression in thymus of six month old AKR mice. (5) Determine biological and biochemical character and significance of MuLV isolated from AKR mice in (3) above. (6) Use cell fractionation techniques to identify the cellular source of various classes of MuLV from thymus of different age AKR mice.

Major Findings: The contractor terminated the studies described during the approximately two and one-half months of funding during FY 82.

Intrathymic injection of SMX-1, a dualtropic MuLV originally derived from MSV stock protected AKR mice from developing MuLV-accelerated and spontaneous leukemia. The thymus of SMX-1 injected mice showed no change in weight, morphology or thymocyte size and quantitative expression of differentiation antigens. Injection of SMX-1 does not prevent X-ray induced leukemias in B6 mice, or MCA induced leukemias in RF mice.

Malignant cells of mouse transformed by a variety of different agents have been found to express high levels of a 55,000 dalton phosphoprotein (p53) with phosphotransferase activity. Little or no p53 can be detected in normal cells with the exception of thymocytes. In view of the fact that several different oncogenic viruses have kinase activity, the association of this activity in p53 is important with regard to the possibility of a common pathway of transformation by diverse agents.

Significance to Biomedical Research and the Program of the Institute: Detailed structural analysis of murine oncornavirus virion proteins has enabled production of highly specific immunological probes for these proteins. The functional role these proteins play in virogenesis and oncogenesis and cellular control of the synthesis and functioning of these proteins can now be probed.

Proposed Course: This contract terminated December 24, 1981.

Date Contract Initiated: September 25, 1978

Current Annual Funding: No funding in FY 82

SOUTHERN CALIFORNIA, UNIVERSITY OF (NO1-CP8-1032)

Title: Immunoprevention of Natural and Induced Tumors in Wild Mice

Contractor's Project Director: Dr. Suraiya Rasheed

Project Officer (NCI): Dr. Garrett V. Keefer

Objectives: By infection with helper amphotropic MuLV, attempt to rescue the transforming genes from chemically transformed cells and chemically induced tumors of wild mice and New World rodents; determine the role of amphotropic virogenes in natural and chemical tumorigenesis of laboratory and wild mice; attempt to prevent chemically induced tumors by passive immunization with anti-amphotropic IgG; determine interaction of amphotropic and ecotropic murine leukemia viruses (MuLV) with lymphoid cells during the pathogenesis of natural and experimental B cell lymphomas of wild mice; study the role of ecotropic MuLV virogenes in slow CNS disease in wild mice and attempt passive immunization with anti-ecotropic IgG; determine the role of recombination of amphotropic and ecotropic MuLV in the genesis of viral isolates with enhanced oncogenicity; attempt to determine the mechanism of control of amphotropic virus expression in tissues of laboratory mice.

Major Findings: This contract effort was active for two months in FY 82. During this time the Principal Investigator was in the process of terminating animal experiments and developing the appropriate reports.

Significance to Biomedical Research and the Program of the Institute: Development of methodology for derepressing the genes governing expression of the complete retrovirus from laboratory mouse strains could be applied to the rescue of a human virus. Further, the lymphomas associated with the wild mouse type C virus are of a B cell type, whereas lymphomas in laboratory mouse models, T cell lymphomas are more common. Human lymphomas are also of B cell type; therefore, the outbred wild mouse model has great relevance for study of the human disease.

Proposed Course: This contract terminated November 30, 1981.

Date Contract Initiated: September 16, 1978

Current Annual Funding: No funding in FY 82

STANFORD UNIVERSITY (NO1-CP9-1011)

Title: Isolation and Characterization of T Lymphoma Cells and Normal Cell Receptors for Thymotropic Murine Oncornaviruses

Contractor's Project Director: Dr. Irving L. Weissman

Project Officer (NCI): Dr. Garrett V. Keefer

Objectives: Develop methods for the isolation of T lymphoma receptors for T-MuLV, compare the receptors from several T lymphomas which bind the same T-MuLV and compare receptors from a single tumor having the capacity to bind several different T-MuLVs. Compare receptors with other known gene products expressed on the surface of murine lymphocytes, determine if T-MuLV binding is by recognition of the viral env gene products gp70 and p15E. Compare T lymphoma receptor specificity with peptide maps of the bound ligands (e.g., gp70).

Major Findings: The identity of BCL_1 lymphoma derived immunoglobulins and associated proteins have been examined for the molecules bearing idiotypic determinants detected by rat antibodies designed to see idiotypic markers. By preparing hybridomas from the spleen of the rat producing anti-idiotypic antisera, several clones of cells producing monoclonal antibodies directed against BCL_1 surface determinants were obtained. Several antibodies detected idiotypic determinants on immunoglobulin, while one that had appeared to be idiotypic-like detected a type specific determinant in the BCL_1 murine leukemia virus envelope, a not surprising finding given that the BCL_1 immunoglobulin was postulated to bind to these envelope gene products. True anti-idiotypic antibodies block binding of virus both to the isolated immunoglobulin, and to BCL_1 lymphoma cells. Other B cell lymphomas with crossreactive-idiotypic determinants also had diminished binding of retroviruses to their surface receptors by pretreatment with these anti-idiotypic antibodies. Two of the entire set of monoclonal idiotypic antibodies detected surface markers on a minor subset of selected T lymphomas, and such anti-idiotypic antibodies block cognate retrovirus binding to these cells also.

While screening several T cell and T lymphoma lines for the possibility that they have rearrangement of their genomic immunoglobulin genes, an apparent rearrangement involving kappa light chain genomic sequences was discovered in the Lyt-2,3 T cell lymphoma, RadLV induced VL3. By northern mRNA analysis, kappa related sequences are expressed in these cells although they do not bear surface antigenic determinants of the constant region of the kappa light chain. The cDNA library from these cells revealed a clone bearing sequences cross-hybridizing with both RadLV and two variable regions of the kappa chain. Of specific interest is the possibility that the immunoglobulin related sequence in this particular clone may represent downstream readings of the RadLV promoter which was inserted during the development of the RadLV lymphoma. This gains great interest when one considers the similarity to the independent promoter-insertion and receptor-mediated leukemogenesis hypothesis. Portions of these studies are continuing under grant 1 R01 CA 32031-01.

Significance to Biomedical Research and the Program of the Institute: It has been determined that the envelope protein of the virion is the virus receptor molecule; specific antibody to this protein may prevent development of cancer. However, the cooperating receptor molecule has not been identified. Information relevant to the initial virus-cell interaction is basic to determining the complete course of events leading to malignant transformation.

Proposed Course: This contract terminated December 15, 1981.

Date Contract Initiated: December 15, 1978

Current Annual Funding: No funding in FY 82

STANFORD UNIVERSITY (NO1-CP9-1044)

Title: Virologic, Biologic and Immunologic Characterization of Hodgkin's Disease and Other Human Malignant Lymphomas

Contractor's Project Director: Dr. Henry Kaplan

Project Officer (NCI): Dr. Garrett V. Keefer

Objectives: To collect Hodgkin's disease tissue and serological samples for study. To cultivate human lymphoma tissue in vitro and characterize the established cell lines and clones derived from them. To investigate cultures for presence of virus, and characterize any viruses detected. To characterize, immunologically, the cells in relation to the host autoimmune responses.

Major Findings: Concentrated supernatant culture fluids of SU-DHL-1 human histiocytic lymphoma cell line, previously shown to contain low levels of a retrovirus with little or no infectivity, were inoculated into 6 newborn marmosets and 3 newborn rhesus monkeys. No clinical evidence of lymphoma or leukemia had developed in any of the animals after about 9 months of observation.

Attempts have been made to establish additional permanent cell lines from a large number of tissue specimens and effusion fluids from patients with Hodgkin's disease and other lymphomas, and occasional leukemias. One cell line believed to be derived from the neoplastic giant cells in a spleen involved by Hodgkin's disease has been established and partially characterized, and a mouse monoclonal antibody has been raised to one of its cell membrane antigens.

Further experience has been gained with a liquid culture system for the long-term cultivation of normal and leukemic bone marrow.

Continued work on the purification of the serum T cell inhibitor previously detected in the sera of patients with untreated Hodgkin's disease has revealed that it is a low molecular weight glycolipid or glycolipopeptide.

Significance to Biomedical Research and the Program of the Institute: Hodgkin's disease has the attributes of an infectious process. This project focuses available technology to detect evidence for a virus etiology in this malignancy. These studies may provide new information applicable to diagnosis, prognosis and treatment of Hodgkin's disease.

Proposed Course: This contract terminated December 15, 1981.

Date Contract Initiated: April 1, 1974

Current Annual Funding: No funding in FY 82

SUMMARY REPORT

RESEARCH RESOURCES

The Research Resources component of the Biological Carcinogenesis Branch (BCB) is responsible for planning, initiating and maintaining a coordinated program of research material support to meet the needs of extramural investigators funded by the Branch as well as other investigators in cancer research. This coordinated program includes initiation, development, maintenance and management of resource contracts and the responsibility for the day-to-day general management and direction of all resources distribution. Since the leadership for this component is vacant, the management of this activity currently resides in the Office of the Chief under the direction of the Deputy Chief.

Laboratory investigations carried out under the sponsorship of the BCB depend on the availability of adequate quantities of viruses, viral reagents, antisera, animals and clinical and laboratory materials of optimal purity, viability and potency, some of which are not available from the commercial sector. The BCB resources component provides these research materials and other supporting activities through contract operations representing four general areas. These include: activities directed toward production, characterization and distribution of purified viruses, viral reagents and appropriate antisera; activities concerned with animal resources, including production of pathogen-free species of animals, breeding of cotton-topped marmosets, maintenance of animal colonies including primates, and containment-type primate holding facilities; activities directed toward the provision of specialized testing services for the examination of experimental materials; and activities concerned with the storage, inventory and distribution of normal and malignant human specimens.

During this report period, twenty resource contracts were active. Virus production and antisera preparation efforts were shared by a total of six contracts whose funding represented 33% of the total Resources budget. The animal resources area (eight contracts) accounted for 29% of the budget, provision of testing and service efforts (five contracts) accounted for 37% of the budget and human specimen acquisition and distribution (one contract) received no funds in FY 1982.

In addition, the research resources component of the Branch has coordinated the distribution of a variety of resources to Russian, French and Japanese scientists in keeping with formal U.S. or NCI international agreements with these countries covering the mutual exchange of cancer research materials. Materials supplied include purified and concentrated viruses, specialized viral proteins and antigens, normal and infected tissue culture cell lines, and a wide range of antisera and substantial amount os AMV reverse transcriptase enzyme.

CONTRACT INDEX

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BECTON-DICKINSON AND COMPANY (NO1-CP9-1004)

Title: Preparation of Antisera to Oncogeneic or Potentially Oncogenic Viruses.

Contractor's Project Director: Dr. Roger Wilsnack

Project Officer (NCI): Dr. John S. Cole, III

Objectives: The objectives of this contract have been to develop, produce, characterize and distribute antisera to oncogenic viruses and their components for use in the biological carcinogenesis program. In addition, antisera to immunoglobulins of various animal species and the T-antigen of SV40 are produced.

Major Findings: During the contract year, 170 reagent shipments, consisting of 97 different types of immunoreagents, were prepared and delivered to 131 investigators.

Immunization schedules and reagent characterization activities were phased out in January 1982. Validation of the total reagent inventory was completed in February 1982, during which period copies of all associated computer programs, data files, and code tables were made available to the Biological Carcinogenesis personnel at NCI. In March 1982, all activities related to reagent distribution were completed. Additionally, bulk inventories of FITC-conjugated and anti-species reagents as well as aliquoted volumes of specific lots of anti-viral reagents were transferred to the NCI Repository. A Final Inventory report has been submitted to NCI. The contract ended June 16, 1982.

Significance to Biomedical Research and the Program of the Institute: The contractor served as a centralized source of potent and specific antisera developed for use in cancer research. The close collaboration of the project with BCB research programs resulted in significant usefulness not only to program but to the entire research community.

Proposed Course: This contract expired June 16, 1982. A recompetition is in progress to provide a successor contract to provide heterologous, tumored and monoclonal antibodies.

Date Contract Initiated: October 1, 1978

Current Annual Level: No funds in FY '82

CHILDREN'S HOSPITAL OF MICHIGAN (NO1-CP9-1003 AND NO1-CP2-1017)

Title: Inter-and Intraspecies Identification of Cancer Cells In Vitro

Contractor's Project Director: Dr. Ward D. Peterson, Jr.

Project Officer (NCI): Dr. Jack Gruber

Objectives: This contract provides the general scientific community with a resource service facility for the interspecies and intraspecies identification of cell cultures.

Major Findings: During this report period this project was reinitiated through the competitive selection process and the incumbent was again selected for award. Under the new effort the contractor will have the capability of identifying up to 20 cell cultures per month on a continuing basis using immunologic, biochemical, and morphologic techniques. Additionally, the resources "payback" system is being applied to this effort and will be phased-in during the first year. This new project has been underway for an insufficient period of time for a significant report.

Significance to Biomedical Research and the Program of the Institute: In the search for oncogenic viruses, many cell cultures from the same or different species are used concurrently, which offer frequent opportunities for cross contamination. In multiple-species tumor transplantations, the species derivation of induced tumors sometimes comes into question. Additionally, the significance of virus presence in tissue cells, the ability to grow virus, or the validity of virus isolation systems are all dependent upon the assurance of the identity of the cell cultures used.

Proposed Course: This effort will continue to provide a service for cell culture identification.

Date Contract Initiated: April 1, 1982

Current Annual Level: $204,386

CORNELL UNIVERSITY (NO1-AI-02651)

Title: Breeding Facility for Woodchucks (Marmota monax)

Contractor's Project Director: Dr. B. C. Tennant

Project Officer (NIAID): Dr. Frank Tyeryar

Objectives: This transfer of funds enables the National Institute for Allergy and Infectious Diseases to produce captive-born woodchucks and to evaluate their suitability as models for studies of human hepatitis and hepatocellular carcinoma.

Major Findings: The major findings thus far can be summarized as follows: It is possible to breed the animals in captivity by use of appropriate husbandry techniques; the animals can be infected

with woodchuck hepatitis virus during a very limited period in their early life; shipping of the animals in this time interval usually results in their death from maternal cannibalism; thus, an on site facility for research as well as breeding is indicated.

Significance to Biomedical Research and the Program of the Institute: This activity may furnish a valuable model for studies in the development of hepatocellular carcinoma in humans. It is apparently superior to the duck in that histopathologic characteristics of hepatoma has been demonstrated in the woodchuck.

Proposed Course: The contractor will continue with the breeding effort to produce seronegative woodchucks, and efforts to demonstrate their usefulness will increase as the supply of woodchuck pups increases.

Date Contract Initiated: May 27, 1980

Current Annual Level: $225,444

ELECTRO-NUCLEONICS LABORATORIES, INC. (NO1-CP9-1001)

Title: Large-Scale Production of Oncogenic or Potentially Oncogenic Viruses

Contractor's Project Director: Mr. John Lemp, Jr.

Project Officer (NCI): Dr. John S. Cole, III

Objectives: To provide for the isolation, large-scale production, concentration, assay and distribution of murine endogenous and xenotropic oncogenic viruses. Production and quality control involve tissue culture, electron microscopy, immunology, and various biochemical/biophysical techniques.

Major Findings: During this past year the contractor processed 930 liters of virus-containing fluids harvested from several xenotropic and one ecotropic virus/tissue culture systems and, as directed by the Project Officer, distributed the purified virus concentrates and cells to the BCB repository and to individual investigators involved in a variety of research projects. The flexibility of the program was emphasized in several ways. The production of two xenotropic viruses -- BALB virus 2 and NZB:MuLV -- was continued at varying levels consistent with the needs for virus resources. The production of murine leukemia virus (Rauscher) was reactivated. Using procedures for obtaining AKR virus with a high yield of gp-70 previously developed at ENLI, high yields of virus envelope gp-70 from the MuLV (Rauscher) were obtained and 318 mls of the virus concentrate was sent to the Repository. The xenotropic viruses BALB: virus 2 and NZB:MuLV comprised the contractor's total required production volume until the Contract Project Officer requested the special gp 70-containing MuLV (Rauscher) preparations for the last two months of the contract.

Significance to Biomedical Research and the Program of the Institute: In order to carry out research on the biochemistry and biophysics of oncogenic animal viruses, it has been necessary to provide large quantities of concentrated virus. With the emphasis on cloned fragment of nucleic acids and recombinant DNA technology, needs for these products have greatly decreased.

Proposed Course: This contract terminated January 7, 1982.

Date Contract Initiated: January 8, 1979

Current Annual Level: No funds in FY'82

ELECTRO-NUCLEONICS LABORATORIES, INC. (NO1-CP-01009)

Title: Production of RNA Avian Oncogenic Viruses

Contractor's Project Director: Mr. John Lemp, Jr.

Project Officer (NCI): Dr. John S. Cole, III

Objectives: To produce, purify, characterize and distribute Rous sarcoma Virus, Prague c strain (RSV-Pr. c) to collaborating investigators funded by the BCB. Production and quality control involve tissue culture, electron microscopy, immunology and various biochemical/biophysical techniques.

Major Findings: During this contract year, the contractor produced Rous Sarcoma virus of the following types: Schmidt-Ruppin D strain, seed stock Dr. P. Vogt; Carr-Zilber strain, NCI Repository seed stock CZ-2; 559 strain, NCI Repository seed stock TV-65; Harris strain, NCI Repository seed stock HA-12.

The new virus seed stocks were completely quality-controlled and frozen as infected primary chicken embryo fibroblast cultures. The virus produced was of high quality, and has been shipped to a number of laboratories.

Significance to Biomedical Research and the Program of the Institute: Large quantities of concentrated and well characterized virus have been utilized to conduct research on the biophysics and biochemistry of oncogenic viruses. Changes in research directions and advances in technology have markedly decreased the needs for these items.

Proposed Course: This effort terminated November 6, 1981

Date Contract Initiated: November 7, 1979

Current Annual Level: No funds in FY'82

EMORY UNIVERSITY, YERKES PRIMATE CENTER (NO1-CP3-3343)

Title: Maintenance of a Colony of Irradiated, Aging Rhesus Monkeys

Contractor's Project Director: Dr. Harold McClure

Project Officer (NCI): Dr. Garrett V. Keefer

Objectives: To monitor the incidence of tumors in a unique group of irradiated, aging rhesus monkeys and to supply tissue from tumors to the Biological Carcinogenesis Branch (BCB) collaborators for transplantation, tissue culture, and virus isolation studies.

Major Findings: Triannual physical examinations have been conducted on each of the 49 rhesus monkeys included in this study. The periodic examinations concentrate on the detection of developing neoplasms. Most of the animals remain in fair to good physical condition. During the most recent survey, 43 animals were classified as being in fair to good physical condition, 3 animals were relatively thin, and 3 animals were moderately to severely emaciated. Skin and/or subcutaneous nodules or masses are present in 17 animals. Most of these consist of small papilloma-like lesions of the skin or small subcutaneous masses. One animal has a large basal cell carcinoma of the skin of the upper arm. Four animals have small to large masses in the lower abdomen in the region of the uterus. During the most recent hematologic survey white blood cell counts ranged from 2,600/cmm to 12,400/cmm. None of the animals showed any significant degree of anemia.

During the past year, three animals in the group died. One animal, a 26-year-old nonirradiated control, died as the result of intestinal blockage from disseminated endometriosis. Another animal which was exposed to 512 rem of mixed-spectrum irradiation from an atomic bomb detonation in 1957 died as the result of a salivary gland adenoma, a pheochromocytoma of the adrenal, and an adenoma of the pituitary. Still another animal that was exposed to 32 rep neutron irradiation in 1955 died as the result of an oral squamous cell carcinoma. Tumor specimens from the squamous cell carcinoma and the salivary gland adenoma were shipped to other investigators in the Biological Carcinogenesis Program. Three animals presently in the colony have tumors, or have had life threatening malignancies surgically removed. These include a basal cell carcinoma of the skin, an adenocarcinoma of the duodenum, and a leiomyosarcoma of the colon wall.

Observations in this group of animals have confirmed their value as a source of nonhuman primate tumor material, as 22 of 47 (47%) animals which have died have had neoplasms. In addition, 3 animals with neoplasms are currently present in the colony. Twenty-two of the 25 (88%) neoplasms encountered in the colony have been in irradiation-exposed animals.

Significance to Biomedical Research and the Program of the Institute: The BCB 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, irradiated subhuman primates for research sponsored by the BCB. Malignant changes in these irradiated primates may provide useful information which might be applied to humans, who are also subjected to similar physical stresses.

Proposed Course: This contract terminated April 30, 1982.

Date Contract Initiated: May 1, 1971

Current Annual Level: No funding in FY 82

INFORMATION MANAGEMENT SERVICES, INC. NO1-CP1-1014

Title: Computer Support for Resources Management

Contractor's Project Director: Mr. Robert W. Burton

Project Officer (NCI): Ms. Wilma L. Varrato

Objectives: To assist in processing, storage, and retrieval of data associated with research resource materials of the Biological Carcinogenesis Branch.

Major Findings: During the last year, the contractor provided computer support to the Biological Carcinogenesis Branch. Efforts were directed toward: a) the design and implementation of a combined distributions and inventory single file system for the management of the collection, storage, and distribution of research materials; b) the design and implementation of a Query system for the location of available resources; c) data entry to support the existing systems; d) the development and production of reports; and e) documentation of revisions to current and newly developed systems and programs.

Significance to Biomedical Research and the Program of the Institute: Computerization of resources data makes it possible for the Biological Carcinogenesis Branch to exercise close control over the inventory of viruses, sera, human tissues, and other materials provided by the NCI and used in cancer research. In addition, computerization makes it possible to rapidly obtain information necessary to determine availability, location, quantity, etc. of all resources within its jurisdiction; thereby, permitting rapid response to needs of the Program while avoiding resource excesses or shortages.

Proposed Course: This effort will continue to provide computer support to the Biological Carcinogenesis Branch.

Date Contract Initiated: July 1, 1981

Current Annual Level: $241,828

LIFE SCIENCES, INC. NO1-CP1-1013

Title: Production of Avian Myeloblastosis Virus and AMV Reverse Transcriptase

Contract's Project Director: Dr. Joseph W. Beard

Project Officer (NCI): Dr. John S. Cole, III

Objectives: The objectives of this project are the large-scale in vivo production of BAI strain A avian myeloblastosis virus (AMV) and the pre-

paration and distribution of significant quantities of AMV reverse transcriptase enzyme. The "payback" for resources has been instituted with the initiation of this contract.

Major Findings: In the past year, this contract has operated under a system (the "payback" system) in which the requestor pays for the material which he requests as well as the shipping costs. The charges imposed during the first year have been 7 cents per unit of transcriptase and $1,000 per gram of viruses. While the amount of transcriptase shipped has decreased, the number of shipments has remained about the same as last year (588 vs. 587 last year). It is expected that approximately 5,000,000 units of transcriptase will be shipped, and that $310,000 in payback funds will be realized. In addition, approximately $40,000 in gratis shipments will be made to participants in various bilaterial scientific agreements involving the United States and other countries (France, Canada, Italy, etc.). Over 300 shipments have been made to laboratories in the U.S., and over 200 shipments have been made to foreign laboratories. Although the funds taken in did not completely offset contract costs in the first year, it is anticipated that reduction in the size of the effort coupled with future collections will eliminate the need for future government funds to continue the effort. The current annual level shown does not reflect the operation of the payback system.

Significance to Biomedical Research and the Program of the Institute: An important aspect of studies of biological carcinogenesis involves studies on the interaction of cDNA copies of oncornaviral genomes with cellular protein synthesis. Such studies could aid in determining and assigning functions to various parts of the viral genome and in determining the possible function of postulated "src" or "onc" genes. For these studies, a large and consistantly active supply of "reverse transcriptase" is vital. In addition to these areas, reverse transcriptase is of great importance to certain recombinant DNA studies. A third area of importance is the provision of large (multi-gram) quantities of avian myeloblastosis virus for studies on the mechanism of induction of avian tumors.

Proposed Course: This contract was subject to competitive continuation in FY 81. The incumbent, as the successful awardee, will continue to meet requests for avian myeloblastosis virus and will continue the preparation and distribution of large quantities of AMV reverse transcriptase.

Date Contract Initiated: May 19, 1981

Current Annual Level: $559,564

LIFE SCIENCES, INC. (NO1-CP6-1005)

Title: Production and Maintenance of Selected Reagent Grade-Specific Pathogen-Free Animals

Contractor's Project Director: Dr. Wendall M. Farrow

Project Officer (NCI): Dr. John S. Cole, III

Objectives: To produce specific pathogen-free (SPF) animals for cancer 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: An outbred SPF, leukosis-free flock of Japanese quail produced 16,000 fertile or embryonated eggs for investigators. A supply flock of avian pathogen-free White Leghorn chickens supplied 15,000 C/E, fertile eggs. Additionally, 3,000 embryonated eggs (9-11 days incubation) and 32 chicks 1-3 days of age were produced for recipients. To maintain definitive status of the White Leghorn flock, 100 single embryos were phenotyped and monitored for avian oncornavirus GS-1, chick helper factor and RIF. Monitoring of sera from retired pedigreed chickens indicated no evidence of avian pathogens, including avian leukosis and Marek's herpesvirus. Approximately $10,000 was recovered under the "payback" system for this effort.

Significance to Biomedical Research and the Program of the Institute: This contract provided investigators funded by the Biological Carcinogenesis Branch 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: Based on changing needs and the commercial availability of the mice supplied, only the quail egg production portion of this service-type contract for the production of reagent grade SPF animals will be continued. This will reduce both the size and cost of future years of the effort.

Date Contract Initiated: February 8, 1968

Current Annual Level: $120,000

LITTON BIONETICS, INC. (NO1-CP9-1022)

Title: Operation of a Facility to Maintain Nonhuman Primates for Cancer Research

Contractor's Project Director: Dr. Marion Valerio

Project Officer (NCI): Dr. Garrett V. Keefer

Objectives: The objective of this contract is to provide a facility for holding nonhuman primates for cancer research

Major Findings: This contract effort supports a biohazard containment area that housed 99 *Macaca mulatta* (rhesus), 5 *M. fascicularis*

(cynomolgus) 6 M. arctoides (stump-tailed macaque), 2 M. nemestrina (pig-tailed macaque), 10 Papio sp. (baboons), 27 Aotus tribirgatus (owl monkeys), 8 Saguinus fusciollis (white-lipped marmosets), 6 Saimiri sciureus (squirrel monkey), 3 Hylobates lar (white-handed gibbon) and 4 Pan troglodytes for a total of 169 monkeys. During the past year twenty-six special studies were active involving eleven different cancer researchers (one Russian, eight NCI intramural, two extramural). However, seventeen studies have been terminated, thereby reducing the number of animals to 40 and the number of studies to nine at the end of the fiscal year.

Significance to Biomedical Research and the Program of the Institute: Inasmuch as experimentation for the biological activity of candidate human cancer viruses will not be carried out on humans, 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: This contract will continue the long-term holding and study of experimental animals inoculated by collaborating investigators.

Date Contract Initiated: January 1, 1979

Current Annual Level: $273,379

MASON RESEARCH INSTITUTE (NO1-CP6-1052)

Title: Role of Hormonal Factors in the Induction of Mammary Tumors in Rhesus Monkeys Infected with Mason-Pfizer Monkey Virus

Contractor's Project Director: Dr. Arthur E. Bogden

Project Officer (NCI): Dr. Garrett Keefer

Objectives: To provide support for the holding and observation of virus infected nonhuman primates.

Major Findings: MPMV, originally isolated from a spontaneous breast tumor in a rhesus monkey, has been described as a prototype virus of the type D retroviruses. Active infection in the majority of MPMV-inoculated subhuman primates being held at the contractor's laboratory and monitored for mammary tumor induction has been confirmed by radioimmunoassay screening for the specific MPMV core protein p27 in the serum. Cytopathology of breast aspirates has been completed and the results will be presented in the final report.

Significance to Biomedical Research and the Program of the Institute: MPMV was recovered from a primate mammary cancer, it possesses characteristics common to known oncogenic viruses, and cross-reactions have been observed between the viral antigens and antigens present in human breast cancer specimens. Investigation for possible oncogenic properties in a primate host is valuable as a potential model for human breast cancer.

Proposed Course: This contract terminated June 8, 1982

Date Contract Initiated: June 9, 1979

Current Annual Level: No funding in FY 82

MELOY LABORATORIES, INC. (NO1-CP-01020)

Title: Large Scale Tissue Culture Virus Production For Cancer Research

Contractor's Project Director: Mr. George Gray

Project Officer (NCI): Dr. John S. Cole, III

Objective: The objectives of this contract are to provide for the large-scale production and distribution of viruses of continuing interest to BCB funded investigators. There viruses include RD114 virus, Baboon Endogneous virus and Mason-Pfizer monkey viruses and the mouse mammary tumor virus in the C3H, GR and RIII cell systems.

Major Findings: This contract has produced RD114 virus, Baboon Endogenous virus, and Mouse Mammary Tumor virus in the C3H, GR and RIII cell lines. The C3H cell line has been found to produce substantial amounts of C type virus in addition to the desired B type. Other cell lines are being evaluated as replacements for the C3H line, and production of MMTV in this line has been stopped.

Significance to Biomedical Research and the Program of the Institute: This production effort for type B, C and D retroviruses provides sufficient quality of these agents for ongoing and planned research activities carried out by qualified investigators.

Proposed Course: This contract will expire 9/23/82.

Date Contract Initiated: September 24, 1980

Current Annual Level: $336,873

MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES (NO1-CP6-1038)

Title: Acquisition of Human Tumor Specimens For Cancer Research

Contractor's Project Director: Dr. Yashar Hirshaut

Project Officer (NCI): Dr. Jack Gruber

Objectives: To collect sera and tissues from human subjects with neoplastic tumors to be used in cancer research studies.

Major Findings: The Tumor Procurement Center at Memorial Hospital for Cancer and Allied Diseases is a major facility for the collection and distribution of specimens used in cancer research. For the duration of the project, which terminated in February, 1982, the Center continued to supply

the tissue and sera specimens required. During the period October 1, 1981, through February 28, 1982, 1,646 specimens were obtained comprised of 1,319 tissues and 327 sera.

Significance to Biomedical Research and the Program of the Institute: A continuous supply of appropriate human tissues from patients with cancer is vital to cancer research investigations. In order to carry out important studies on the biochemistry and immunology of suspected oncogenic human viruses, it is important that large quantities of human malignant tissues be available for analysis.

Proposed Course: This contract terminated on February 28, 1982

Date Contract Initiated: March 1, 1978

Current Annual Level: $125,000 (No funds from FY 1982)

MICROBIOLOGICAL ASSOCIATES, INC. (N01-CP1-1000)

Title: Operation of a Repository and Distribution Center for Biological Materials

Contractor's Project Director: Ms. Cynthia McKinney

Project Officer (NCI): Ms. Wilma L. Varrato

Objectives: This contract provides a secure low temperature storage facility for biological reagents and clinical specimens prepared for the BCB. The facility receives, inventories, stores, and distributes these materials to both domestic and foreign recipients as authorized by the NCI Project Officer. Accurate computerized inventories and records of shipment are provided to the Branch.

Major Findings: This effort made over 65 shipments to domestic laboratories and 11 shipments to foreign laboratories of viral reagents, antisera and human speciments in the past year. The contractor received 37 shipments, consisting primarily of 6000 milliliters of viral reagents and approximately 3,500 human specimens.

Significance to Biomedical Research and the Program of the Institute: The storage and shipping facilities operated under this contract enable grantees and contractors of the BCB to have access to a large inventory of special research materials.

Proposed Course: To continue the operation of a repository for viruses, viral products and human specimens.

Date Contract Initiated: March 1, 1981

Current Annual Level: $264,246

MICROBIOLOGICAL ASSOCIATES, INC. (NO1-CP3-3288)

Title: Development of Laboratory Animal Virus Diagnostic Reagents and Operation of a Service Laboratory

Contractor's Project Director: Dr. Michael Collins

Project Officer (NCI): Dr. John S. Cole, III

Objectives: To develop reagents and tests for the detection of rodent viruses; to apply these and other tools in the determination of the importance of the endogenous viruses in experimental systems; to study means for elimination of viruses from laboratory animal populations; and to assist in the characterization of the gene dependent expression of murine leukemia.

Major Findings: The contractor operated a murine virus serodiagnostic and viral diagnostic laboratory for the Biological Carcinogenesis Branch, NCI. During this report period, over 27,000 serological tests were performed on sera from mice, rats, hamsters or guinea pigs. A total of 145 animal tissues, tansplantable tumors, ascites, cell cultures, and viral reagents were tested for murine viral contamination by the mouse antibody production (MAP) procedure. Special tests for the detection of lactic dehydrogenase (LDH) virus were conducted on 164 tumor or oncogenic viral preparations and 3,434 XC plaque assays were conducted for the detection of murine leukemia in cell cultures and animal tissues. The contract produced and maintained an inventory of 36 different viral diagnostic reagents. Production included 815 ml of viral reagents and 125 ml of specific antisera. These reagents are available and were supplied on request to 20 investigators.

Significance to Biomedical Research and the Program of The Institute: These virus diagnostic capabilities provide the NCI with the ability to monitor laboratory rodent 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 lymphocytic choriomeningitis (LCM) in rodent systems.

Proposed Course: Due to the very low utilization of these services by DCCP funded investigators (less than 10% of the total contract) and the commercial availability of many of these services, this contract expired on its anniversary date of January 31, 1982.

Date Contract Initiated: April 10, 1961

Current Annual Level: $425,166

NAVY, DEPARTMENT OF (YO1-CP8-0500)

Title: Development and Characterization of Cell Substrates for Use in Cancer Research

Contractor's Project Director(s): Dr. Neylan Vedros
Dr. David Kingsbury

Project Officer (NCI): Dr. Jack Gruber

Objectives: The objective of this project is the short-term continuation of an interagency agreement with the Department of the Navy for the maintenance of the Cell Culture Department (CCD) at NBL. In the past, this project had concerned two main activities: a cell bank for establishment and distribution of a wide variety of normal and malignant human and animal cell lines, and also a cytogenetics karyology reference laboratory for the characterization of cells in culture. For the basic one year renewal of this project only the maintenance of the cell bank is being continued, and that continuation as a terminating caretaker activity.

Major Findings: This final year of support is making possible the completion of cell line characterizations already in progress; the sorting and assembly of the essential documentation concerning each culture, such as origin and identity, subculture history, growth characteristics, viability, morphology, karyology, virus susceptibility, etc.; and the time for the staff at NCI to arrange for an appropriate transfer and physical relocation of the extremely valuable cell line collection of this facility. Additionally, NBL has attempted to fill, as is possible, outstanding requests for cell cultures from investigators in the general scientific community.

Significance to Biomedical Research and the Program of the Institute: An obvious approach to experimental human cancer research is the study of cultured human tumor cells. Many experimental techniques in virology, immunology, cell biology, chemical carcinogenesis, metabolism, and biochemistry require large amounts of cells or the continuous availability of standard cell substrates, with the result that established human cell lines are of critical importance. When established cell lines can be developed, thorough characterization becomes possible and deviations from the standard can then be measured. This project concerned a research support service facility for the development, characterization, repository, and distribution of a wide variety of cell substrates for use in cancer research.

Proposed Course: This contract terminated on September 30, 1982

Date Contract Initiated: October 1, 1977

Current Annual Level: $200,000

OAK RIDGE ASSOCIATED UNIVERSITIES (NO1-CP-21004)

Title: Marmoset Colony for Cancer Research

Contractor's Project Director: Dr. Harry E. Walburg

Project Officer (NCI): Dr. Garrett V. Keefer

Objectives: The aim of this contract is the development and maintenance of a marmoset breeding colony in order to ultimately have appropriate

numbers of these animals available for experimental use, and to provide a marmoset facility that includes a support laboratory for the inoculation and monitoring of animals under study as well as an adequate animal containment-holding area.

Major Findings: One hundred and forty-six (146) marmosets including 67 cotton-topped breeders, 25 cotton-topped stock, and 44 experimental marmosets were moved to this facility during the month of May 1982, for the continuation of this effort. Oak Ridge Associated Universities was the successful awardee in response to RFP #NCI-CP-21004-76.

Significane to Biomedical Research and the Program of the Institute: Inasmuch as experimentation for the biological activity of candidate human viruses will not be carried out on humans, it is imperative that another system be developed for these determinations and subsequently for the evalution 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. The marmoset appears to be especially suitable for use as a comparative model system. To date, at least five and possibly six virus tumor models, including Epstein-Barr and Herpesvirus saimiri viruses, have been established in marmoset monkeys. In addition, because of its small size, the marmoset is economical to house yet it is large enough for routine surgical procedures and serological monitoring.

Proposed Course: Continuation of services as described.

Date Contract Initiated: July 1, 1982

Current Annual Level: $235,392

RUSH-PRESBYTERIAN-ST. LUKE'S MEDICAL CENTER (NO1-CP7-1014)

Title: Marmoset Colony for Cancer Research

Contractor's Project Director: Dr. Richard Massey

Project Officer (NCI): Dr. Garrett V. Keefer

Objectives: The aim of this contract is the development and maintenance of a marmoset breeding colony in order to ultimately have appropriate numbers of these animals available for experimental use, and to provide a marmoset facility that includes a support laboratory for the inoculation and monitoring of animals under study as well as an adequate animal containment-holding area.

Major Findings: This program provides a resource of marmosets and the technical expertise for performance of experimental studies. The marmoset colony currently numbers 236 animals: 120 breeders, 44 experimental animals, and 72 young uninoculated animals. The breeding colony contains 67 cotton-topped (Saguinus oedipus), 53 white-lipped (Saguinus sp.). The colony served as a resource in support of six research projects approved by the Research Resources Primate Utilization Review Group. Virology, clinical

pathology and pathology services were supplied for performance of certain studies. In May 1982 the white-lipped marmoset breeders (53) and stock animals (37) were transferred to another government supported colony, leaving a total of 146 marmosets.

Significance to Biomedical Research and the Program of the Institute: Inasmuch as experimentation for the biological activity of candidate human viruses will not be carried out on humans, 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. The marmoset appears to be especially suitable for use as a comparative model system. To date, at least five and possibly six virus tumor models, including Epstein-Barr and Herpesvirus saimiri viruses, have been established in marmoset monkeys. In addition, because of its small size, the marmoset is economical to house yet it is large enough for routine surgical procedures and serological monitoring.

Proposed Course: The contract terminated May 31, 1982

Date Contract Initiated: April 1, 1977

Current Annual Level: $49,940 (Two months of funding in FY 82)

SHOWA UNIVERSITY RESEARCH INSTITUTE (NO1-CP1-1012)

Title: Production, Purification, and Concentration of Potentially Oncogenic DNA Viruses

Contractor's Project Director: Dr. Meihan Nonoyama

Project Officer (NCI): Dr. Maurice Guss

Objectives: The objectives of this contract are to prepare, process, and purify high quality Epstein-Barr virus (EBV) of both B95-8 and P3HRI strain, and to prepare 55S EBV DNA for biochemical/molecular biology studies of the role of EBV in human cancer.

Major Findings: This is a successor contract to one previously held by Life Science, Inc. (NO1-CP8-1023). The "payback" system is in effect for this project. Production was delayed initially because of problems associated with transfer of equipment and materials from the previous contractor. The contractor is processing 30 liters per week of EBV and producing 10 µg per week of characterized EBV DNA.

Proposed Course: The contractor will continue the production of EBV and EBV DNA. The contract level of effort and funding will be modified in accord with the "payback" system and EBV product demand.

Date Contract Initiated: August 31, 1981

Current Annual Level: $295,597

SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH (NO1-CP7-1003)

Title: Influence of Virus-Related Genes on Susceptibility to Cancer

Contractor's Project Director: Dr. Edward A. Boyse

Project Officer (NCI): Dr. John S. Cole, III

Objectives: (1) Breeding, maintenance, and distribution of congenic strains of mice which differ from their partner strains in expression of genes concerned with production or control of leukemia virus, and (2) testing these paired strains for their susceptibility to spontaneous and induced malignancy.

Major Findings: During the past year, the contractor continued to supply breeding pairs of congenic mice to foreign and domestic grantees and contractors of the BCB. More than 40 percent of the recipients have published papers utilizing the progeny of these animals. Efforts continue to be directed to attaining the 20 generations of mice necessary to a defined genetic background.

Significance for Biomedical Research and the Program of the Institute: Genetic control of susceptibility to spontaneous and viral-induced leukemia in mice has been well documented. However, the mechanism of control by the several loci involved has only recently received attention. The present study has been designed to assign specific functions to each controlling genetic allele. Identification of genetic control mechanisms in murine strains should form the groundwork for identifying similar controlling factors in other species, including man.

Proposed Course: "Quartets" of four strains, representing two base strains and two corresponding congenic strains in which the differentiating alleles have been switched both ways, will be developed and supplied to all qualified, interested investigators. Seven quartets are planned covering the H-2, Fv-1, PC, Tia and GiX loci. The influence of each of the alleles on expression of virus, host response to viral products, and occurrence of leukemia will be investigated by monitoring virological, immunological and pathological parameters during the life span of these strains.

Making use of congenic stocks already established, selected 'double' congenic strains will be made in which alleles of two loci will be substituted on a genotypic background common to that on which each allele was individually isolated in the first place. This will permit assessment of the joint action of alleles of two genes, as compared with either alone, on virus-related characteristics and on susceptibility to neoplasia and other diseases associated with type C virus. The contract will continue with the development and provision of these genetically defined mice.

Date Contract Initiated: December 15, 1980

Current Annual Level: $140,000

BIOLOGICAL CARCINOGENESIS BRANCH

GRANTS ACTIVE DURING FY82

DNA VIRUS STUDIES

Investigator/Institution/Grant Number	Title
ALONI, Yosef Weizmann Institute of Science 5 R01 CA 14995-08	Control of Gene Expression in Tumor Viruses and Cells
ALWINE, James C University of Pennsylvania 5 R01 CA 28379-02	Regulation of DNA Tumor Virus Gene Expression
BASILICO, Claudio New York University 5 R01 CA 11893-12	Cellular and Viral Control of Oncogenetic Transformation
BASILICO, Claudio New York University 2 P01 CA 16239-08	Biosynthesis in Normal and Virus-Transformed Cells
BAUM, Stephen Yeshiva University 5 R01 CA 10945-12	Interaction of Oncogenic Viruses (Adenovirus and SV40)
BENJAMIN, Thomas L Harvard University 5 R01 CA 19567-06	Mechanism of Cell Transformation by Polyoma Virus
BENJAMIN, Thomas L Harvard University 5 R01 CA 25390-04	Effects of HR-T Mutations on Polyoma Gene Expression
BERG, Paul Stanford University 5 R01 CA 15513-08	Basic Mechanisms in Viral Carcinogenesis
BERG, Paul Stanford University 1 R01 CA 31928-01	Transduction of Genetic Information Related to Cancer
BERK, Arnold J University of California (LA) 5 R01 CA 25235-04	Biosynthesis of Adenovirus Early RNAs
BLACK, Paul H Boston University 5 R01 CA 28107-04	Mechanisms of Transformation by Oncogenic Viruses

BOTCHAN, Michael R
University of California (Berkeley)
5 RO1 CA 30490-02

Transformation of Cells by SV40 Virus

BROCKMAN, William W
University of Michigan
5 RO1 CA 19816-06

Role of SV40 Gene A in Cellular Transformation

BROKER, Thomas R
Cold Spring Harbor Lab.
1 R13 CA/AI/TW 32922-01

International Conference on Papilloma Viruses

BUTEL, Janet S
Baylor College of Medicine
5 RO1 CA 22555-05

Biological Properties of SV40 Early Proteins

BUTEL, Janet S
Baylor College of Medicine
5 RO1 CA 25215-03

Tumor Virus Effects on Mouse Mammary Epithelial Cells

CALNEK, Bruce W
Cornell University
5 RO1 CA 06709-20

Avian Leukosis Complex

CARMICHAEL, Gordon G
University of Connecticut
1 RO1 CA 32325-01

Regulation of Polyoma Early Gene Expression

CARROLL, Robert B
New York University
5 RO1 CA 20802-06

Biochemical and Functional Properties of SV40 T Antigen

CARTER, Timothy H
St. John's University
5 RO1 CA 25757-04

The Regulation of Adenovirus Gene Expression

CHINNADURAI, G
St. Louis University
1 RO1 CA 31719-01

Genetic Analysis of AD2 Early Genes

CHINNADURAI, G
St. Louis University
2 RO1 CA 33616-04

Adenovirus in Locus: Role in Ocogenic Transformation

CLOUGH, Wendy G
University of Southern California
5 RO1 CA 23070-05

EBV DNA Synthesis in Transformed Lymphocytes

CONLEY, Anthony J
St. Louis University
1 RO1 CA 33101-01

Regulatory Functions of Herpes Simplex Virus Gene Expression

CONSIGLI, Richard A
Kansas State University
3 RO1 CA 07139-18S1

Studies in Polyoma Transformed Cells---Virion Proteins

COOPER, Neil R Scripps Clinic 2 RO1 CA 14692-09	Humoral Immunity to Viruses and Virus-Infected Cells
COURTNEY, Richard J University of Tennessee 2 RO1 CA 24564-05	Studies of Purified Herpes Simplex Virus Glycoprotein
COURTNEY, Richard J University of Tennessee 5 RO1 CA 27870-02	Proteins of HSV-Infected and Transformed Cells
CROCE, Carlo M Wistar Inst. of Anatomy and Biology 5 RO1 CA 16685-07	Mapping of Tumor Virus Genomes in Transformed Cells
DANNA, Kathleen J University of Colorado (Boulder) 5 RO1 CA 24924-03	SV40 Early Proteins--Possible Roles in Oncogenesis
DARNELL, James E, Jr Rockefeller University 5 RO1 CA 16006-09	RNA and Growth Control in Animal Cells
DE MARCHI, Jeanette M Vanderbilt University 5 RO1 CA 20806-05	Induction by Cytomegalovirus of Cell DNA Synthesis
DE PAMPHILIS, Melvin L Harvard University 5 RO1 CA 15579-08	Tumor Virus DNA Replication: A Probe into Oncogenesis
DEROSIERS, Ronald C Harvard Medical School 1 RO1 CA 31363-01	Molecular Basis for Herpesvirus Saimiri Oncogenicity
DIAMANDOPOULOS, George T Harvard University 5 RO1 CA 08731-15	Viral Carcinogenesis with Special Reference to SV40
DI MAYORCA, Giampiero College of Medicine & Dentistry, NJ 5 RO1 CA 25168-03	Transformation Genes of Simian Virus 40
DI MAYORCA, Giampiero College of Medicine & Dentistry, NJ 5 RO1 CA 25169-03	BK Virus, A Human Papovavirus
ECKHART, Walter Salk Institute for Biological Studies 5 RO1 CA 13884-10	Viral Gene Functions and Regulation of Cell Growth
EGGERDING, Faye A University of California (LA) 5 RO1 CA 25545-02	Regulation of Adenovirus 2 Transcription

EVANS, Mary J
N Y State Department of Health
5 RO1 CA 22655-03

DNA Polymerase(s) of the Replication SV40 Chromosomes

FALK, Lawrence A, Jr
Harvard University
5 RO1 CA 27225-03

Study of Human and Simian Lymphotropic Herpesviruses

FAREED, George C
University of California (LA)
5 RO1 CA 20794-06

Simian Virus 40, DNA Replication and Transformation

FLUCK, Michele M
Michigan State University
5 RO1 CA 29270-02

Control of Gene Expression on Viral Transformants

FOLK, William R
University of Michigan
2 RO1 CA 13978-10

Mammalian Cell Transformation by Oncogenic Viruses

FRENKEL, Gerald D
New York State
Department of Health
2 RO1 CA 28084-02

Inhibition of Cellular DNAses by DNA Tumor Viruses

FRIEDMANN, Theodore
University of California (San Diego)
2 RO1 CA 24288-04

Cellular and Papovaviral Gene Expression

GALLOWAY, Denise A
Fred Hutchinson Cancer Research Ctr
5 RO1 CA 26001-04

Herpesvirus Expression in Transformation and Latency

GAYNOR, Richard B
University of California (LA)
1 R23 CA 30981-01

Adenovirus 5 Mutants in Transforming Functions

GHOSH, Prabhat K
Yale University
1 RO1 CA 32799-01

Regulation of Simian Virus 40 Transcription

GINGERAS, Thomas R
Cold Spring Harbor Laboratory
5 RO1 CA 27275-02

DNA Sequence and Computer Analysis of a Tumor Virus

GLASER, Ronald
Ohio State University
1 RO1 CA 29066-01

Epstein-Barr Virus DNA In Transfected Cells

GRALLA, Jay
University of California (LA)
5 RO1 CA 19941-07

Regulation of Transcription by DNA-Protein Complexes

GREEN, Maurice St. Louis University 5 RO1 CA 28689-02	Human Papillomaviruses
GREEN, Maurice St. Louis University 5 RO1 CA 29561-24	Biochemistry of Animal Virus Multiplication
GREEN, Maurice St. Louis University 5 RO1 CA 21824-06	Replication of RNA Tumor Viruses
GREEN, Melvin H University of California (San Diego) 5 RO1 CA 24281-03	The Process and Control of Transcription of SV40
GURNEY, Elizabeth T University of Utah 5 RO1 CA 21797-05	Growth Control and Viral Gene Expression
GUTAI, Mary W New York State Department of Health 5 RO1 CA 28250-02	SV40 DNA Replication and Recombination in Animal Cells
HAGER, Lowell P University of Illinois (Urbana) 5 RO1 CA 17619-07	Biochemical Studies on T Antigen and Transformed Cells
HALLICK, Lesley M University of Oregon Hlth Sci Ctr 5 RO1 CA 24799-03	Replication and Repair of Viral DNA and RNA Complexes
HARTER, Marian L College of Medicine & Dentistry, NJ 5 RO1 CA 28414-02	Functions of Early Proteins Encoded by Adenovirus
HAYWARD, Gary S Johns Hopkins University 5 RO1 CA 22130-05	Structural Organization of Herpes Virus DNA Molecules
HAYWARD, Gary S. Johns Hopkins University 5 RO1 CA 28473-02	Cellular Transformation by DNA of Human Herpesviruses
HAYWARD, S. Diane Johns Hopkins University 1 RO1 CA 30356-01	EBV Genome Expression: Localization of Specific Functions
HELD, William A New York State Dept. Health 5 RO1 CA 27647-02	TK-Mutants of Herpes Virus and Their Suppression

HINZE, Harry C University of Wisconsin (Madison) 5 RO1 CA 21195-04	Vaccination Against an Oncogenic Herpes Virus
HIRSCH, Martin S Massachusetts General Hospital 5 RO1 CA 12464-11	Immune Reactivity and Oncogenic Virus Infections
HORWITZ, Marshall S Yeshiva University 5 RO1 CA 11512-12	Adenovirus DNA Synthesis and Polypeptide Assembly
HOWETT, Mary K Pennsylvania State Univ (Hershey) 2 RO1 CA 25305-04	In Vivo Cocarcinogenesis of Chemicals and Viruses
HSU, Ming-Ta Rockefeller University 5 RO1 CA 19073-06	Basic Mechanism of Viral Oncogenesis
HUANG, Eng-Shang University of North Carolina (Chapel Hill) 5 RO1 CA 21773-04	Cytomegaloviruses and Human Malignancy
HUNTER, Anthony R Salk Institute for Biological Studies 5 RO1 CA 17096-07	Macromolecular Synthesis and Cell Growth Control
HUNTER, Anthony R Salk Institute for Biological Studies 5 RO1 CA 28458-02	Viral Transforming Proteins
HYMAN, Richard W Pennsylvania State Univ Hershey Med Ctr 5 RO1 CA 16498-08	Malignancy and DNA Homology Among the Herpes Viruses
ISOM, Harriet C Pennsylvania State Univ Hershey Med Ctr 5 RO1 CA 23931-04	Regulation of Differentiation in Hepatocytes in Vitro
KASAMATSU, Harumi University of California (LA) 5 RO1 CA 21768-05	Centriolar Antigens and Involvement in Cell Proliferation
KELLEMS, Rodney E Baylor College of Medicine 5 RO1 CA 24618-03	Control of Host Gene Expression by DNA Tumor Viruses
KETNER, Gary W Johns Hopkins University 2 RO1 CA 21309-04	Transformation and Gene Regulation by Adenoviruses
KIEFF, Elliott D University of Chicago 5 RO1 CA 17281-07	EBV Interaction with Lymphoblasts In Vitro & In Vivo

KIT, Saul Baylor College of Medicine 5 RO1 CA 06656-20	Biochemical Aspects of Viral Carcinogenesis
KLEIN, George Karolinska Institutet 1 RO1 CA 28380-01	Epstein-Barr Virus Determined Nuclear Antigen (EBNA)
KLEIN, George Karolinska Institutet 1 RO1 CA 30264-01	Immune Effector Mechanisms in EBV-Carrying Patients
KNIPE, David M Harvard University 5 RO1 CA 26345-03	Genetics of Herpes Virus Transformation
KOWALSKI, David Roswell Park Memorial Institute 5 RO1 CA 23996-03	Role of DNA Relaxing Enzyme in SV40 DNA Replication
LANCASTER, Wayne D Georgetown University 3 RO1 CA 32603-01S2	Role of Papillomavirus DNA in Cell Transformation
LANCASTER, Wayne D Georgetown University 7 RO1 CA 32638-01	Papillomavirus DNA and Antigens in Human Neoplasms
LEBOWITZ, Jacob University of Alabama (Birmingham) 5 RO1 CA 17077-08	Circular DNA--Implications for Cancer and Drug Resistance
LEHMAN, John M University of Colorado Medical Center 2 RO1 CA 16030-07	Pathology of Neoplastic Transformtion
LEVINE, Arnold J State Univ. of New York (Stony Brook) 5 RO1 CA 28127-02	SV40 Cellular Antigens and Host Range
LEWIS, James B Fred Hutchinson Cancer Res. Ctr. 1 RO1 CA 29600-01A1	Functions of Adenovirus Proteins in Transformation
LIVINGSTON, David M Sidney Farber Cancer Institute 2 RO1 CA 15751-08	Structure and Function of SV40 Non Virion Proteins
LIVINGSTON, David M Sidney Farber Cancer Institute 2 RO1 CA 24715-04	Isolation and Function of Small SV-40 T Antigen
LUFTIG, Ronald B University of South Carolina 5 RO1 CA 28078-03	Viral Interaction of Microtubules in Cancer Cells

MC DOUGALL, James Fred Hutchinson Cancer Res. Cen. 5 RO1 CA 29350-02	The Biology of Transformation Herpesviruses
MANN, Kristine E University of Alaska 5 RO1 CA 26048-02	SV40 Tumor Antigen in SV40 Nucleoprotein Complexes
MARTIN, Jonathan Tulane University 1 RO1 CA 29631-01	Molecular Analysis of JC Virus Interaction With Cells
MILLER, I George, Jr Yale University 5 RO1 CA 12055-11	Studies of Epstein-Barr Virus
MILLETTE, Robert L Wayne State University 5 RO1 CA 21065-07	Herpes Simplex Virus Gene Regulation
MUNNS, Theodore W Washington University 5 RO1 CA 27801-04	Characterization of RNA/DNA in Oncogenic Systems
NATHANS, Daniel Johns Hopkins University 5 PO1 CA 16519-07	Program on Molecular Biology of Viral Tumorigenesis
NONOYAMA, Meihan SHOWA University Research Institute 7 RO1 CA 31949-01	Marek's Disease Virus: Transformation and Oncogenesis
NONOYAMA, Meihan SHOWA University Research Institute 5 RO1 CA 31950-02	Oncogenicity of Epstein-Barr Virus
NOONAN, Christine Baylor College of Medicine 1 RO1 CA 30925-01	Interaction of Human Wart Virus With Cultured Skin Cells
OZER, Harvey L Hunter College 5 RO1 CA 23002-06	Host Functions Related to Tumor Virus Infection
PAGANO, Joseph S University of North Carolina 5 PO1 CA 19014-06	DNA Virus Genomes, Oncogenesis and Latency
PASS, Franklin University of Minnesota (Minneapolis) 5 RO1 CA 25462-03	Human Papilloma Virus and Malignant Disease

PEARSON, Gary R
Mayo Foundation
5 RO1 CA 20679-06

Biochemistry of Herpes Virus-Induced Membrane Antigens

PEARSON, GARY R
Mayo Foundation
5 RO1 CA 24123-03

Humoral Response to Herpes & Epstein-Barr Virus

PEARSON, Gary R
Mayo Foundation
1 R13 CA/TW 33410-01

Fourth International Symposium on Nasopharyngeal Carcinoma

PEARSON, George D
Oregon State University
2 RO1 CA 17699-07

Replication Map of an Oncogenic Virus

POLLACK, Robert E
Columbia University
5 RO1 CA 25066-04

Oncogenic Virus Gene Control of Cell Growth and Shape

PRIVES, Carol L
Columbia University
5 RO1 CA 26905-03

Function and Expression of SV40 Viral Tumor Antigens

RAPP, Fred
Pennsylvania State University
5 PO1 CA 27503-02

Herpesviruses and Neoplasia

RASKA, Karel, Jr.
Rutgers Medical School
5 RO1 CA 21196-06

Low Molecular Weight RNA in Adenovirus Infections

RAYMENT, Ivan
Brandeis University
5 R23 CA 27260-03

X-Ray Diffraction Studies on Polyoma Virus

REKOSH, David M
State Univ of New York (Buffalo)
5 RO1 CA 25674-04

Adenovirus Early Gene Function and DNA Replication

RICCIARDI, Robert
Wistar Institute
5 RO1 CA 29797-02

Organization and Expression of Adenovirus Genes

ROBERTS, Bryan E
Harvard University
2 RO1 CA 27447-04

Organization and Expression of Genes in Viral DNAs

ROBERTS, Thomas
Sidney Farber Cancer Institute
1 RO1 CA 30002-01A1

Isolation of Polyoma T Antigens Synthesized in

ROEDER, Robert G Washington University 5 RO1 CA 16640-08	Regulation of Adenovirus Gene Expression
ROIZMAN, Bernard University of Chicago 5 RO1 CA 08494-17	Mechanisms of Viral Infection in Relation to Cancer
ROMAN, Ann Indiana Univ. (Indianapolis) 5 RO1 CA 29318-02	Regulation of Papovavirus Replication
ROSENTHAL, Ken S Northeastern Ohio Universities 5 R23 CA 28342-02	Herpes Simplex Virus Glycoproteins and Infection
ROTHSCHILD, Henry Louisiana State University 5 RO1 CA 27943-02	Structure and Function of an SV40 Thermoinducible Mutant
RUNDELL, Mary K Northwestern University 5 RO1 CA 21327-05	Structural Analysis of the SV40 Tumor Antigen
SAMBROOK, Joseph Cold Spring Harbor Laboratory 2 PO1 CA 13106-11	Cold Spring Harbor Laboratory Cancer Research Center
SCHAFFER, Priscilla A Sidney Farber Cancer Institute 5 RO1 CA 20260-06	Transforming Genes of Herpes Simplex Virus
SCHIERMAN, Louis W University of Georgia 5 RO1 CA 30109-02	Immunogenetic Study of a Herpes Virus Induced Lymphoma
SHAH, Keerti V Johns Hopkins University 2 RO1 CA 13478-10	Investigation of SV40-Related Infections of Man
SHENK, Thomas E State Univ. New York (Albany) 5 RO1 CA 28919-02	Structure and Function of DNA Tumor Virus Genomes
SILVERSTEIN, Saul J Columbia University 5 RO1 CA 17477-07	Molecular Biology of Herpes Virus
SIMMONS, Daniel T University of Delaware 5 RO1 CA 25942-03	Origin and Function of SV40-Specific TAU Antigens

SPEAR, Patricia G
University of Chicago
5 R01 CA 21776-06

Herpesvirus Gene Expression in Transformed Cells

SPELSBERG, Thomas C
Mayo Foundation
5 R01 CA 25340-03

A New Class of Epstein-Barr Virus Nuclear Antigen

ST. JEOR, Stephen C
University of Nevada
5 R01 CA 28089-03

Herpesvirus-Induced Malignancy

STEINBERG, Mark L
New York University
5 R23 CA 27869-02

Viral Transformation of Human Keratinocytes

STEVENS, Jack G
University of California (LA)
5 R01 CA 18151-06

Analysis of the Shope Papilloma-Carcinoma System

STROMINGER, Jack L
Sidney Farber Cancer Institute
5 P01 CA 21082-06

Molecular Basis of Viral Oncogenesis

STROMINGER, Jack L
Sidney Farber Cancer Institute
5 R01 CA 24926-03

Study of Epstein-Barr Nuclear Antigen

SUMMERS, William C
Yale University
2 R01 CA 13515-09

Genetic Study of Animal Viruses

TAMM, Igor
Rockefeller University
5 R01 CA 18608-23

Virus Induced Alterations in Animal Cells

TEGTMEYER, Peter J
State Univ New York (Stony Brook)
5 R01 CA 18808-07

Mechanisms of Viral Oncogenesis: SV40 Gene Function

TEGTMEYER, Peter J
State Univ. New York (Stony Brook)
5 P01 CA 28146-02

Tumor Virus-Host Interaction

TENEN, Daniel G
Sidney Farber Cancer Institute
5 R23 CA 26018-03

SV40 T Antigen Binding Sites in Cellular DNA

TEVETHIA, Mary J
Penn State Univ Hershey Med Ctr
2 R01 CA 24694-04

Mutagenesis of Specific Regions of the SV40 Genome

TEVETHIA, Satvir S Penn State Univ Hershey Med Ctr 5 RO1 CA 25000-04	Biology of SV40 Specific Transplantation Antigen
THEIS, Gail A New York Medical College 5 RO1 CA 18904-06	Responses of Lymphocytes to an Oncogenic Herpes Virus
THORLEY-LAWSON, David Sidney Farber Cancer Institute 5 RO1 CA 28737-02	Epstein Barr Virus Membrane Antigen
TIBBETTS, Clark J Univ of Connecticut Health Center 2 RO1 CA 17130-07	Adenovirus Genome Expression--Physical Mapping Studies
TJIAN, Robert University of California (Berkeley) 2 RO1 CA 25417-04	The SV40 Tumor Antigen
TOPP, William C Cold Spring Harbor Laboratory 5 RO1 CA 24830-03	SV40 Early Gene Products and Viral Oncogenesis
TROY, Frederic A University of California (Davis) 5 RO1 CA 17327-07	Membrane Bound Enzymes, Tumor Antigens and Malignancy
ULTMANN, John E University of Chicago 5 PO1 CA 19264-06	Viral Oncology Research Center Program
VARSHAVSKY, Alexander Mass. Institute of Technology 5 RO1 CA 30376-02	Studies on the SV40 Virus Structure & Replication
VELICER, Leland F Michigan State University 5 RO1 CA 23408-03	Marek's Disease Herpes Virus Antigens
VILLARREAL, Luis P Univ of Colorado Medical Center 5 RO1 CA 25819-04	Gene Expression of a Small DNA Tumor Virus: SV40
WAGNER, Edward K University of California (Irvine) 5 RO1 CA 11861-13	Viral RNA Synthesis Control in Herpes Virus Infection
WEISSMAN, Sherman M Yale University 5 PO1 CA 16038-08	Program on the Molecular Basis of Viral Transformation

WEISSMAN, Sherman M Yale University 5 RO1 CA 16106-06	Function of SV40 Gene Products
WENTZ, William B Case Western Reserve 1 RO1 CA 31973-01	Sexually Transmitted Disease in Uterine Carcinogenesis
WETTSTEIN, Felix O University of California (LA) 2 RO1 CA 18151-07	Analysis of the Shope Papilloma-Carcinoma Style
WILLIAMS, James F Carnegie-Mellon University 5 RO1 CA 21375-05	Genetic Analysis of Adenoviruses
WILLIAMS, James F Carnegie-Mellon University 1 RO1 CA 32940-01	Study of Adenoviru Trans-formation - Defective Mutants
WILSON, John H Baylor College of Medicine 5 RO1 CA 15743-09	Oncogenic Mechanisms: SV40 and Host Genetic Analysis
WOLD, William S St. Louis University 5 R23 CA 24710-03	Adenovirus 2 Coded Early Glyco-protein
WU, Guang-Jer Emory University 5 RO1 CA 25270-03	Regulation of Transcription
ZIMMER, Stephen G University of Kentucky 1 RO1 CA 33434-01	Analysis of Defined AD2 Transformed Cell Revertants

BIOLOGICAL CARCINOGENESIS BRANCH

GRANTS ACTIVE DURING FY 82

RNA VIRUS STUDIES (I)

Investigator/Institution/Grant Number	Title
ARLINGHAUS, Ralph B. Univ. of Texas System Cancer Ctr. 5 RO1 CA 25465-03	Biosynthesis and Characterization of Murine Oncornavirus
BACHELER, Lee T. Temple University 5 RO1 CA 29519-03	Organization and Expression of Leukemia Virus Genomes
BALTIMORE, David Massachusetts Institute of Technology 5 PO1 CA 26717-03	Molecular Analysis of Oncogenic Viruses
BEDIGIAN, Hendrick G. Jackson Laboratory 1 RO1 CA 31102-01	A New Murine Model for the Study of Nonthymic Leukemia
BURNS, William H. Johns Hopkins University 1 RO1 CA 30090-01	Role of Thymic Epithelium in Viral Leukemogenesis
CARDIFF, Robert D. Univ. of California (Davis) 5 RO1 CA 21454-06	MTV Gene Amplification and Expression
CASEY, James W. Louisiana State University 1 RO1 CA 31702-01	Molecular Mechanisms of Retroviral Induced Leukemia
CERNY, Jan Univ. of Texas Medical Br. (Galveston) 7 RO1 CA 32591-01	Regulatory Mechanisms of Neoplasia
COGGIN, Joseph H., Jr. University of South Alabama 2 RO1 CA 23491-05	Etiology of a Lymphoma Epizootic in Hamsters
COHEN, J. Craig Tulane University School of Medicine 5 RO1 CA 30358-02	Role of Endogenous Viruses in Mammary Carcinogenesis
COMPANS, Richard W. University of Alabama (Birmingham) 2 RO1 CA 18611-08	Molecular Studies of Oncorna and Arenaviruses

COPELAND, Neal G. Jackson Laboratory 1 R01 CA 32324-01	Ecotropic MuLVs of Normal and Mutant Strains
DARNELL, James E., Jr. Rockefeller University 5 P01 CA 18213-07	Correlated Program in Viral Oncology
DATTA, Syamal K. New England Med. Ctr. Hosp. 5 R01 CA 31789-03	Genetic-Viral-Immunologic Studies in New Zealand Mice
DAVIDSON, Norman R. California Institute of Technology 5 R01 CA 25991-03	Sequence Organization of Integrated Tumor Virus Genomes
DINA, Dino Yeshiva University 2 R01 CA 24223-04A1	Regulation of Murine RNA Tumor Virus Gene Expression
DURAN-REYNALS, Maria L. Yeshiva University 2 R01 CA 07160-17	Possible Neoplastic Effects of Non-Neoplastic Viruses
ECKNER, Robert J. Boston University 5 R01 CA 19562-07	Biological and Physical Properties of Friend Virus
ELDER, John H. Scripps Memorial Hospital 2 R01 CA 25533-04	Sequence Studies of the gp70's of Recombinant Retrovirus
ESSEX, Myron E. Harvard University 5 R01 CA 13885-08	Oncornavirus-Associated Cell Membrane Antigens
ESSEX, Myron E. Harvard University 5 R01 CA 18216-06	Study of (FLV) Leukemia Virus
FAMULARI, Nancy G. Sloan-Kettering Mem. Inst. for Cancer Res. 5 R01 CA 27950-03	Influence of MuLV env and gag genes in leukemogenesis
FAN, Hung Y. University of California (Irvine) 7 R01 CA 32455-02	Expression and Localization of C-Type Virus Genes
FAN, Hung Y. University of California (Irvine) 2 R01 CA 32454-01	Studies of Murine Leukemia Virus Integration

FLEISSNER, Erwin J.
Sloan-Kettering Inst. for Cancer Res.
5 R01 CA 15297-09

Viral and Mouse Genes in Leukemia Virus Infection

FLEISSNER, Erwin J.
Sloan-Kettering Inst. for Cancer Res.
5 P01 CA 16599-08

Oncogenic Viruses Program Project

FRIEND, Charlotte
Mount Sinai School of Medicine
5 R01 CA 10000-17

Filterable Agents and Tumor Induction in Mice

GARDNER, Murray B.
University of California (Davis)
1 R01 CA 30912-01A1

Mammary Tumorigenesis in Hosts Lacking MuMTV DNA

GARDNER, Murray B.
University of California (Davis)
1 R01 CA 31619-01

Genetic Control of Ecotropic Retrovirus in Wild Mice

GIRARDI, Anthony J.
Institute for Medical Research
5 R01 CA 24940-04

Immunologic Studies in Mouse and Human Breast Cancer

GOFF, Stephen P.
Columbia University
5 R01 CA 30488-02

Construction and Analysis of Retrovirus Mutants

GREENBERGER, Joel S.
Sidney Farber Cancer Institute
5 R01 CA 26785-03

Requirements for Spontaneous Leukemogenesis In Vitro

HAAS, Martin
Salk Institute for Biological Studies
5 R01 CA 30146-02

Viral Malignant Lymphomagenesis in X-Irradiated mice

HASELTINE, William A.
Sidney Farber Cancer Institute
2 R01 CA 19341-06A1

The Molecular Biology of Replication RNA Tumor Viruses

HASELTINE, William A.
Sidney Farber Cancer Institute
5 R01 CA 29294-02

Molecular Biology of Leukemia and Sarcoma Retroviruses

HAYS, Esther F.
Univ. of California (Los Angeles)
5 R01 CA 12386-10

Development of Lymphoma in the Thymus

HOOVER, Edward A.
Colorado State University
7 R01 CA 32552-01

Pathogenesis of Animal Leukemia

HOPKINS, Nancy H. Massachusetts Institute of Technology 2 RO1 CA 19308-07	Endogenous Viruses of BALB/c Mice
HUNTER, Eric University of Alabama (Birmingham) 5 RO1 CA 27834-02	Genetics of Primate "D" Type Retroviruses
KABAT, David Univ. of Oregon Hlth. Sciences Ctr. 2 RO1 CA 25810-04	Leukemogenesis by Friend Spleen Focus Forming Virus
KAPLAN, Henry S. Stanford University 5 RO1 CA 03352-26	Biological Aspects of Carcinogenesis by Radiation
KAPLAN, Henry S. Stanford University 5 RO1 CA 29079-02	Studies of Retroviruses from Human Lymphoma Cells
LERNER, Richard A. Scripps Clinic and Research Fdn. 5 PO1 CA 27489-03	Immunological and Pathological Consequences of Endogenous Retroviral Expression
LEVY, Jay A. Univ. of California (San Francisco) 1 RO1 CA/AM 33137-01	Role of Endogenous Xenotropic Viruses
LILLY, Frank Yeshiva University 5 RO1 CA 19873-06	Genetic Control of Resistance to Friend Virus Disease
LILLY, Frank Yeshiva University 5 RO1 CA 19931-06	Mechanism of the H-2 Effect on Viral Leukemogenesis
LILLY, Frank Yeshiva University 5 RO1 CA 26010-03	Genetic Resistance to Spontaneous Leukemia in Mice
LUCE, Judith A. University of California (San Francisco) 1 R23 CA 32844-01	Characterization of FBJ Murine Osteosarcoma Virus
LUFTIG, Ronald B. University of South Carolina 2 RO1 CA 28077-04	Assembly of Murine Leukemia Viruses
MARCUS, Stuart L. Sloan-Kettering Inst. for Can. Res. 3 RO1 CA 18369-06S1	RLV DNA Polymerase Enzymology and Radioimmunoassay

MERUELO, Daniel New York University Medical Center 5 RO1 CA 22247-06	Genetic Control of Virus-Induced Leukemia
MERUELO, Daniel New York University Medical Center 1 RO1 CA 31346-01A1	Loci Affecting Radiation/RadLV Induced Leukemogenesis
MODAK, Mukund J. Sloan-Kettering Institute 5 RO1 CA 21404-06	Molecular Effects of Enzymatic DNA Synthesis
O'DONNELL, Paul V. Sloan-Kettering Inst. for Can. Res. 1 RO1 CA 31491-01	Kinetic Study of Virus-Accelerated Leukemia in AKR Mice
OLSEN, Richard G. Ohio State University 5 RO1 CA 30338-02	FeLV Leukemogenesis and Preneoplastic Lesions
OZANNE, Bradford W. Univ. of Texas Hlth. Sci. Ctr. (Dallas) 5 RO1 CA 23043-05	Peptide Transforming Factors from Transformed Cells
PARKS, Wade University of Miami 5 RO1 CA 27890-03	MMTV in Spleen Lymphoid Cells: Immunologic Effect
PETERSON, David O. Texas A & M Research Foundation 1 RO1 CA 32695-01	Genetic and Molecular Analysis of Steroid Responsiveness
PIKO Lajos Veterans Administration Hospital 5 RO1 CA 24989-04	Gene Expression in Early Mouse Development
PROFFITT, Max R. Cleveland Clinic Hospital 2 RO1 CA 20242-07	Autoimmunity and Virus-Induced Leukemia
RASCHKE, William C. La Jolla Cancer Research Foundation 5 RO1 CA 30903-02	Characterization of the Abelson Leukemia Virus
RASHEED, Suraiya University of Southern California 5 RO1 CA 27246-03	Leukemia and Sarcoma Genes in Cellular Transformation
ROSENBERG, Naomi E. Tufts University 2 RO1 CA 24220-04	Abelson Leukemia Virus Transformation of Lymphocytes

ROSNER, Marsha A. Massachusetts Institute of Technology 1 R01 CA 32267-01	Isolation and Characterization of Retrovirus Receptors
ROY-BURMAN, Pradip University of Southern California 5 R01 CA 26809-03	Oncodevelopmental Gene Expression in Feline Leukemia
SARKAR, Nurul H. Sloan-Kettering Inst. for Can. Res. 5 R01 CA 17129-08	Components of the Mouse Mammary Tumor Virus
SCHWARTZ, Robert S. Tufts University 1 R01 CA 31849-01	Experimental Model of Malignant Lymphoma
SOMERS, Kenneth D. Eastern Virginia Medical School 5 R01 CA 28474-03	Cellular Transformation by MSV
SPIEGELMAN, Sol Columbia University 5 P01 CA 23767-04	Molecular Virology
STEFFEN, David L. Worcester Fdn. for Experimental Biology 5 R01 CA 30674-02	Mechanisms of Viral and Nonviral Leukemogenesis
THACH, Robert E. Washington University 5 R01 CA 13008-11	Replication of Virulent and Oncogenic Viruses
THOMAS, Christopher Y. University of Virginia 1 R01 CA 32995-01	Molecular Genetics of Leukemia Viruses of Inbred Mice
VAIDYA, Akhail Hahneman Medical College and Hospital of Philadelphia 5 R01 CA 22413-06	Etiological Studies of Mammary Carcinoma
VARMUS, Harold E. Univ. of California (San Francisco) 5 R01 CA 19287-07	Molecular Biology of Mouse Mammary Tumor Virus
VERMA, Inder M. Salk Institute for Biological Studies 5 R01 CA 16561-08	Reverse Transcriptase from RNA Tumor Viruses
VERMA, Inder M. Salk Institute for Biological Studies 5 R01 CA 21408-06	Genetic Organization of RNA Tumor Viruses

VOGT, Marguerite M. Salk Institute for Biological Studies 5 R01 CA 13608-10	Viral Gene Functions Involved in Transformation
WATSON, James D. Cold Spring Harbor Laboratory 5 R13 CA 02809-27	Support for Symposia on Quantitative Biology
WEINBERG, Robert A. Massachusetts Institute of Technology 5 R01 CA 17537-08	Interaction of Sarcoma and Leukemia Genomes
WEISSMAN, Irving L. Stanford University 1 R01 CA 32031-01	The Receptor-Mediated Leukemogenesis Hypothesis
WHEELOCK, E. Frederick Hahneman Medical College and Hospital of Philadelphia 5 R01 CA 32575-02	Role of Endogenous Viruses in Tumor Dormant States
WITTE, Owen N. University of California 5 R01 CA 27507-03	Transformation by Abelson Murine Leukemia Virus
YAMAMOTO, Keith Univ. of California (San Francisco) 5 R01 CA 20535-06	Mechanisms of Gene Regulation by Steroid Receptor Proteins
YANG, Wen K. Oak Ridge National Laboratory 5 R01 CA 30308-02	Mechanism of Fv-1 Restriction of Murine Leukemia Viruses

BIOLOGICAL CARCINOGENESIS BRANCH

GRANTS ACTIVE DURING FY 82

RNA VIRUS STUDIES (II)

Investigator/Institution/Grant Number	Title
ARMENTROUT, RICHARD W. University of Cincinnati 1 RO1 CA 31797	Viral Probe of Tumor-Specific DNA Replication Factor
ASTRIN, Susan M. Inst for Cancer Research, Philadelphia 5 RO1 CA 27797-02	Control of Expression of Endogenous Viral Genes
AXEL, Richard Columbia University 5 RO1 CA 16346-07	Molecular Control of Chromatin Transcription
BALUDA, Marcel A. Univ of California (Los Angeles) 5 RO1 CA 10197-15	Tumor Induction by Avian Myeloblastosis Virus
BEEMON, Karen L. Johns Hopkins University 1 RO1 CA 33199-01	Location and Function of M6A in Retrovirus RNAS
BISHOP, John M. Univ of California (San Francisco) 5 RO1 CA 12705-11	Rous Sarcoma Virus: Replication and Cell Transformation
BOETTINGER, David E. University of Pennsylvania 5 RO1 CA 16502-08	Genetic Analysis of RNA Tumor Viruses
BOETTINGER, David E. University of Pennsylvania 5 RO1 CA 30383-02	Virus Induced Myeloid Leukemia
BOSE, Henry B. University of Texas 5 RO1 CA 27003-03	Virus Sequences In REV-Transformed Cells
BREWER, John I. Northwestern Univ. 1 RO1 CA 29461-01	Trophoblastic Tumors: New Organism/Immunology/Therapy
BRILES, Worthie E. Northern Illinois University 5 RO1 CA 12796-10	Immunogenetics of Tumor Related Alloantigens

BRUGGE, Joan S. SUNY--at Stony Brook 5 R01 CA 27951-02	The ASV Transforming Protein and its Cellular Homologue
CARBON, John A. U. California (Santa Barbara) 5 R01 CA 11034-14	Studies on Gene Organization and Expression
CASPAR, Donald L. Brandeis University 5 R01 CA 15468-09	Assembly of Viruses, Membranes and Tissues
CHIRIKJIAN, Jack G. Georgetown University 5 R01 CA 16914-06	RNA Modifying Enzymes in Normal and Neoplastic Cells
COFFIN, John M. Tufts University 5 R01 CA 17659-07	Relationship of Avian Tumor Virus RNA and Host Genome
COFFIN, John M. Tufts University 5 R01 CA 27108-03	Mechanism of Variability of Tumor Virus RNA
COLLETT, Marc S. University of Minnesota 5 R01 CA 29041-02	Structure and Function of the ASV Transforming Protein
COLLINS, Carolyn J. University of Virginia 5 R01 CA 24137-03	Integration of RNA Tumor Viruses in Mammalian Cells
COOPER, Geoffrey M. Sidney Farber Cancer Inst 5 R01 CA 18689-06	Infectious DNA for Endogenous RNA Tumor Virus Genes
CRITTENDEN, Lymen B. U.S. Dept. of Agriculture 1 R01 CA 29656-01	Avian Endogenous Retroviral Gene Expression
DAHLBERG, James E. University of Wisconsin (Madison) 5 R01 CA 15166-08	Small RNAs of RNA Tumor Viruses
DUESBERG, Peter H. University of California (Berkeley) 5 R01 CA 11426-13	Molecular and Genetic Analyses of RNA Tumor Viruses

EHRLICH, Melanie Tulane Medical School 2 ROl CA 19942-05	5-Methylcytosine in DNA: Cancer and Development
EISENMAN, Robert N. Fred Hutchinson Cancer Research Cen 5 ROl CA 20525-05	Control Mechanisms in Avian Oncornavirus Replication
ERIKSON, Raymond L. University of Colorado Medical Cen 5 ROl CA 21117-17	Biosynthesis of Viral RNA
FARAS, Anthony J. University of Minnesota (Minneapolis) 5 ROl CA 18303-08	RNA-Directed DNA Polymerase and 70S RNA of Oncornavirus
FARAS, Anthony J. University of Minnesota (Minneapolis) 5 ROl CA 26387-03	Reversion of Rous Sarcoma Virus-Transformed Cells
GOLDBERG, Allan R. Rockefeller University 5 ROl CA 13362-10	RSV Functions Involved in Transformation
GOULIAN, Mehan University of Calif. (San Diego) 5 ROl CA 11705-12	DNA Synthesis Studies
GRANDGENETT, Duane P. St. Louis University 5 ROl CA 16312-08	Avian Retrovirus DNA Synthesis and its Regulation
GRANOFF, Allan St. Jude's Children's Res. Hosp. 5 ROl CA 07055-19	Studies of Lucke Tumor Associated Virus
GRAY, Horace B. University of Houston 5 ROl CA 11761-11	Hydrodynamics of Circular DNA Forms
GUNTAKA, R. V. Columbia University 5 ROl CA 28990-02	Synthesis Structure and Function --Avian Tumor Virus DNA
HALPER, Michael The Wistar Institute 1 ROl CA 31514-01	Oncornavirus-Induced Immunosuppression

HANAFUSA, Hidesaburo Rockefeller University 2 ROl CA 14935-09	Cellular Alteration Induced by Rous Sarcoma Virus
HARRISON, Stephen C. Harvard University 5 ROl CA 13202-10	Virus Structure and Assembly
HAYWARD, William S. Rockefeller University 2 ROl CA 16668-08	RNA Tumor Virus Gene Expression
HALOWCZAK, John A Rutgers Medical School 5 ROl CA 11027-14	Transcription and Translation in Pox Virus Infected Cells
HOLTZER, Howard University of Pennsylvania 5 ROl CA 18194-07	Conversion of Embryonic Cells into Transformed Cells
HUMPHRIES, Eric H. Univ of Texas (Dallas) 5 ROl CA 23041-03	Endogenous Avian Retrovirus in Non-Permissive Cells.
HUNTER, Anthony B. Salk Institute Bio. Studies 1 ROl CA 17096-07	Macromolecular Synthesis and Cell Growth Control
HUNTER, Eric University of Alabama 5 ROl CA 29884-02	Site Specific Mutagenesis in the ENV Gene of RSV
KAJI, Akira University of Pennsylvania 5 ROl CA 19497-05	Replication of RNA Tumor Virus
KOPROWSKI, Hilary Wistar Institute 5 POl CA 21124-05	Genetics and Virology of Cancer
KUNG, Hsing-Jien Michigan State University 5 ROl CA 24798-03	Recombination and Replication of Avian Sarcoma Viruses
KUNG, Hsing-Jien Michigan State University 1 ROl CA 33158-01	Erythroleukemia: Oncogene Activation by Retrovirus
LAI, Michael M. University of Southern California 2 ROl CA 16113-07	Structure and Replication of RNA Tumor Viruses

LEIS, Jonathan P. Case Western Reserve University 5 ROl CA 27414-03	Processing and Translation of Tumor Virus RNA In Vitro
LINIAL, Maxine L. Fred Hutchinson Cancer Research Ctr 2 ROl CA 18282-07	Viral Coded Functions in Rous Sarcoma Virus
LUTWICK, Bruce E. Maimonides Medical Center 7 ROl CA 32566-01	Hepatitis B Virus and Hepatocellular Carcinoma
MAGUN, Bruce E. University of Arizona 5 ROl CA 20913-05	Growth Regulatory Mechanisms in Viral Transformation
MARCUS, Philip I. University of Connecticut 5 POl CA 14733-09	Gene Expression, Virus Replication and Cell Growth
MARTIN, G. Steven University of California (Berkeley) 5 ROl CA 25464-03	Transformation of Differentiating Cells
MARTIN, G. Steven University of California (Berkeley) 2 ROl CA 17542-07	Genetics of RNA Tumor Viruses
MASON, William S. Institute for Cancer Research 5 ROl CA 26012-03	Replication of Rous Sarcoma Virus
MAYOR, Heather D. Baylor College of Medicine 5 ROl CA 14618-08	Growth and Maturation of Adenoassociated Satellite Viruses
MENKO, Sue University of Minnesota 1 R23 CA 29289-02	The Effect of SRC on Cytoskeletal Functions
MILMAN, Gregory Johns Hopkins University 5 ROl CA 21650-07	Biochemistry of Mutation in Human Cells
MOSCOVICI, Carlo University of Florida 5 ROl CA 10697-15	Specificity of Avian Myeloblastosis Virus
NADAL-GINARD, Bernardo Yeshiva University 5 ROl CA 26860-02	Transforming Gene of Rous Sarcoma Virus

NEIMAN, Paul E.
Fred Hutchinson Cancer Research Center
5 R01 CA 20068-06

Molecular Mechanisms in Neoplasia

NEIMAN, Paul E.
Fred Hutchinson Cancer Research Center
5 P01 CA 28151-02

Retroviruses and Cancer

PARSONS, J. Thomas
University of Virginia
5 R01 CA 27578-03

Mechanisms of RNA Tumor Virus DNA Integration

PARSONS, J. Thomas
University of Virginia
5 R01 CA 29243-02

Sarcoma Virus Specific Tumor Antigens

PENHOET, Edward E.
U. California (Berkeley)
5 R01 CA 20357-06

Control of RNA Synthesis in Eukaryotic Cells

PERDUE, Michael L.
University of Kentucky
5 R01 CA 26170-03

Avian Oncornavirus Messenger DNA

POGO, Beatriz G.
Mt. Sinai School of Medicine
5 R01 CA 29262-02

The expression of Oncogenicity of Shope Fibroma Virus

QUIGLEY, James P.
Downstate Medical Center
2 R01 CA 16740-06

Proteases in Growth Control and Malignant Transformation

RHODE, Solon L., III
Inst of Med Res of Bennington
7 R01 CA 26801-04

Replicon Control in Normal and Transforming Cells

RHODE, Solon L. III
Inst. of Med. Res. of Bennington
5 R01 CA 25866-04

Role of Latent Viruses In Resistance

ROBBINS, Phillips W.
Mass. Institute of Technology
5 R01 CA 14142-19

Cell and Virus Glycoproteins-Synthesis and Function

ROBINSON, Harriet L.
Worcester Fdn for Exper Biology
5 R01 CA 23086-05

Inheritance and Expression of Avian C-Type Viruses

ROBINSON, Harriet L.
Worcester Fdn for Exper Biology
5 R01 CA 27223-03

Avian Leukosis Viruses and Cancer

ROHRSCHNEIDER, Larry R. Fred Hutchinson Cancer Research Center 5 ROl CA 20551-06	Mechanisms of Oncornavirus-Induced Transformation
RUECKERT, Roland R. University of Wisconsin (Madison) 5 ROl CA 08662-16	Structure and Synthesis of Avian RNA Tumor Viruses
RUTTER, William J. Univ of Calif (San Franansico) 1 ROl CA 32797	Molecular Analysis of Hepatitis B. Virus
SCOTT, June R. Emory University 5 ROl CA 11673-12	Lysogeny and Bacteriophage Pl
SEFTON, Bartholomew Salk Institute 2 ROl CA 17289-07	Viral Membranes: Structure and Biosynthesis
SHAFRITZ, Davis Albert Einstein College of Medicine 1 ROl CA 32605-01	Hepatitis B Virus - Chronic Hepatitis Liver Change
SHALLOWAY, David. I Penn State Univ. 1 ROl CA 32317-01	Role of PP60c-SRC, Homolog of the RSV Oncogenic Protein
SHANK, Peter R. Brown University 1 ROl CA 32980-01	Stability and Disease Trophisms of Avian Proviral DNAS
SIDDIQUI, Aleem University of Colorado 1 ROl CA 33135-01	Expression of Hepatitis B Virus Genes and Hepatoma
SMITH, Ralph E. Duke University 5 ROl CA 12323-11	Biochemistry of RNA Tumor Virus Replication
STAVNEZER, Edward Sloan Kettering Inst for Can Res 5 ROl CA 24163-03	Synthesis and Processing of Avian Retrovirus RNAs
STAVNEZER, Edward Sloan-Kettering Inst. for Can Res 1 ROl CA 32817-01	The Origin, Structure, and Biological Activity of SKVS
STOLTZFUS, Conrad M. University of Iowa 5 ROl CA 28051-03	Retroviruses RNA Metabolism

TATTERSALL, Peter J. Yale University 1 ROl CA 29303-01	Molecular Basis of Parovirus Target Cell Specificity
TAYLOR, John M. Institute for Cancer Research 5 ROl CA 22651-04	Early Events in Avian Retrovirus Replication
TEMIN, Howard M. University of Wisconsin (Madison) 5 POl CA 22443-05	Molecular Biology and Genetics of Tumor Viruses
TEREBA, Allan M. St. Jude Children's Research Hospital 5 ROl CA 28221-02	Localization and Mechanism of Retrovirus Integration
VANAMAN, Thomas C. Duke University Med. Center 5 POl CA 30246-02	Regulatory Functions of Protein-Nucleic Acid Interactions
VIOLA, Michael V. U. of Connecticut 5 ROl CA 27792-02	Pathogenesis of Paget's Disease of Bone
VOGT, Peter K. University of Southern California 5 ROl CA 13213-09	Interactions Between Avian Tumor Viruses and Their Hosts
VOGT, Peter K. University of Southern California 5 ROl CA 29777-02	Avian Retrovirus Structure
VOGT, Volker M. Cornell University 5 ROl CA 20081-05	Avian Retrovirus Structure in Assembly
WANG, Lu-Hai Rockefeller University 5 ROl CA 29339-02	Transforming Genes of Avian Sarcoma Viruses
WATSON, Kenneth F. Univ. of Montana 2 ROl CA 19729-06	Mechanism of Viral RNA-Directed DNA Polymerization
WEBER, Michael J. U. Illinois (Urbana) 5 ROl CA 12467-10	Early Cellular Changes in Viral Oncogenesis
WEINTRAUB, Harold M. Fred Hutchinson Cancer Research Center 5 ROl CA 26663-04	Cell Transformation by RSV

WEISS, Gary B.
University of Texas
1 R01 CA 31800-01

Infidelity of Human RNA
Directed DNA Polymerase

WELLS, Robert D.
U. of Wisconsin (Madison)
5 R01 CA 20279-05

DNA Structure and Gene Regulation

SUMMARY REPORT
CHEMICAL AND PHYSICAL CARCINOGENESIS BRANCH

The Chemical and Physical Carcinogenesis Branch (1) plans, coordinates, and administers a national extramural program of basic and applied research consisting of grants and contracts, collectively concerned with the occurrence and the inhibition of cancer, caused or promoted by chemical or physical agents acting separately or together or in combinations with biological agents; (2) plans, organizes, and conducts meetings of scientists and otherwise maintains contacts with scientists-at-large, to identify and evaluate new and emergent research in and related to the fields of chemical and physical carcinogenesis; (3) provides a broad spectrum of information, advice, and consultation to scientists and to institutional science management officials, relative to NIH and NCI funding and scientific review policies and procedures, preparation of grant applications, and choice of funding instrument, based on individual need; (4) plans, develops, maintains, and allocates research resources necessary for the support of carcinogenesis research of high programmatic interest; and (5) provides NCI management with recommendations concerning funding needs, priorities, and strategies relative to the support of chemical and physical carcinogenesis research, consistent with the current state of development of individual research elements and the promise of potential, new initiatives.

Research and related activities supported under this program bear upon a broad range of subject-matter areas, with principal emphasis on environmental carcinogenesis, mechanisms of action of chemical and physical carcinogens, DNA damage and repair in carcinogenesis, inter- and intra-species comparisons in the response to carcinogen exposure, the role of tumor promoters, hormones, and other cofactors in cancer causation, experimental approaches to the inhibition of carcinogenesis, and in vitro carcinogenesis studies on human cells, tissues, and subcellular fractions. The program also supports the synthesis, acquisition, and distribution of a considerable spectrum of chemical substances, critically needed in the field of carcinogenesis research.

Grants and contracts administered by the Staff of this Branch, support five complementary categories of chemical and physical carcinogenesis research and associated resources: Molecular Carcinogenesis, Carcinogenesis Mechanisms, Biological and Chemical Prevention, Special Projects, and Research Resources. Molecular Carcinogenesis focuses on changes in physiological compounds and processes associated with exposure to carcinogens, effects of carcinogens on cell structure, ultrastructure, and function, DNA damage and repair following exposure to carcinogens, identification of biochemical and molecular markers of malignant transformation of cells, the development of analytical procedures for the identification and quantitation of carcinogens present in biological specimens, and studies on enzymes characteristically associated with the carcinogenesis process. The Carcinogenesis Mechanisms category relates to the absorption and body distribution of carcinogens, metabolism, activation and inactivation of carcinogens, identification of proximate and ultimate carcinogenic forms, molecular structure-carcinogenicity relationships, carcinogen-mutagen relationships, isolation, identification, and synthesis of suspect carcinogens and their metabolites, and factors which alter carcinogen activity. Biological and Chemical Prevention is concerned with the experimental inhibition of carcinogenesis caused by chemical, physical, and biological agents. Efforts are devoted to the identification, development, and testing (both in vitro and in vivo) of agents intended to inhibit carcinogenesis. Areas of prime interest include mechanisms of action of candidate

preventive agents, binding proteins and receptors, structure-function relationships, and the experimental use of combinations of preventive agents. The Special Projects category is characteristically concerned with a scrutiny of the broad domain of carcinogenesis research, with the object of identifying areas of research of manifest or emergent importance, which would appear to merit an initial or increased measure of encouragement and support. In this context, current interests are focused on interspecies comparisons in carcinogenesis, the role of tumor promoters, hormones, and other cofactors in human cancer causation, and the use of human cells, tissues, and subcellular fractions in carcinogenesis research. Additionally, grants concerned with cancer-related endocrinology and the Program Projects within this Branch, are assigned to the Special Projects category. The Research Resources category, supported solely by contract, is principally concerned with the synthesis and distribution of selected chemical carcinogens and certain of their metabolites, with particular reference to polynuclear hydrocarbon carcinogens, their metabolic intermediates, and analagous heterosubstituted compounds, as well as the synthesis and distribution of retinoids, including radiolabeled forms.

TABLE I

CHEMICAL AND PHYSICAL CARCINOGENESIS BRANCH

	FY 1982			
	CONTRACTS		GRANTS	
	No. of Contracts	$ (Millions)	No. of Grants	$ (Millions)
Carcinogenesis Mechanisms	1	.00	60	5.48
Biological & Chemical Prevention	16	.60	34	2.66
Molecular Carcinogenesis	14	.11	135	12.02
Research Resources	10	.82	0	.00
Special Projects	5	.00	116	17.24
Frederick Cancer Research Facility	1	2.50	0	.00
TOTALS	47	4.03	345	37.40

Note: Individual projects have been adjusted to show annualized levels of effort and may not correspond to fiscal year budget amounts shown in tables and pie charts.

TABLE II

CHEMICAL AND PHYSICAL CARCINOGENESIS BRANCH
(Extramural Activities - FY 1982)

	No. of Contracts/Grants	$ (Millions)
Research Contracts	36	0.71
Research Grants	289	31.37
Traditional Project Grants (269 grants; $25.43 million)		
Conference Grants (3 grant; $.03 million)		
New Investigator Research Grants (7 grants; $.38 million)		
Program Project Grants (10 grants; $5.53 million)		
Interspecies Comparisons (RFA)	16	2.12
Mechanisms of Chemoprevention (RFA)	15	1.23
Tumor Promoters, Hormones & Other Cofactors in Human Cancer Causation (RFA)	25	2.68
Research Resources Contracts	10	0.82
Frederick Cancer Research Facility	1	2.50
TOTALS	392	41.43

Carcinogenesis Mechanisms

Grants in the Carcinogenesis Mechanisms Program include studies relating to metabolism and mechanisms of action of carcinogens and their metabolites; isolation, identification, and synthesis of known and suspect carcinogens and their metabolites; molecular structure-activity relationships; and carcinogen-mutagen relationships. The grants have been divided into four groups determined by the carcinogenic agent under study. These are polycyclic aromatic hydrocarbons, aromatic amines, alkylating agents and other agents. This last category includes fiber studies, compounds that do not fit into the above groups such as carbon tetrachloride, chloro-olefins, malondialdehyde plus others, and studies that involve compounds from more than one group. Each group covers the entire spectrum of research since many grants studying metabolism, for example, are also studying mechanisms of action and structure-activity relationships. A discussion of each group follows.

Polycyclic Aromatic Hydrocarbons

This group comprises approximately 25% of the grants in the Carcinogenesis Mechanisms Program. A variety of studies are supported in this area. Included are development of new synthetic methods, kinetic studies, metabolism and chemical and biological mechanisms of action.

A number of grants involve synthesis of fluorinated, hydroxylated and methylated derivatives of a variety of polycyclic aromatic hydrocarbons. These derivatives are tested for biological activity usually in collaborating laboratories, as part of long-range programs studying the relationship of molecular structure to biological activity. Other examples include synthesis of suspected metabolites in the benzacridene series and development of stereo-and regio-chemically controlled synthetic methods for bay region diol-epoxide metabolites in the benzanthracene series.

The majority of the grants support studies of metabolic pathways, identification of metabolites and mechanisms of action of a variety of polycyclic aromatic hydrocarbons. One example is a study of the metabolic activation of dibenz(a,c)anthracene (DBA) (Hewer et al., 1981). Although DBacA is only a relatively weak tumor initiating agent, the authors found the mechanisms by which it may be activated by metabolism to be of interest, partly because of its relevance to the structure-activity relationships that exist within the hydrocarbons as a class of chemical carcinogens. The metabolism of ^{3}H-labeled DBacA to products that react with nucleic acids has been examined in mouse skin in vivo, in hamster embryo cells in culture and in rat liver microsomal preparations that contained added DNA. Hydrolysates of DNA that had been isolated from each of the biological systems were examined by chromatography on columns of Sephadex LH20 that were eluted with water/methanol gradients. The chromatographic characteristics of some of the adducts formed when ^{3}H-DBacA or ^{3}H-DBacA 10,11-diol was incubated with rat liver microsomal preparations in the presence of DNA or with hamster embryo cells were compared with the characteristics of the hydrocarbon-deoxyribonucleoside adducts that are formed in reactions of anti-DBacA 10,11-diol 12,13-oxide with DNA. While the DBA metabolites involved in the formation of most of the hydrocarbon-deoxyribonucleoside adducts have not been identified, evidence was obtained that

implicates the anti-DBacA 10,11-diol 12,13-oxide in the metabolic activation of DBacA in these two systems. Although only relatively low levels of reaction occur between DBacA metabolites and DNA in hamster embryo cells, the structure of the adducts that are formed is of interest since this hydrocarbon may be activated like benz(a)anthracene through formation of vicinal diol-epoxides that are not of the bay-region type. The authors caution that it must be remembered that other unidentified metabolites react with DNA in these systems. Further studies will include efforts to identify these metabolites. In the in vivo study, the authors were unable to demonstrate the presence of hydrocarbon-deoxyribonucleoside adducts on hydrolysates of DNA obtained from mouse skin treated with DBA. This is an interesting finding, according to the authors, since it is known that DBA does become covalently bound to the DNA of mouse skin, and it does initiate tumors in mouse skin. They intend to further explore this observation (CA 21959).

Two studies concerning the effects of weakly carcinogenic polycyclic aromatic hydrocarbons on more potent polycyclic aromatic hydrocarbons were recently described. In the first (Baird et al., 1981) the effects of benzo(e)pyrene (BeP) on the metabolism of benzo(a)pyrene (BaP) were studied in hamster embryo cell cultures to determine if BeP alters the pathways of a carcinogenic hydrocarbon. The weakly carcinogenic BeP acts as a cocarcinogen when applied with BaP. The authors found that at doses equimolar to or 4-fold higher than BaP, BeP had little or no effect on the total amount of BaP metabolized, but the proportion of BaP metabolites recovered as the 9,10-and 7,8-diols was greater in the BeP-treated groups. A ten-fold excess of BeP inhibited BaP metabolism. In an experiment where BaP metabolism was measured at 6, 24, and 72 hours in the presence of BeP, the 9,10- and 7,8-diols represented a higher proportion of the BaP metabolites at all time points when compared with control groups treated with BaP alone. The authors conclude that these results demonstrate that the cocarcinogenic hydrocarbon BeP alters the metabolism of BaP with the result that the proportion metabolized to diols is increased, and the proportion metabolized to water soluble metabolites is decreased (CA 28825).

In the second study the effects of BeP and DBacA on the skin tumor initiating activities of several methyl substituted polycyclic aromatic hydrocarbons were examined (DiGiovanni et al., 1982). The differential effects of BeP on skin tumor initiation by dimethylbenzanthracene (DMBA) and BaP had suggested that the presence of a methyl substituent might play an important role in the type of response elicited under the influence of a particular modifier. The data obtained indicate that the effect of BeP or DBacA on skin tumor initiation cannot be predicted. They also indicate that weak or non-carcinogenic polycyclic aromatic hydrocarbons such as BeP and DBacA modify the response to more strongly carcinogenic polycyclic aromatic hydrocarbons by affecting the tumor initiation phase. Tumor initiation by DMBA and DBahA was consistently inhibited by both DBacA and BeP. 3-Methylcholanthrene (MCA) tumor initiation was inhibited by DBacA but not by BeP, and BaP tumor initiation was reproducibly potentiated by BeP. In these studies, BeP and DBacA were applied 5 minutes prior to the initiators. The authors also found that the time of application of BEP and DBacA, relative to initiation, was extremely critical for the type of modifying effect observed. If BeP was applied 24 hours prior to or 24 hours after initiation with DMBA or BaP, little or no effect was observed. DBacA, when applied 24 hours prior to initiation with BaP, effectively inhibited papilloma formation. In an effort to understand the differential effect of BeP on tumor initiation with DMBA or BaP, the covalent binding of these two hydrocarbons to epidermal DNA was examined under the influence of the modifier. Single topical applications of 20 or 200 nmol BeP reduced the covalent binding of ^{3}H-DMBA to 47% or 22% respectively of the control value. In contrast, single topical applications of

200 or 2000 nmol BeP had no effect on the covalent binding of ^{3}H-BaP to epidermal DNA. The authors suggest that DBacA may act as an inhibitor of arylhydrocarbon hydroxylase (AHH) activity, while both the inhibitory and potentiating effects of BeP could be explained on the basis of competition for metabolism (CA 20076).

Finally, one grant concerns interesting studies of microbial and fungal metabolism of polycyclic aromatic hydrocarbons. A recent paper from this group describes the fungal oxidation of 3-MCA (Cerniglia et al., 1982). The authors found that the filamentous fungus, Cunninghamella elegans, metabolized 3-MCA to 1-hydroxy-3-MCA, 2-hydroxy-3-MCA,1-keto-3-MCA, 2-keto-3-MCA and trans-9,10-dihydrodiols of 1-hydroxy-3-MCA. In addition, several unidentified derivatives of 1-hydroxy-3-MCA were found. The metabolic profile of 3-MCA from C. elegans was similar to those reported for the formation of these compounds by mammalian hepatic microsomes. No metabolism was found at the K region of the 3-MCA molecule. Earlier studies on the fungal metabolism of benzpyrene and benzanthracene also found no evidence of metabolism at the K region of the compounds. The regio selectivity in the metabolism of polycyclic aromatic hydrocarbons by C. elegans suggests differences in the fungal cytochrome P-450 dependent monooxygenase from the cytochrome P-450 purified from liver microsomes of rats, rabbits, and mice. Fungal studies by this group indicate that the enzymes involved appear to show almost the same relative and enantiomeric specificity as the analogous enzymes in mammals. Thus C. elegans has the potential of serving as a suitable model for the arylhydrocarbon hydroxylase of mammalian liver (CA 19078).

Aromatic Amines

Included in this group, comprising about 14% of the grants, are studies ranging from development of new synthetic methods for possible metabolites to biological studies designed to identify and characterize metabolites of carcinogenic arylamines in vivo and in vitro, in efforts to better understand the mechanism of action of this class of carcinogens. An important element in metabolic studies is the identification of metabolites which require availability of unequivocally synthesized standards for identification. One project is developing new and hopefully easier synthetic routes to N-aryl and N-vinyl hydroxylamines. Another is synthesizing substituted 2-(N-hydroxyacetamide) fluorines and N-hydroxytrans-stilbenylacetamides to be used as substrates to study bioactivation by mammalian arylhydroxamic acid-N,O-acyltransferase.

In one interesting project, the chemistry of aryl hydroxylamines and their derivatives is being studied in aqueous solution. The studies are designed so that basic information relevant to metabolic pathways may be obtained. It was observed by the Principal Investigator, that aryl hydroxylamines were highly reactive in in vitro metabolic studies in mammalian liver preparations and that the reactivity in large part did not appear to depend on the presence of active enzyme or proteinaceous material. A study of the chemistry of a representative aryl hydroxylamine, phenylhydroxylamine, (PHA) in aqueous environment in the physiologically relevant pH range was performed (Becker and Sternson, 1981). It was known that PHA is unstable in solution. It is oxidized to nitrosobenzene which then condenses to azoxybenzene. In oxygenated aqueous solution, it is also oxidized to nitrobenzene. At pH 5.8 or less, p-nitrosophenol is also formed. The kinetics of PHA disappearance and product formation was studied in phosphate buffer and in cacodylate buffer. The rate constants for PHA disappearance were approximately equal to those for nitrobenzene formation in both buffers. There were differences in product distribution for the two buffers. Data obtained suggested that rates and products were determined in different processes and that there must be one

intermediate in the reaction sequence. The authors observe that the overall mechanism is very complicated and cannot yet be defined. From these experimental data, the authors suggest that a reactive intermediate, perhaps one forming between O_2 and PHA is responsible for generating nitrobenzene and p-nitrosophenol and propose a possible reaction sequence. The authors conclude that the study has shown that, once formed, aryl hydroxylamines degrade spontaneously under aerobic conditions to form potentially carcinogenic products through intermediates that are also potentially carcinogenic. These reactions are buffer catalyzed and the occurrence of catalysis by phosphate, a physiological buffer, suggests that similar acceleration of aryl hydroxylamine oxidation may occur in vivo (CA 28782).

Biological studies include two projects studying conversion of aromatic nitrogen containing compounds to potentially toxic hydroxamic acids by marine organisms and by selected microorganisms. Others are looking at mechanisms involved in the biological activation of selected arylamines. A couple of projects involve studies of N-2-fluorenyl acetamide (2-AAF) in mammary gland. In one of the projects, a study of mammary carcinogenesis of arylamines in lactating rats and their offspring, the investigators were able to induce cytochrome P-450 with 3-MCA and β-naphthoflavone (β-NP) in the mammary gland microsomes of the lactating rats (Ritter and Malejka-Giganti, 1982). Earlier attempts to detect this enzyme on rat mammary gland microsomes had been unsuccessful due to low level of enzyme and spectral interference by contaminants. Improved preparation procedures and a more sensitive spectrophotometer eliminated this problem. The authors found that the hydroxylation of BaP and 2-AAF increased markedly in the mammary gland and liver microsomes of lactating rats after pretreatment with 3-MCA or β-NF. The metabolic assays indicated that mammary gland microsomes from 3-MCA treated rats and especially those of β-NP treated lactating rats had a substantial capacity for hydroxylation of the carcinogens. Only the overall conversion of BaP and 2-AAF to hydroxymetabolites were determined, thus it is not known if any of the hydroxy products formed are carcinogenic. The authors state that it is of particular interest to answer this question in the case of 2-AAF, since the conversion of this compound to N-hydroxy-2-AAF determines its carcinogenicity for the rat mammary gland. The authors have also found that mammary gland microsomes of lactating rats, chemically treated with 2-AAF have a low capacity for N-hydroxylation of this compound (CA 28000).

Alkylating Agents

Almost all of the grants in this group are concerned with nitroso compounds. Most are studying mechanisms of action either in vivo or in vitro. Two projects are concerned with the chemistry of nitrosamines and determining the relevance of the observed chemical reactivity to the biological system. Two groups are pursuing studies to determine whether N-nitroso compounds can be formed in man. Most of the remainder are studying metabolism and mechanisms of action. Two examples of the types of studies supported in this area are discussed below.

In the first, the extent of α-hydroxylation in the metabolism of two nitrosamines, dimethylnitrosamine (DMN) and N-nitroso-N-methylaniline (NMA) by rat liver S9 fraction was determined (Kroeger-Koepke et al., 1981). Evolution of $^{15}N_2$ labeled molecular nitrogen was used to measure the extent of α-hydroxylation during rat liver homogenate metabolism of doubly ^{15}N-labeled DMN and NMA. These measurements were correlated with the extent of total metabolism as measured by the disappearance of the nitrosamines and by the formation of formaldehyde. In the course of the experiments, the authors found that formaldehyde formation, as usually measured, underestimated the extent of nitrosamine metabolism. One of the problems encountered was that semicarbazide, which is frequently used to suppress

formaldehyde loss, is a substrate for the hepatic enzymes and gives rise to a small amount of formaldehyde and a significant amount of molecular nitrogen. Thus the authors used a much less precise but more accurate estimate of direct nitrosamine loss. Their results indicate that the α-hydroxylation pathway accounts for about 34% of the reduction of DMN and about 19% of the reaction of NMA when uninduced Fischer 344 rat liver S9 is used for metabolism. The results found in these experiments are roughly halfway between those obtained by earlier workers. The authors note that considerable methodologic differences exist between their experiments and the others and that better controls existed in theirs. They suggest that their results reflect the true picture more accurately and conclude that the rest of the metabolism of these nitrosamines must occur by different pathways (CA 23451).

Another group has been continuing its studies on the metabolism and mechanism of action of N-nitrosodipropylamine (NDPA) and its β-oxidized derivatives N-nitroso-2-hydroxypropylpropylamine (NHPPA) and N-nitroso-2-oxopropylpropylamine (NOPPA) (Leung and Archer, 1981). Urinary metabolites of these compounds were determined in Sprague-Dawley rats. The major excretion product in each case was found to be the β-glucuronide of NHPPA. Exhaustive hydrolysis of 24-hour urine samples with β-glucuronidase yielded an amount of NHPPA corresponding to approximately 5% of the NDPA administered. During the first 24 hours following administration of NHPPA, approximately 80% of the administered dose appeared in the urine, mostly in the form of conjugated NHPPA. The 24-hour urine sample from rats treated with NOPPA yielded approximately 50% of the administered dose as conjugated NHPPA. Generally only low levels of the unchanged nitrosamines or their unconjugated metabolites were detected. Nor were the authors able to detect N-nitrosomethylpropylamine or DMN in the urine of any of the animals. N-Nitrosomethyl-propylamine has been proposed as the hypothetical methylating agent derived from metabolism of NDPA by some investigators. The results confirm earlier predictions that NDPA is metabolized in part by β-oxidation. Urinary excretion of large amounts of conjugated NHPPA following administration of NDPA also confirms the authors' in vitro experiments in which they showed that NOPPA was readily reduced to NHPPA by both soluble and microsomal enzymes from rat liver. The mechanism of methylation of DNA by NDPA in the rat still remains unclear (CA 26651).

Other Agents

This group is the largest and most varied comprising about 44% of the grants in this program. Included are projects studying compounds that do not belong in the other three groups, projects investigating compounds from more than one group, foreign body tumorigenesis, fiber tumorigenesis, metal carcinogenesis and synthesis of compounds not belonging to the three previously described groups. Two groups are doing complementary studies on malondialdehyde, using purified malondialdehyde. One is studying the mutagenicity and the other is performing an in vivo carcinogenicity study in an effort to unequivocally determine whether or not malondialdehyde is a carcinogen or a mutagen. Foreign body tumorigenesis is the subject of another study. Three projects are addressing various aspects of fiber carcinogenesis. Another is attempting to elucidate the mechanism of action of carcinogenic nickel compounds. Studies on metabolism and mechanism of action of nitrofurans, chloroolefin compounds, and nitroamidazole compounds are being supported. One group is studying the effects of carbon tetrachloride and polychlorinated biphenyls on adrenocortical function, another is studying the genetic basis for the variation in aflatoxin B_1 metabolism observed between different strains of mice. A variety of synthesis studies are represented as well as molecular-structural determinations of selected carcinogens and carcinogen-nucleoside adducts using x-ray

crystallography. The above is a very brief non-exhaustive overview of the studies included in the group. A few specific examples are discussed below.

In a study attempting to relate structure to mutagenic activity, the mutagenicity of 12 cycloaliphatic epoxides was investigated using the Ames Salmonella assay without the addition of liver homogenate fractions (Frantz and Sinsheimer, 1981). In general, these dicyclic oxiranes possess a cis-1,2-disubstituted stereochemistry about the epoxide ring. Results obtained confirmed the authors' earlier observations of weak mutagenic response at high dose levels for their compounds. They found that while mutagenicity decreased with expanding ring size, inhibition of bacterial growth increased with increasing ring size. They also found that toxicity accompanying the required high doses for demonstration of mutagenicity for these compounds, prevented the establishment of meaningful dose-response ranges for the remaining epoxides listed. While it is well established that cyclic aliphatic epoxides resulting from metabolism of BaP are highly mutagenic, little had been reported concerning the mutagenicity of less complex cycloaliphatic epoxides. Earlier work by this group had indicated that among the limited numbers of 1,2 disubstituted epoxides tested, only structures having a cis configuration about the oxirane ring were mutagenic, hence, this study to investigate the oxirane series (CA 25770).

A recent publication from a group studying the effects of exposure to carcinogens on blastocytes described the effects of exposure of mouse blastocytes to N-methylnitrosourea (NMU) and MCA on ability to implant to surrogate mothers and ability to incorporate radiolabeled precursors of macromolecular synthesis (Iannaccone et al., 1982). The results indicate that the direct acting carcinogen, NMU, can alter the incorporation of precursors of macromolecular synthesis in blastocytes that are viable following exposure. Incorporation of ^{3}H-thymidine, ^{3}H-uridine and ^{3}H-leucine were measured either immediately after exposure and after 18 hours of culture. The reduction in incorporation of uridine and thymidine immediately following exposure was of much lower magnitude than that observed 18 hours following exposure. The effect in leucine incorporation was seen immediately after exposure. Removal of the zonae pellucidae prior to NMU exposure had no significant effect on the magnitude of the reduction in thymidine incorporation determined following exposure. The authors state that this indicates that the zonae pellucidae does not provide a protective barrier against NMU. No demonstrable effect was observed on the incorporation of leucine determined immediately following a 1-hour exposure or on uridine and thymidine incorporation determined after 18 hours of exposure to MCA. MCA requires metabolic activation to be effective as a carcinogen or as a mutagen. A 50% reduction in normal implantation rate was observed as a result of exposure to 100 mg. of NMU per ml., but a 96% decrease in birth rate was observed at that concentration. The authors say that this may indicate that the viable blastocytes have deficits which are expressed later in gestational development. They conclude by saying that their results suggest there is a potential effect of exposure to NMU in early preimplantation embryos. Their experimental system of in vitro exposure of blastocytes to carcinogens offers many advantages. It allows study of the compound without the modifying effect of maternal tissues and can be used to evaluate the relative importance of various tissues on activation of these compounds by reconstituting systems in vitro. Toxic concentrations of such compounds may be related to concentrations affecting implantation rate and birth rate with this approach (CA 29675).

Two groups in a collaborative effort recently published a study showing inhibition of BaP-phenol production by ethanol in perfused rat liver (Reinke et al., 1982).

The authors developed a method to monitor BaP-phenol production in perfused rat liver. Phenols were detected in liver perfusate within 3 minutes after initiation of BaP infusion into the liver of a 3-MCA-treated rat. The release of phenols into the perfusate increased steadily until the maximum rate of approximately 45 nmol per g. liver per hour was reached after 25 to 30 minutes. Approximately 80% of the phenols were conjugated. When the infusion was discontinued, the rate of phenol release decreased rapidly for 8 to 10 minutes before stabilizing at a rate of 13 to 15 nmol per g. per hour during the remainder of the assay period. In liver from control animals, phenols were released soon after the addition of BaP, however maximal rates of only 8 to 9 nmol per g. per hour were observed. The rate of phenol release did not diminish when the infusion of BaP was discontinued. Fasting had no effect on phenol production in livers from fasted 3-MCA treated rats. However, when BaP was infused into liver of similarly treated rats in the presence of 20 mM ethanol, the rate of phenol release was inhibited by approximately 50%. Interestingly, ethanol at concentrations up to 100 mM did not affect the production of benzo(a)pyrene phenols by isolated microsomes from 3-MCA-treated rats.

In analyzing the kinetics of phenol release, the authors suggest that the data indicate that hydroxylation, enzymatic conjugation reactions, and release of phenols across cell membrane occur rapidly. The rapid decline in the rate of phenol release when BaP was removed from the extracellular medium suggest that BaP may not penetrate rapidly into the cells. The slow elution of phenol after BaP infusion was terminated is probably due to a slow diffusion of stored BaP to the site of mixed function oxidation rather than a gradual release of preformed phenols. The effect of ethanol on the release of phenols was compared with the effect of ethanol on metabolism of other carcinogens. Evidence indicates that ethanol does not inhibit arylhydrocarbon hydroxylase activity. The authors suggest that ethanol inhibits a metabolic pathway which generates the NADPH required for BaP activation in livers from fasted rats. The exact nature of these interactions as well as possible effects of ethanol on other BaP metabolites remain to be elucidated (CA 20876, CA 23080).

A group that has been studying mechanisms of action of a variety of carcinogens recently published a paper on the metabolism and nucleic acid binding of 7-fluoro-2-acetamidofluorene (7 fluoro-AAF) in rats (Scribner et al., 1982). In these experiments, Sprague Dawley and Fischer rats were fed 7-fluoro-AAF. One important observation was the presence of 7-hydroxy-AAF as a metabolite of 7-fluoro-AAF. While this had been shown previously, the amount of 7-hydroxy-AAF isolated in these experiments was comparable to that found when AAF itself is administered. Thus, the authors observe that it seems questionable to infer that alterations in biological activity of compounds induced by fluorine substitution are due to blockage of metabolism at the substituted position. Another interesting observation was that total binding of AAF to both DNA and RNA in female Fischer rats was several-fold higher than in female Sprague-Dawley rats; although the susceptibility of the females in the two strains appears comparable. The authors observe that this is only another example of the failure to establish a broad correlation between carcinogenicity of compounds and the degree to which they attack nucleic acids in their target tissue. The binding of 7-fluoro-AAF to nucleic acids of female Sprague-Dawley rats is higher than the binding of AAF, but not by an amount corresponding to the marked difference in tumor response to the two compounds. Comparison of the binding levels of the common substrate 7-fluoro-AAF to rats fed either AAF or 7-fluoro-AAF for 4 weeks before the injection of 7-fluoro-AAF suggests that the quite different effects of the two compounds on the activation system for peaks II and III, when added to the initial differences in binding would indeed

account for the observed difference in carcinogenicity between the two compounds. The adduct distribution was also interesting. The proportion of acetylated adduct was higher in RNA than in DNA, and it is lacking in the DNA of Sprague-Dawley females altogether.

The authors found 8-AAF-guanine or 8-(7-fluoro-AAF)-guanine adducts in the nucleic acids of Fischer females. They observed that since the two strains appear to have comparable susceptibilities to hepatic carcinogenesis by AAF, it would seem that the appearance of this particular adduct is not essential for hepatocarcinogenesis in these animals. In Sprague-Dawley males, the ability to generate this adduct disappears only after 4 weeks of feeding either AAF or 7-fluoro-AAF. The authors suggest that the activation process, rather than the repair process has been altered. After discussing at length the metabolic similarities and differences, the authors come to the following conclusions. "Previous studies have shown depression of acetylated adducts as a result of prolonged feeding of AAF, but this appears to be the first demonstration that the same feeding has effects on levels of diacetylated adducts which (1) are dependent on the aryl group of the aromatic amide, (2) may be opposite in males and females, and (3) are closely correlated with the relative hepatocarcinogenicities of the compounds fed." (CA 23712).

Molecular Carcinogenesis

Research grants in the Molecular Carcinogenesis Program area support studies focused on the characterization of carcinogen-macromolecule interactions; changes in biological macromolecules, cell structure, ultrastructure, and functions as a result of carcinogen or cocarcinogen exposure; the identification of biochemical and molecular markers and properties of cells transformed by carcinogens; the genetic and other mechanisms of cell transformation; the development of carcinogenicity/-mutagenicity testing procedures; the mechanisms of carcinogen-induced mutagenesis and genetic damage; enzymes associated with carcinogenesis induced by chemical and physical carcinogens; and the role of DNA repair in carcinogenesis. Expanded descriptions of individual subject areas along with examples of research accomplishments are provided below.

Carcinogen-Macromolecule Interactions

The projects in this subject area include studies on the identification and quantitation of adducts to DNA mainly but also to RNA and protein. Carcinogen-DNA adducts are characterized by such techniques as high pressure liquid chromatography, absorption and fluorescence spectroscopy, and nuclear magnetic resonance spectroscopy to give information on the preferred sites of binding of the carcinogen, the structures of carcinogen-adducts, the conformation of covalent adducts, and the conformational changes induced in nucleic acids as a result of carcinogen binding. In the majority of the studies, the polycyclic aromatic hydrocarbon carcinogens with benzo(a)pyrene and its metabolites as model compounds are used. This is due to the extensive knowledge on the metabolic pathways and the identity of the proximate and ultimate reactive forms of this carcinogen. Other carcinogens widely used in these studies include various nitrosamines, N-2-acetylaminofluorene and its derivatives and aflatoxin B_1. Miscellaneous compounds used include azodyes, diethylstilbestrol, other alkylating agents such as the nitrosoureas, and coumarins.

The polycyclic aromatic hydrocarbon carcinogens have been shown to be metabolized in vivo to a number of highly reactive epoxide derivatives with the ultimate

carcinogenic form of benzo(a)pyrene (BaP) which is found bound to cellular macromolecules being a diol epoxide. There is a preferred formation of certain forms of stereoisomeric BaP diol epoxides in cells and binding to DNA occurs with high stereoselectivity with the most reactive BaP diol epoxide being the 7R enantiomer of 7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene (BaPDE). Geacintov et al., 1982, have studied the effects of hydroxyl groups in the substituted benzo ring and the position of this benzo ring on the pyrene nucleus in two structurally related molecules BaP and benzo(e)pyrene (BeP) on the physico-chemical properties and conformations of the DNA adducts formed. The specific compounds used were BaPDE, the structurally similar epoxide 9,10-epoxy-7,8,9,10-tetrahydro-BaP (BaPE) which lacks OH groups in the 7 and 8 positions and 9,10-epoxy-9,10,11,12-tetrahydro-BeP (BePE) which is analogous to BaPE except for the position of the substituted benzo ring on the pyrene nucleus. The conformation of the covalent adducts derived from the reactions of the compounds with calf thymus DNA in aqueous buffer systems were investigated and compared by means of absorption and fluorescence spectroscopy and electric linear dichroism techniques. Two types of conformations are recognized, one involving the intercalation of the compound between the DNA bases, and the second involving the external location of the compound probably in one of the grooves of the DNA molecule. The BaPDE-DNA complex was found to display predominantly an external binding conformation in which i^+ is bound primarily to the N^2 atom of guanine in the minor groove of DNA. The BaPE- and BePE-DNA complexes displayed both an intercalative and an external binding conformation with the intercalative conformation predominating. The lack of hydroxyl groups in BaPE and BePE results in a loss in stereospecificity in adduct formation. The presence of the 7 and 8 hydroxyl groups in BaPDE appear to reduce the probability of formation of intercalation-type covalent adducts by exerting steric hindrance effects and appear to be, at least in part, responsible for the enantiomeric stereospecificity in the covalent reaction between BaPDE and DNA. BaPDE, however, has been shown to bind to DNA in a non-covalent intercalation complex (Geacintov et al., 1981). The major reaction pathway at this intercalation site is the hydrolysis of BPDE to its nonreactive tetraol. Studies such as these aid in the understanding of the biological activity of these compounds. (CA 20851).

Nitrosamines, such as dimethylnitrosamine, have been shown to be activated by oxidative metabolism to form an unstable hydroxylated derivative which decomposes to yield a highly reactive alkylating agent. Alkylation products in the reaction of nitrosamines with DNA have been identified with the predominant species being 7-alkyl-guanine, O^6-alkylguanine, 3-alkyladenine and 7-alkyladenine. There is substantial evidence suggesting that the formation of O^6-alkylguanine in DNA and its persistence through cell division are important in tumor initiation by nitrosamines. Also, since metabolism is obligatory for the carcinogenic action of nitrosamines and in light of the unstability of the metabolic product, it is thought that only those tissues possessing the capacity to activate nitrosamines are at risk for tumor development. Not all cells within a tissue need to possess a metabolic capacity, however. Umbenhauer and Pegg, 1981, have shown that freshly isolated rat hepatocytes were able to metabolize μM concentrations of dimethylnitrosamine to a methylating agent which methylated hepatocytes DNA completely within 2 hours. Where extracellular calf thymus DNA was added to the incubated hepatocytes, the extracellular DNA also became methylated showing that the methylating species formed by the hepatocytes was sufficiently stable to pass out of the cells in substantial amounts. These results support the possibility that alkylation of liver cells would not be confined to those cells metabolizing dimethylnitrosamine but could be extended to those cells which are in close proximity to the activating cells. In other experiments, dose levels of 1 μg/kg to 10 mg/kg of dimethylnitrosamine were

administered to rats either orally or by i.v. injection (Pegg and Perry, 1981). The amount of 7-methylguanine and O^6-methylguanine was measured in DNA from liver and kidney. The results confirm the rapidity of metabolism of low doses of dimethylnitrosamine and that the alkylation of DNA is complete within a few minutes of exposure. In comparing levels of adducts in liver and kidney DNA, the results suggest that at the lower doses, the dimethylnitrosamine absorbed from the intestine into the portal blood supply is metabolized sufficiently rapidly by the liver that little is available for metabolism in the kidney. The kidney was shown to be less able than liver to remove O^6-methylguanine from DNA at either high or low dose levels. Thus, the kidney may be more at risk for tumor initiation. This has been demonstrated by this laboratory and others since single large doses of dimethylnitrosamine produce exclusively kidney tumors in the rat while chronic exposure in the diet produces liver tumors but no kidney tumors. These results indicate the importance of the route of administration in determining the interaction of dimethylnitrosamine with various organs and suggest that the formation of alkylated bases in extrahepatic tissues may depend on the dose and on the rate of absorption of the carcinogen. The very high activity of the liver in removing O^6-methylguanine from DNA may provide a protective mechanism against tumor initiation. (CA 18137).

Studies on the antibiotic streptozotocin were conducted by Bennett and Pegg, 1981. This antibiotic is widely used for induction of diabetes in experimental animals and for the treatment of pancreatic neoplasms. Structurally, streptozotocin is a 2-deoxyglucose derivative of the carcinogen N-methyl-N-nitrosourea. This compound was shown to be a potent methylating agent reacting with DNA in vitro to form methylated purines similar in extent and relative proportions to that produced by N-methyl-N-nitrosourea. The DNA methylation products, 7-methylguanine, O^6-methylguanine, 3-methyladenine, and 7-methyladenine, were formed in liver, kidney, intestine, and pancreas of rats administered streptozotocin. N-methyl-N-nitrosourea produced approximately equal amounts of methylation in DNA of liver, brain, and kidney. Streptozotocin, however, caused virtually no methylation in brain DNA, but both liver and kidney DNA were alkylated to a greater extent than with N-methyl-N-nitrosourea. The methylation of kidney DNA may account for the ability of streptozotocin to induce renal tumors. Also, the significant methylation of pancreatic DNA produced by streptozotocin, if it is concentrated in the β-cells, may account for their destruction. If rats are pretreated with nicotinamide (100 mg/kg), the extent of methylation of pancreatic DNA was reduced by about 40%, but the extent of methylation of liver and kidney DNA was not affected. The reduction in alkylation could then permit the survival of a greater fraction of the methylated cells which could go on to develop tumors.

Exciting results, concerning a new unusual form of DNA, have recently been published. This new form of DNA, called Z-DNA, has a left-handed helical conformation as opposed to the right-handed conformation of the conventional B-DNA. This was discovered by accident by Alexander Rich and his associates at the Massachusetts Institute of Technology while doing x-ray diffraction studies on crystals of self-complementary DNA tetramers consisting mostly of deoxyguanine and deoxycytosine. In left-handed Z-DNA, the Watson-Crick base pairs are found on the outside of the molecule, and the guanine residues are in the <u>syn</u> conformation. Also, the molecule has only one deep groove instead of the two grooves in the right-handed B-DNA form. The B-DNA to Z-DNA transition was shown to occur at high salt concentrations. When poly(dG-dC)•poly(dG-dC) was methylated at the N-7 position of guanine by the alkylating agent dimethyl sulfate, the B- to Z-DNA transition was facilitated (Möller et al., 1981). Increasing levels of methylation decreased the

salt concentration required to convert the polymer to the Z-form. When 100% of the guanine residues are methylated, the modified polymer is fully converted to Z-DNA in a physiological salt solution. The presence of methylated bases markedly accelerates the speed of the conversion of B-DNA to Z-DNA. The laboratory of Gary Felsenfeld at NIH has also observed the B- to Z-DNA transition of poly(dG-dC)•poly(dG-dC) copolymers at physiological salt concentrations, but with methylation of cytosine residues at the C-5 position. This effect is somewhat greater than that observed for N-7 guanine methylation. Since many carcinogens and mutagens alkylate DNA, its effect may be to modify the B to Z conformations in the favor of Z-DNA. As it is known that the methylation of cytosine-guanosine sequences of eukaryotic DNA is tied to gene expression, the genes being inactive if the sequences are methylated, it is suggested that Z-DNA is involved in gene regulation.

Considerable interest in the molecular details of the covalent binding of activated derivatives of chemical carcinogens exists, since these reactions may constitute critical events in the process of carcinogenesis. The reaction of N-acetoxy-N-2-acetylaminofluorene (N-AcO-AAF) at the C(8) position of deoxyguanosine residues in native B-DNA presents certain steric problems due to the anti conformation of these residues. There is evidence that the reaction of N-AcO-AAF with DNA is associated with a distortion of the DNA helix, termed base displacement, in which the AAF-modified deoxyguanosine residue assumes the syn conformation. Since the deoxyguanosine residues are already in the syn conformation in Z-DNA, and the C(8) position is exposed on the outer surface of the DNA molecule, experiments were conducted to examine DNA modified by treatment with N-AcO-AAF (Santella et al., 1981a). Poly(dG-dC)•poly(dG-dC), which can exist in the Z form, and poly(dG)•poly(dC), which cannot be converted to the Z form, were used. Conformations were examined by circular dichroism and susceptibility to nuclease S1 digestion which degrades single strand regions. The data show that AAF modification of poly(dG-dC)•poly(dG-dC) to a 20-30% level favors the transition of the polymer from the B to the Z conformation. Poly(dG)•poly(dC) did not show any large conformational changes with high AAF modification. Poly(dG-dC)•poly(dG-dC) modified by AAF to an extent of 28% was almost completely resistant to nuclease S1 digestion. However, both poly(dG)•poly(dC) and DNA modified to similar levels by AAF were highly susceptible to nuclease S1 digestion, showing that AAF modification resulted in the disruption of the double-stranded structure. The reactions were also studied by determining reactivity with anti cytidine antibodies and by computer analysis using minimized potential energy calculations (Santella et al., 1981b). The results obtained were consistent with the induction of the Z-DNA conformation in AAF-modified poly(dG-dC)•poly(dG-dC). Using minimized potential energy calculations on the dCpdG-AAF dimer as a model for the modified polymer, it was shown that the proposed Z-DNA conformation is energetically stable. A model for an AAF modified tetramer, dGpdCpdGpdC, is proposed in which the AAF is external to the Z-DNA duplex. The results suggest that if, in vivo, a region of alternating guanosine-cytidine sequence were modified by AAF, this would favor its transition from the B to the Z conformation. This marked change in nucleic acid conformation could then induce important functional changes in that region of the cellular genome. (CA 29753, CA 21111, CA 28038).

The relevance of Z-DNA to biological systems has been demonstrated by Dr. Alexander Rich and his colleagues. Using antibodies raised in rabbits which are specific to the Z-DNA conformation, the presence of Z-DNA has been demonstrated in the interband regions of Drosophila polytene chromosomes. This study was the first identification of the Z-DNA conformation in material of biological origin. The role of Z-DNA in the control of gene expression and in carcinogenesis has yet to be

elucidated, but the results obtained so far suggest that it may be a significant one.

Changes in Cellular Macromolecules and in Cell Functions

The types of research activities in this subject area include studies on changes in the composition and amounts of various proteins and small molecules and changes in the pattern of DNA methylation in cells induced by carcinogens to the preneoplastic or neoplastic state. The proteins studied include nonhistone chromosomal proteins, membrane glycoproteins, and the enzymes involved in DNA synthesis or RNA transcription such as the DNA polymerases, DNA ligases, DNA methylases, and RNA polymerases. In addition, there are several studies in which the focus is on the effects of carcinogen exposure on DNA replication, RNA transcription, or protein translation in cells. The rat or mouse liver is the system most widely used. Cultured fibroblasts of mouse or human origin are also used in several of the studies. The carcinogens employed in these studies include aflatoxin B_1, N-2-acetylaminofluorene (AAF), the azodye 3'-methyl-4-dimethylaminoazobenzene, carcinogenic metals, polycyclic aromatic hydrocarbons, and nitrosamines.

The role of methylation of nuclear DNA in eukaryotic cells is not yet known. The site of methylation has been shown to be almost exclusively at the 5' position of cytosine, and the cytosine residue is usually contained in the sequence CpG. This methylation involves a postreplication DNA modification and is proposed to function in many ways, including control of transcription, maintenance of chromosome structure, repair of DNA, establishment of preferred sites for mutation, oncogenic transformation and, in certain systems, protection of DNA against enzymatic degradation. There is evidence demonstrating a correlation between gene expression and hypomethylation of DNA. This relationship has stimulated interest in determining the levels of DNA methylation of malignant tissues. The 5-methylcytosine (m^5C) content of DNA in premalignant nodules and primary hepatocellular carcinomas induced in rats by exposure to AAF or diethylnitrosamine was found to be less than that in normal livers or in regenerating livers. In order to determine if this reduced m^5C content of tumor DNA is a result of a deficiency in DNA methylase or an alteration in its specificity, the enzyme in a transplantable hepatocellular carcinoma cell line was studied (Walker and Becker, 1981). Its activity was compared to that of a DNA methylase purified 200-fold from nuclei of regenerating rat liver. The results showed that the larger number of unmethylated sites in DNA from transplantable hepatocellular carcinoma cells was not due to a deficiency in DNA methylase, since the level of methylase activity of nuclear extracts from hepatocellular carcinoma cells was 2.7 times that of normal liver and 1.5 times that of regenerating liver. The methylases from the three sources had similar rates of reaction with different DNA substrates thus showing that the DNA methylase from hepatocellular carcinomas did not have an altered substrate specificity. Similar results were obtained when normal and malignant mouse liver tissue was examined (Lapeyre et al., 1981). In this case, DNA from spontaneously arising tumors and hepatocellular carcinomas arising in three strains of mice exposed to AAF, chlordane or 3'-methyl-4-dimethylaminoazobenzene were compared with DNA from normal mouse liver. Of several possible explanations for the decreased methylation of tumor DNA, the most likely one appears to involve the nature of chromatin composition or configuration or both. The differences in tumor nonhistone chromosomal protein complement when compared to that of normal cells could be involved. (CA 20657).

An investigation of the nature and role of nonhistone chromosomal protein-DNA complexes in cellular differentiation and hepatocarcinogenesis by chemicals is being

conducted in the laboratory of Dr. Hnilica. Cell and tissue specific markers consisting of antigenic nonhistone chromosomal DNA-protein complexes are used. The immunospecificity of these complexes were shown to change with tumorigenesis or during tissue differentiation. Protein components partially responsible for the immunologic activity of the antisera have been separated. Antisera to three partially purified Novikoff hepatoma protein antigens were used to characterize their tissue specificity and subcellular distribution (Schmidt et al., 1981). The three proteins have molecular weights of about 39,000, 49,000, and 56,000 and are designated p39, p49, and p56. The p56 protein was found to be present in normal rat liver, 24 hour regenerating rat liver, fetal rat liver, or kidney, although in much smaller amounts as found in Novikoff hepatoma. The p49 and p39 antigens were specific for Novikoff hepatoma. All three protein antigens were found to be present in the cytoplasmic fractions as well as in isolated chromatin. Further characterization of the three antigens has established their identity as cytokeratins (Schmidt et al., 1982). Using two dimensional gel electrophoresis, each of the three proteins was found to exist in several isoelectric forms, all characteristic of the keratins. The p39 and p56 antigens had amino acid compositions compatible with this family of proteins. The antigens were localized by immunofluorescence microscopy on elaborate filament arrays characteristic of the cytokeratins. The immunologic specificity for at least the p39 antigen suggests that this polypeptide may offer distinct possibilities as a probe for neoplasia. (CA 26412).

Other key functions of cells, such as DNA, RNA, and protein synthesis, have been shown to be affected by carcinogen exposure. The mycotoxin aflatoxin B_1 (AFB_1) has been shown to have profound affects on the synthesis of macromolecules in rat hepatocytes. The nature of transcription and translation processes during early stages of AFB_1 carcinogenesis has been studied using hepatocytes isolated following the administration of a single 6 mg/kg dose of AFB_1 to rats. (Emeh et al., 1981). The results show that within about 3 hours after AFB_1 administration, both the heterogeneous nuclear RNA transcription and cytoplasmic translation are inhibited by greater than 80% and that these processes are rapidly restored in about 24 to 30 hours. The results obtained also suggest that some classes of transcription and translation products may be affected preferentially during AFB_1 carcinogenesis. In addition to the nuclear compartment, the synthesis of macromolecules takes place in the mitochondria of cells. A number of studies have shown that the mitochondrial content, structure, and function are altered in a variety of tumor cells. Mitochondria from tumor cells have been shown to have altered ultrastructural organization, membrane composition, abnormal ion transport, and altered biochemical properties. Thus mitochondria may be one of the cellular targets of attack by carcinogens. Mitochondria were recently shown to possess a cytochrome P450-type of monooxygenase system capable of activating AFB_1. The administration of AFB_1 (6 mg/kg) to rats results in the covalent binding of AFB_1 to liver mitochondrial DNA at levels three to four times higher than to nuclear DNA. (Niranjan et al., 1982). The AFB_1 adduct concentration in mitochondria remained unchanged, even after 24 hours, suggesting a lack of excision repair in mitochondria. Similarly, RNA and protein synthesis in mitochondria remain inhibited up to 24 hours suggesting that AFB_1 exposure exerts long-term effects on the mitochondrial genetic system. Although the precise role of mitochondrial genes in the carcinogenic process remains unknown, it is suggested that altered mitochondrial biosynthetic processes affecting mitochondrial oxidative function and varied metabolic activities of the cell may directly or indirectly contribute to the neoplastic outcome. (CA 22762).

In contrast to the effect of AFB_1, polycyclic aromatic hydrocarbons such as 3-methylcholanthrene (3-MC) have been shown to stimulate RNA synthesis in tissues and cells administered this carcinogen. Its effect as an inducer of the mixed function oxidase system is well-known. Studies were undertaken to further elucidate the effects of the mixed function oxidase inducing agents, 3-MC and phenobarbital, at the transcriptional level (Liberator and Bresnick, 1981a). Aggregate RNA polymerase activity as assayed in whole liver nuclei isolated from rats treated in vivo with 3-MC was stimulated by 33% over control activity. RNA polymerase I activity was shown to be maximally increased by 70% at about 16 hours post 3-MC administration while RNA polymerase II activity was stimulated by 33%. The kinetics of stimulation differed in that RNA polymerase I activity increased earlier and peaked later. Phenobarbital was shown to have no effect on the activity of hepatic RNA polymerases. Partial purification of the RNA polymerases allowed for a comparison of the treated and control activities using an exogenous template. While no qualitative difference was evident, the RNA polymerases I and II isolated from 3-MC-treated rats demonstrated more activity than control enzymes. This indicates an effect of the polycyclic hydrocarbon 3-MC at the level of the enzyme. In a further characterization of the effects of 3-MC administration on hepatic RNA polymerases I and II, studies were conducted which were designed to distinguish between three possible explanations for the increase in RNA polymerase activities (Liberator and Bresnick, 1981b). The three general mechanisms proposed involve: (1) an increase in the net number of enzyme molecules; (2) the interconversion between "free" and "template-engaged" enzyme pools; or (3) the manipulation of RNA polymerase activities by some regulatory component. The results from this study have provided evidence against the first two possibilities. During the purification of RNA polymerase I, the presence of a low molecular weight stimulatory component was demonstrated. Further work is needed, however, to clarify whether a stimulatory component is indeed present or whether an alternate explanation of the data is correct. (CA 20711).

Markers and Properties of Transformed Cells

Research included in this subject area involves studies on various growth and functional properties of initiated cells, preneoplastic cells, and fully transformed cells, and the identification of biochemical and molecular markers for distinguishing these altered cell types from normal cells. The development of most cancers is believed to involve a multistep process in which cells progress from normal to initiated, preneoplastic, and premalignant stages to the end point of malignant neoplasia. A detailed analysis of the sequence of relevant biochemical and biological alterations associated with the development of chemically-induced carcinogenesis is needed in order to characterize cells at each stage. To achieve this purpose, a variety of model systems of chemical carcinogenesis are being used or are being developed. The predominant model system which is being used by several investigators involves the induction of hepatocarcinogenesis in rats by a variety of chemicals and employing various treatment regimens depending on the type of study being done. Either a chronic or intermittent carcinogen exposure regimen can be used. Other treatment regimens start with a single exposure to a carcinogen followed by applying a cell proliferative stimulus and a treatment to apply a selective pressure for the initiated cell. The sequential appearance of focal and nodular changes in hepatocytes in the development of hepatocellular carcinoma can be observed and analyzed. Support for studies on other model systems is limited to a single or very few laboratories for each type of system. For example, one laboratory is developing models of renal carcinogenesis in which adenocarcinomas or mesenchymal tumors are selectively induced after a single dose of dimethylnitrosamine. Cell culture models of renal carcinogenesis are also being

developed to allow correlation of in vitro events with those known to occur in vivo. A mammary gland organ culture system is being developed in another laboratory. The stages of oral carcinogenesis are being sequentially analyzed using hamster buccal pouch epithelium as the experimental system. The analysis of the development of respiratory carcinogenesis is being conducted using a rat tracheal implant/short-term organ culture/cell culture experimental system. The in vitro transformation of cells in culture occupies the focus of several other research groups. Human fibroblasts and epithelial cells, rat hepatocytes, and rat urothelial cells are being used in those studies. Altered growth properties of transformed cells allows their selective proliferation in these systems. One study is concerned with the role of liver necrosis and the role of calcium ions in necrosis in the development and expression of hepatocellular carcinoma. Several biochemical markers have been used to identify transformed, preneoplastic and neoplastic cells. The acquisition of gamma-glutamyltranspeptidase (GGT) activity and the loss of glucose-6-phosphatase and ATPase activities are the most common markers used for the histochemical identification of carcinogen-altered cells in liver or in other organ sites. For cells in culture properties such as altered morphology or the ability to grow in soft agar (anchorage independent growth) are the most common measures of cell transformation. Other potentially useful markers and properties are being evaluated for potential use.

The laboratory of Dr. Emmanuel Farber at the University of Toronto has provided results which have had seminal influence in establishing working models and hypotheses for aspects of epithelial carcinogenesis. The liver model system of carcinogenesis may serve as a paradigm for other epithelial tissues. The model developed by this group involves the induction by an initiating dose of a carcinogen of altered hepatocytes resistent to the inhibitory effects of AAF on cell proliferation. Such resistant hepatocytes are selectively stimulated to proliferate rapidly to produce nodules with a selective pressure such as a short exposure to AAF in combination with a stimulus for hepatocyte proliferation such as a partial hepatectomy or CCl_4 administration. In one study, experiments were done to assess the role of liver necrosis in the induction of early steps in liver carcinogenesis (Ying et al., 1981). A necrogenic dose of diethylnitrosamine was given to rats and the appearance of foci of resistant hepatocytes that stain for GGT was measured. The number of enzyme-altered foci was decreased by up to 62% by post-treatment with diethyldithiocarbamate, a derivative of disulfiram which was shown earlier to prevent liver cell necrosis without inhibiting at least some of the known interactions of nitrosamines with cellular nucleic acids. This post-treatment also decreased the cumulative labeling index of hepatocytes by 78% demonstrating the reduction in regenerative hepatocyte proliferation. The effects of post-treatment with diethyldithiocarbamate could be reversed by performing a partial hepatectomy up to 68 hours after the post-treatment. Non-necrogenic doses of diethylnitrosamine or dimethylnitrosamine did not induce foci of resistant cells, but did so when coupled with a cell proliferation stimulus. The results of this study clearly establish an important role for liver cell necrosis in the production of early steps in liver carcinogenesis in this model. The mechanism for this effect is suggested to be the induction of compensating liver cell proliferation. In an attempt to further elucidate the role of cell proliferation in hepatocarcinogenesis, a study was conducted to investigate whether two different types of delays in the cell cycle would influence the induction of hepatic preneoplasia and neoplasia by 1,2-dimethylhydrazine in a similar fashion (Ying et al., 1982). The corticosteroid, hydrocortisone, which is a potent inhibitor of DNA synthesis in the liver, was used as well as delaying the time of partial hepatectomy after 1,2-dimethylhydrazine administration in order to accomplish delaying cell cycle progression. The results

of this study show that a delay in the cell cycle with or without temporary interruption, significantly decreased the initiation of liver carcinogenesis and the induction of early presumptive preneoplastic hepatocyte alterations with 1,2-dimethylhydrazine. These findings also confirm previous data on the need for properly timed cell proliferation in the induction of early hepatocyte alterations by several carcinogens. It is noted that 1,2-dimethylhydrazine is predominantly a carcinogen for the colon and to a lesser degree for the vascular endothelium of the liver but not for hepatocytes, even though it extensively methylates the DNA of liver. The results of this study suggest that the reason for the ineffectiveness of this carcinogen in inducing liver cell cancer may be the lack of a measurable degree of necrosis and subsequent cell proliferation with the doses used. Finally, the nature of the time dependence for cell proliferation in its role in the induction of early lesions by carcinogens are suggested to be related to the need for time to repair or remove carcinogen-induced lesions. (CA 21157).

In a different study on the role of cell proliferation in the induction of hepatocarcinoma in rats, the effects of feeding a choline-devoid diet on liver cell proliferation and whether any such effect is modified by inclusion of phenobarbital in the diet was investigated (Abanobi et al., 1982). The feeding of a choline-devoid diet and dietary phenobarbital administration have been shown to be efficient promoters of liver carcinogenesis in the rat. Also, the combination of phenobarbital in a choline-devoid diet resulted in a synergistic effect. In this study, liver DNA synthesis and liver cell proliferation was studied in rats fed a choline-devoid diet, a choline-supplemented diet, or the same diets with the inclusion of 0.06% phenobarbital. In rats fed the choline-devoid diet, both DNA synthesis and cell proliferation were shown to be increased over those present in rats fed the choline-supplemented diet. The inclusion of phenobarbital in the choline-devoid diet, however, caused the inhibition of DNA synthesis and cell proliferation. The results obtained indicate that stimulation of cell proliferation, although necessary, may not be a sufficient condition for an agent to be an efficient promoter of liver carcinogenesis. As a possible explanation for the results, the investigators suggest that a choline-devoid diet and phenobarbital may act by a mechanism of having differential effects on initiated and noninitiated cells. Future studies are being directed to test this hypothesis. (CA 23449).

There are recent reports which show that there is an increase in epoxide hydrolase activity in both hyperplastic nodules and hepatocellular carcinomas and an indication that this enzyme is one form of "preneoplastic antigen." Epoxide hydrolase has been implicated in the activation and inactivation of some types of carcinogens, including polycyclic aromatic hydrocarbons, and a possible role has been suggested for this enzyme in the development of resistance to cytotoxic effects of carcinogens in carcinogen-altered hepatocytes. Thus, this study was undertaken to determine its presence in various cell populations, which appear during experimental liver carcinogenesis, using an immunoperoxidase technique (Enomoto et al., 1981). The very early foci which were positive for GGT, showed very variable staining for epoxide hydrolase in comparison to the surrounding hepatocytes which were markedly increased. As the foci enlarged to become nodules, a much more uniform and increased staining intensity for epoxide hydrolase was demonstrated as the staining in the surrounding parenchyma returned to control levels. Two overall patterns of staining occurred in hyperplastic nodules. A minority of persistent nodules showed a uniformly high intensity of staining for both epoxide hydrolase and GGT, while the majority of nodules, as they underwent remodeling to normal-looking liver, showed a progressive loss of staining for both enzymes. The staining patterns, however, exhibited a dissociation in that the areas that retained GGT lost

epoxide hydrolase activity and vice versa. High activity for epoxide hydrolase was demonstrated in the majority of hepatocellular carcinomas, but there was heterogeniety in different parts of the same neoplasm as well as between neoplasms. The results of this study suggest that epoxide hydrolase may become an additional useful phenotypic marker for both the persistent and remodeling hyperplastic nodules and for the majority of hepatocellular carcinomas. (CA21157).

There are several research groups which have documented a heterogeneity of histochemical phenotypes in foci or islands of benign hyperplastic hepatocytes that appear in rat liver during or following exposure to hepatic carcinogens. The new traits constituting these abnormal phenotypes are also frequently acquired by larger carcinogen-induced hyperplastic nodules and hepatocellular carcinomas. Traits singled out for particular study due to their frequency in the induced lesions includes the acquisition of GGT activity and the loss of glucose-6-phosphatase and ATPase activities. Although the quantitation of island heterogeneity has received considerable attention in recent years, a lack of such information concerning enzyme histochemical changes in the fully developed primary hepatocellular carcinoma is noted. This prompted a histochemical study of these characteristics in primary hepatocellular carcinomas that were induced by feeding AAF to rats (Goldfarb and Pugh, 1981). The results showed that all seven possible combinations of the three abnormal traits were represented, with the carcinomas showing two or three of the enzyme histochemical changes being much more prevalent than those with a single alteration. The distribution of histochemical phenotypes in the carcinomas differs greatly from that reported for enzyme-altered hyperplastic islands induced by carcinogens. The significance of these differences is not clear at the present time. (CA 15664).

In the laboratory of Dr. Ronald Lindahl, studies on the elucidation and characterization of the mechanisms involved in the expression of a series of tumor-specific aldehyde dehydrogenase isozymes are being conducted. In normal rat liver aldehyde dehydrogenase activity is distributed among three isozymes found in mitochondria and microsomes. These have differing substrate and coenzyme preference, substrate Km, immunochemical properties and sensitivity to inhibitors. Hepatomas induced in male Sprague-Dawley rats by AAF have been shown to have a unique aldehyde dehydrogenase phenotype, which is characterized by an increase in total aldehyde dehydrogenase activity due to the appearance of several cytosolic isozymes not detectable in normal liver. These tumor isozymes have different biochemical, biophysical, and immunochemical properties from normal liver isozymes. To identify the mechanism underlying the expression of the tumor-specific aldehyde dehydrogenases, the time course of appearance of the new phenotype was followed during hepatoma formation in Sprague-Dawley rats following brief dietary exposure to AAF of 0.02% for 32 days (Lindahl et al., 1982). Tumor promotion by phenobarbital was also used to compare the effects of a variety of tumor induction protocol on the aldehyde dehydrogenase phenotype. No change in the aldehyde dehydrogenase phenotype was detected until tumors were grossly observed in liver, and the phenotypic change is limited to the tumor. There was no correlation between tumor size or the histology of the various tumors observed and the degree of deviation of the aldehyde dehydrogenase phenotype from normal. The investigators concluded that the tumor specific aldehyde dehydrogenase phenotype is not associated with altered liver metabolism due directly to carcinogen or promoter exposure. They propose that the mechanism of this phenotypic change requires that transformation-associated, stable genetic changes occur in initiated cells that are later expressed as the tumor aldehyde dehydrogenase phenotype. The possible physiological roles for the tumor-specific isozymes in tumor metabolism are being investigated. This

observed change in aldehyde dehydrogenase activity may be a useful marker for one or more changes occurring late in the progression towards neoplasia. (CA 21103).

Following an approach involving the same basic strategy used in developing a model for analyzing the successive stages of liver carcinogenesis, a model for a sequential analysis of the stages of oral carcinogenesis is being developed using hamster buccal pouch epithelium as the experimental system. The utility of using GGT as a histochemical marker for neoplastic lesions induced in the squamous epithelium by topical treatment of 0.5% 7,12-dimethylbenz(a)anthracene (DMBA) in mineral oil for 16 weeks was investigated (Solt, 1981). This flat epithelium has no glandular elements and normally lacks histochemical evidence of GGT activty. At the completion of the DMBA treatment, patchy GGT histochemical activity was detected in areas of dysplasia and in 35 of 106 papillomas and well-differentiated squamous cell carcinomas. GGT activity was not detected in untreated pouches or in mineral oil treated pouches. Using whole mounts of buccal pouch epithelium to detect and quantitate minute populations of GGT-stained cells, multiple discrete GGT-stained areas could be visualized when they were prepared one and six weeks after the final DMBA application. The experimental results obtained were consistent with the hypothesis that the early GGT-stained cell populations are preneoplastic in nature. The intraepithelial plaques of intense GGT activity detected histochemically in whole mounts of hamster buccal pouch epithelium harvested after eight topical DMBA applications over a four week period, represent a very small fraction of the total area of treated epithelium. When DMBA treatment was discontinued, many of the plaques disappeared. The discrete nature of these plaques and their microscopic size suggested to the investigator that each plaque may have originated in a single carcinogen-altered cell. A study was then designed to explore further the possible clonal nature of these lesions and to assess whether the loss of GGT-stained plaques in the post-treatment period represents a material loss of plaques or merely reflects a loss of enzyme expression in plaque cells in the absence of DMBA exposure (Solt and Shklar, 1982). GGT-positive cells could be detected histochemically in whole mounts of pouch epithelium as early as three days after the first appliction of DMBA. Progressively larger GGT-stained epithelial cell populations (plaques) up to 0.5 mm in diameter were observed during three consecutive weeks of DMBA treatment. Twelve weeks after a three-week regimen of six DMBA applications, very few plaques could be detected. Data are presented which suggests that a brief series of three DMBA applications reintroduced GGT histochemical activity in occult (unstained) intraepithelial plaques, which had lost enzyme activity but had persisted over an eleven-week treatment-free interval. The results of this study strongly favor a clonal origin for the GGT-rich lesions. The expression of GGT in a majority of the plaques appears to be a transient metabolic phenomenon associated with DMBA exposure, whereas the induction of plaque cells per se appears to be a persistent and possibly permanent consequence of prior DMBA exposure. These carcinogen-altered cell populations are proposed to be potential precursors for the development of squamous epithelial neoplasia. (CA 28620).

The successful transformation of human diploid cells in culture by chemical carcinogens was not accomplished until relatively recently by the laboratories of Dr. Kakunaga and Drs. Milo and DiPaolo. The importance of this work lies in the need to extrapolate animal transformation and carcinogenesis data to man and to understand the mechanism of human cell transformation by chemicals. In the laboratory of Dr. George Milo, studies were performed to characterize events that occur from time of carcinogen treatment to the expression of neoplastic transformation (Milo et al., 1981a). Several different classes of chemical carcinogens were shown to induce the transformation of human fibroblasts in vitro.

A requirement was shown for the use of synchronized cells with the carcinogen treatment occurring in S phase in order to induce cell transformation reproducibly. The addition of insulin before the cells entered S phase appeared to sensitize the cells to the carcinogen that was added during S phase. The growth of transformed cells but not nontransformed cells was promoted by growth in medium supplemented with 8X nonessential amino acids. Carcinogen transformed cells at an early stage exhibited abnormal colony morphology and an ability to grow in low serum medium. At a later point, the cells lost their density dependent growth characteristics and exhibited anchorage independent growth. These cells were shown to produce undifferentiated mesenchymal tumors in nude mice. Using the protocol developed for the successful transformation of human fibroblasts, the first successful, reproducible transformation of human epithelial cells has been reported (Milo et al., 1981b). The criteria used to assess cell transformation was the property of anchorage independent growth and the ability of the cells to invade chick embryonic skin in vitro. Several carcinogens such as aflatoxin B_1, N-methyl-N'-nitro-N-nitrosoguanidine, propane sultone, β-propiolactone, and UV light were used. The epithelial cells were readily transformed when treated in S phase, and required a much shorter selection period (9 versus 21 population doublings) to exhibit the transformed phenotype. The invasive features of the transformed human epithelial cells in chick embryonic skin in vitro simulated squamous cell carcinoma. (CA 25907).

Due to the importance of understanding the process of malignant transformation of diploid human cells and the significance of a possible connection in human cells between anchorage independent growth and tumorigenicity, the laboratory of Drs. McCormick and Maher has undertaken the reexamination of the role of the various steps prescribed by Drs. Milo and DiPaolo for the induction of anchorage independence by chemical carcinogens. In this study, attempts were made to first repeat, in principal, the protocol of Drs. Milo and DiPaolo, to investigte which steps of their protocol were essential and which were either unnecessary or had only a slightly enhancing effect on carcinogen induction of anchorage independent growth and to determine the kinetics of expression of the anchorage independent phenotype in human cells (Silinskas et al., 1981). A dose-dependent increase in the frequency of diploid human fibroblasts capable of anchorage-independent growth after treatment with the carcinogen propane sultone was demonstrated. The results of this study indicate that the treatment of early-passage randomly proliferating foreskin-derived fibroblasts with doses of carcinogen/mutagen that allow between 10% and 90% survival followed by proliferation of the progeny for 8 to 13 population doublings before selection of anchorage independent growth (optimal time may vary with carcinogen dose) is all that is required for diploid human cells to acquire this phenotype. Procedures such as synchronization of cells and treatment just after the onset of DNA synthesis or the use of special selective medium were judged to be not essential for this induction. A dose-dependent increase in the frequency of 6-thioguanine-resistant cells was also measured. The data suggest that the acquisition of anchorage independent growth in human fibroblasts occurs as the result of a single mutational event. (CA 21289).

Genetics and Mechanisms of Cell Transformation

In the subject area of genetics and mechanisms of cell transformation are studies designed to test the somatic cell mutation hypothesis of cell transformation and to attempt the identification of those specific genes which are responsible or which have an influence on cell transformation. In one study, fish of the genus Xiphophorus are being used in order to evaluate the biochemical genetics of the carcinogenesis process. In another study, the relationship of H-2 haplotypes to

susceptibility to 3-methylcholanthrene-induced tumor induction is being pursued. This is to follow up on preliminary observations suggesting that certain F' hybrids of congenic mice differing only at the H-2 locus were more susceptible than either parent to tumor induction. Newer studies in this area are focused on determining whether a mutation in a specific gene leading to the expression of transformation-specific proteins is the mechanism of chemically-induced neoplastic transformation. In one study of this type, the hypothesis to be tested is that transcriptional activation of genes, which are progenitors of sarcoma virus genes, is required before chemical mutagens can initiate transformation in cultured rat cells, and that initiation involves the production of mutations in at least one copy of the sarcoma virus genes. The comparison of RNA tumor virus-, DNA tumor virus-, and various chemically-transformed cells will be undertaken using somatic cell genetic and two-dimensional gel electrophoresis techniques in order to provide information concerning the nature of the genetic lesion and altered gene expression resulting from the chemically-induced neoplastic transformation of cells. At this point, it should be noted that there has recently been a veritable explosion in new information related to the identification of genes responsible for the transformation of cells to malignancy. Using DNA transfection and gene cloning techniques and restriction endonuclease and Southern blot analysis, several different transforming genes have been isolated from different human tumor cells. The evidence gained so far has led to the hypothesis that each given type of tissue will have a characteristic activated oncogene. In general, investigators have found that cancerous cells of the same type have the same or very similar transforming genes. An example of this is seen in the finding by investigators at the Sidney Farber Cancer Center that the transforming gene of a human mammary carcinoma was very similar to those carried by six mouse mammary carcinomas which were either virally or chemically induced. More recent studies have demonstrated that some of the human transforming genes are homologous to transforming onc genes of RNA tumor viruses. These results provide a link between the transforming genes of viruses and human neoplastic disease. Most of the studies of this type are supported by other Program areas such as Biological Carcinogenesis and Tumor Biology.

The types of mutations leading to the transformation of BHK cells by chemical carcinogens was studied using somatic cell hybridization techniques (Bouck and DiMajorca, 1982). The transformation of BHK cells by carcinogens such as methylnitrosourea and 4-nitroquinoline-N-oxide occurred in a single step and displayed the characteristics expected for a recessive mutation. Dose response studies showed the induction of transformants with kinetics similar to the induction of 6-thioguanine- or ouabain-resistant mutants. Carcinogens causing mainly base change mutations easily induced BHK transformants with temperature-sensitive phenotypes, but frameshift mutagens induced temperature-sensitive transformants with a frequency inversely proportional to its ability to induce frameshift mutations. These results suggest that transformation is due to a somatic mutation. Somatic cell hybrids produced between isogenic lines of chemically or virally transformed BHK cells and normal cells were used to test whether the transformation resulted from a genetic loss. The suppression of the transformed phenotype of the chemically or spontaneously transformed cells in the hybrids while virus-induced transformation could be detected demonstrates that these were recessive to the normal phenotype. (CA 25013 and CA 27306).

A fundamental question in carcinogenesis is whether the initial event leading to the transformation of normal cells into tumor-forming cells results from damage to DNA. The large amount of data demonstrating a high correlation between the mutagenic and carcinogenic properties of various chemicals supports the hypothesis

that somatic mutations are involved in the process leading to neoplasia. A more direct approach to determining whether DNA is the principal target taken by the laboratories of Drs. Maher and McCormick is to compare the frequency of UV-induced neoplastic transformation of normal diploid human fibroblasts with that of xeroderma pigmentosum cells, which are deficient in the rate of excision repair of UV-induced DNA damage, to see if the latter are more sensitive (Maher et al., 1982). Both sets of cells were shown to exhibit a dose dependent increase in transformation which corresponded to a dose-dependent decrease in survival. The transformation frequency data indicate that the XP7BE (xeroderma pigmentosum) cells were significantly more sensitive than normal human fibroblasts to UV-induced loss of anchorage dependence. At doses that caused equal cell killing, the frequency of anchorage-independent cells was approximately equal. The anchorage independent XP7BE and normal cells produced fibrosarcomas in 100% of the athymic mice injected with the cells. The similarity of dose response curves for anchorage independence and mutation to 6-thioguanine resistance which is shown, supports the idea that the acquisition of anchorage independence (transformation) in human cells occurs as the result of a single mutational event. (CA 21289).

Another major part of this subject area includes studies designed to test the cell cycle specificity of the induction of cytotoxicity, mutagenesis, and neoplastic transformation by chemical carcinogens. A substantial amount of information exists which supports the hypothesis of cell cycle specificity. In mouse embryo C3H10T½ cells, G_1 and S phase cells are susceptible to cytotoxicity and mutation, while only the S phase cells (in synchronized cultures) are susceptible to neoplastic transformation by exposure to alkylating agents. In rat liver from adult rats, hepatocytes are generally resistant to carcinogenesis by a single exposure to agents capable of inducing cancer in other tissues. Hepatocyte susceptibility to carcinogenesis can be increased by certain treatments which stimulate the proliferation of damaged cells. Also, it has been observed that hepatocytes were much more sensitive to initiation when treated with methylnitrosourea (MNU) shortly after, rather than before, a two-thirds partial hepatectomy to induce hepatic cell division. In order to study the relationship between the susceptibility of hepatocytes to carcinogenesis in vivo by MNU and the cell cycle phase at which carcinogen-induced damage is incurred, hepatic cell proliferation in juvenile male Fisher 344 rats was charted following a two-thirds partial hepatectomy (Kaufmann et al., 1981). DNA synthesis was shown to occur in hepatocytes in two distinct waves which were followed after 6 to 8 hours by waves of mitotic cell division. Treatment with hydrocortisone, which temporarily and reversibly inhibits hepatocyte proliferation in regenerating livers, after partial hepatectomy altered the kinetic pattern. The initial waves of DNA synthesis and mitosis were each delayed by about 15 hours, and instead of a biphasic response of DNA synthesis and mitoses by hepatocytes, only single peaks were observed during the period of observation. In rats not given hydrocortisone, susceptibility to hepatocarcinogenesis was greatest at 20 hours after partial hepatectomy when the peak fraction of proliferating hepatocytes was in S phase. Hydrocortisone treatments, which shifted the time of onset of DNA synthesis, also shifted the time of greatest sensitivity to MNU with hepatocytes in late G_1 or S phase again the most susceptible. The results illustrate the importance of cell proliferation in carcinogenesis and further point to the specific sensitivity of certain cell cycle phases. (CA 20658).

In another laboratory, important studies are being conducted on the mechanisms of induction of malignant transformation by radiation and its modification by chemical agents in an in vitro system. Previous results from this laboratory have shown that the development of malignant transformation in mammalian cells irradiated in vitro

can be thought of as involving at least two steps: (1) the induction and fixation of the transformed state as a heritable cellular property, and (2) its subsequent expression in terms of morphologically altered cells. Interest in investigating the effects of protease inhibitors on radiation transformation stemed from published reports which demonstrated their ability to inhibit error-prone DNA repair and mutagenesis in bacteria and 12-O-tetradecanoylphorbol-13-acetate (TPA)-enhanced carcinogenesis in vivo. It is hypothesized that the action of an error-prone DNA repair process may be responsible for much of the transformation observed in rodent cell lines in culture. The effects of three protease inhibitors, antipain, leupeptin, and soybean trypsin inhibitor which specifically inhibit different proteolytic enzymes, were investigated for their effects on the induction of oncogenic transformation in mouse C3H10T½ cells by x-rays (Kennedy and Little, 1981). Different patterns of inhibition by the three protease inhibitors was demonstrated. The most effective protease inhibitor was antipain, which was able to completely suppress radiation transformation as well as TPA-enhanced radiation transformation. Its ability to suppress transformation when present for only one day following irradiation suggested that an effect on a DNA repair process was an important part of its action. It is noted that x-ray-induced DNA repair is usually completed within two to four hours of irradiation. Leupeptin was a less effective inhibitor than antipain, and soybean trypsin inhibitor was shown to suppress only the promotional effects of TPA on transformation. The results offer in vitro confirmation for the suppressive effect of protease inhibitors on carcinogenesis in vivo. They also suggest that more than one protease may be involved in the induction of transformation in vitro. (CA 22704).

In a subsequent study, the interaction of TPA and protease inhibitors was examined for their effects on the induction of plasminogen activator activity in normal and transformed mouse C3H10T½ cells (Long et al., 1981). Plasminogen activator is a serine protease which has received considerable attention in recent years due to evidence correlating the appearance of this protease with the neoplastic state. With a few exceptions, a relationship between plasminogen activator activity and in vitro malignant transformation has been shown for a number of cell types. TPA was shown to induce plasminogen activator activity in normal 10T½ cells, which was inhibited by antipain. The plasminogen activator activity of transformed 10T½ cells was high and was not further stimulated by TPA. Antipain also inhibited the plasminogen activator activity of the transformed cells. No effect could be shown with leupeptin and soybean trypsin inhibitor. These results and those showing the suppression of the promotional effects of TPA in x-ray-induced malignant transformation by antipain suggested a definite role for proteases in the transformational event or in the maintenance of the transformed state. (CA 22704).

In a different set of experiments, the effects of the glucocorticoid hormones, dexamethasone and cortisone, with x-ray irradiation on transformation of C3H10T½ cells were studied (Kennedy and Weichselbaum, 1981). On the basis of studies in experimental animals, it has been proposed that sex hormones may act like tumor promoting agents in their ability to enhance the incidence of cancer. But it is noted that dexamethasone has been observed to suppress the enhancement of chemical carcinogenesis in vivo and transformation in vitro resulting from exposure to the tumor promoting agent, TPA. In contrast to these observations, the results reported in this study show that cortisone and dexamethasone do not suppress radiation transformation of C3H10T½ cells in vitro. Cortisone by itself was shown to induce transformation of cells, and it increases the yield of radiation-induced transformants in a synergistic fashion. Dexamethasone did not induce cell transformation. It also increased the yield of radiation-induced transformants, but

the increase was not significantly different from the yield of transformants observed for radiation alone or for the expected additive effect of the combined treatments. These results suggest that promotion in vitro or in vivo that occurs in the presence of TPA is different from the natural expression of carcinogenesis in vivo or transformation in vitro effected by exposure to a high dose of a carcinogen such as x-ray irradiation. From the evidence gathered, the investigators believe that their results may have implications for patients treated with a combination of glucocorticoids and cytotoxic agents. It is noted, as an example, that patients undergoing total nodal radiation and combination chemotherapy (which includes glucocorticoid treatment) for Hodgkin's disease have an elevated incidence of secondary leukemia and lymphoma. The results of this study implicate glucocorticoids in addition to the common view that the cause was due to exposure to combinations of x-rays, alkylating agents, and procarbazine. An additional example is given in that patients who are undergoing chronic immunosuppression by glucocorticoids (organ transplant patients) also have an elevated incidence of malignancy. This malignancy in transplant patients may be due not only to immunosuppression itself, but also to the agents used to achieve immunosuppression. The investigators' results lead them to speculate that when the clinical efficacy of glucocorticoids is similar, dexamethasone may give a decreased risk of tumor formation compared with cortisone. (CA 22704).

Development of Carcinogenicity Test Systems and Mechanisms of Mutagenesis

The development of carcinogenicity test systems subject area includes projects which, for the most part, involve cell culture systems. The differential sensitivity of cells to low or high calcium concentrations in the medium forms the basis for two of the test systems. Most tumor cells are found to be resistant to low calcium concentrations while normal cells are not. In a mouse epidermal cell system, cells treated with chemical carcinogens while growing in low calcium will form colonies of cells which fail to terminally differentiate when transferred to high calcium medium. The calcium resistant epidermal cells can be used as a quantitative assay for carcinogens. Under the mechanism of mutagenesis and genetic damage subject area are projects which form the basis for understanding how mutagenicity test systems work. In several of the projects, the techniques of gene cloning and DNA sequencing are used to gain information concerning the site specificity of any carcinogen-induced damage. Questions concerning both frameshift and point mutations are being investigated. At a higher organizational level are projects attempting to understand the molecular basis of chromosome aberrations or the biological significance of the sister chromatid exchange assay.

One of the test systems being developed involves the use of Chinese hamster V-79 cells grown in suspension culture as multicell spheroids. These spheroids offer the advantage of providing the cell contact, diffusion gradients, and cell cycle alterations characteristic of three-dimensional tissues and, therefore, of being relevant to mutagenesis and carcinogenesis in vivo. The model permits separation of subpopulations of cells such as S-phase from noncycling and outer from inner cells, thus permitting the study of cell cycle and diffusion gradient effects on mutation frequency. In these studies, the nitroheterocycles are used as the test compounds. These compounds are used extensively in the treatment of human infectious disease, although there is now little doubt that most of these drugs are mutagenic and perhaps carcinogenic. The mechanisms of the toxic effects of nitroheterocycles is not well understood, although metabolic reactions of the nitro group are involved. The toxicity of these compounds towards mammalian cells in culture was found to be highly dependent on the extracellular environment during treatment. Cell density during drug treatment may play an important role in the

interpretation of drug toxicity experiments performed in vitro. It was found that Chinese hamster V-79 and mouse L-929 cells exposed to nitroheterocycles under aerobic conditions are considerably more sensitive to the cytotoxic effects of these drugs when incubated at low cell density (10^2 cells/cm^2 or 10^4 cells/ml) than at higher cell density (10^4 cells/cm^2 or 10^6 cells/ml) (Olive, 1981). This is thought to be related to diffusion limitations when cells are in contact and to the ability of dense cell suspension to inactivate drugs. In contrast, when cells are exposed under anaerobic conditions, more toxicity is observed at high cell density than at low cell density. This may be due to local effects of toxic metabolites. From these studies, it is concluded that intracellular drug levels alone determine cell killing and, while anaerobic metabolism drives drug uptake, the mechanism for toxicity is similar under air or nitrogen. In another set of experiments, the effects of the nitroheterocycle AF-2 (furylfuramide; 2-2-(furyl)-3-(5-nitro-2-furyl) acrylamide) on Chinese hamster V-79 spheroids was examined (Olive and Durand, 1981). This compound is an antibacterial nitrofuran which was used for several years as a food additive in Japan. It is a known mutagen and carcinogen and has been shown to be preferentially toxic to hypoxic mammalian cells. Since AF-2 is fluorescent, quantitation of intracellular drug content was performed by flow cytofluorometric analysis after incubation of spheroids under either aerobic or hypoxic conditions. The results demonstrated that for the same level of toxicity, hypoxic spheroids accumulated twice as much AF-2 as aerobic spheroids, but showed less than half the number of mutants resistant to 6-thioguanine. Using velocity sedimentation techniques, it was found that there was a selective increase in the toxic and mutagenic effects of AF-2 for internal versus external cells of the spheroid. When all the cells of the spheroid were used in mutagenicity experiments, however, the response of the external cycling cells predominated over the response of the internal cells. Therefore, these results demonstrate that the cellular environment can modify mutagenesis and also suggest that the growth fraction of the target cells is an important factor to consider in mutation experiments. (CA 28793).

Benzo(a)pyrene (BaP) is converted to several electrophilic metabolites, which can form covalent adducts with cellular DNA, by microsomal cytochrome P-450-linked mixed-function oxidases. In DNA isolated from cells or tissues exposed to BP, two principal covalent ligands are identifiable, the *anti* and *syn* BaP diol epoxides. The predominent covalent DNA modification involves an adduct formed between the 10 position of *anti* BaP diol epoxide and the 2-amino group of guanine. An additional DNA modifying molecule, BaP-9-phenol-4,5-epoxide is found on DNA modified in incubations containing pure microsomes, but not in DNA isolated from cells exposed to BaP. This suggests the relative importance of cellular conjugation pathways in regulating gene modification by carcinogen metabolites. In the study to be described, the quantitative relationship between levels of BaP metabolite-DNA base adducts and the concomitant frequency of reverse mutations in *Salmonella typhimurium* TA98 and TA100 strains used in the Ames assay was determined (Fahl et al., 1981). BaP-induced cytotoxicity and *His+* reverse mutation frequencies were determined. Bacterial DNA hydrolysates, analyzed by chromatography on Sephadex LH-20 columns, showed the presence of three principal adducts formed from the two diastereoisomeric BaP diol epoxides and a 9-hydoxy-BaP metabolite. Bacteria were also incubated with the tritium labeled diol epoxides and 9-hydroxy-BaP. Linear nonsaturating increases in DNA adduct levels were observed up to the highest concentrations used in both TA98 and TA100 cells. The increasing adduct levels were accompanied by linearly increasing mutation frequencies. At equivalent concentrations of the two BaP diol epoxides, an average of 8.2 times more base substitution mutations (TA100) were seen than frameshift mutations (TA98). The results also demonstrate a difference in

"absolute mutagenic efficiency" (i.e., mutations/unit of DNA modification) between the three covalent DNA ligands studied. It is noted that others have shown that anti BaP diol expoide has an 8-fold higher mutagenic efficiency than syn BaP diol epoxide in Chinese hamster V-79 cells, a difference which is also seen in the ability of these two compounds to initiate mouse skin tumors. This is directly opposite to the results found with S. typhimurium TA98 and TA100, the reasons for the difference being unknown. The results of this study, however, help to clearly define a fundamental difference between bacterial and mammalian cells in the ability to translate carcinogen-modified bases into specific genetic mutations. (CA 25189).

In another study, the spectrum of base-pair substitution mutations induced in the lacI gene of a uvrB$^-$ strain of Escherichia coli by BaP diol epoxide and 3,4-epoxycyclopenta(cd)pyrene (CPPE) were determined (Eisenstadt et al., 1982). This study was undertaken because the Ames test, which is a widely used mutagenicity test, detects the ability of chemicals to revert any one of three different point mutations (two frameshift mutations and a base subsitution mutation) but does not yet provide information about the specific changes in DNA sequence that are induced at these loci. It was found that about 10% of all lacI mutations induced by either BaP diol epoxide or CPPE are nonsense mutations, suggesting that base-pair substitutions are a large fraction of the mutational events induced by these chemicals in uvrB$^-$ bacteria. Both carcinogens specifically induced the G•C to T•A and, to a lesser extent, the A•T to T•A transversions. These results suggested a mechanism for transversion induction at G•C sites by BaP diol epoxide. This involves carcinogen binding to the exocyclic N2 amino group of guanine in the template strand followed by a rotation of the modified bases around its glycosylic bond from the anti to the syn conformation. This could allow the specific pairing of modified bases with an imino tautomer of adenine. The model is not directly applicable to the A•T to T•A transversion. A proposed mechanism involves the generation of lesions such as apurinic sites or gaps to account for the occurrence of mutations other than G•C to T•A transversions. (CA 26135).

Enzymes Characteristically Associated with Carcinogenesis

Research projects in this subject area are focused on the characterization and properties of carcinogen metabolizing enzymes such as the cytochrome P-450 monooxygenase system involved in the activation of polycyclic aromatic hydrocarbons and the N-acetyl- and acyl transferases involved in the metabolism of aromatic amines. Also included are studies on the role of the polyamine synthetic enzyme, ornithine decarboxylase (ODC), in UV or chemically-induced carcinogenesis. This enzyme, which forms putrescine by the decarboxylation of ornithine, is the first and probably the rate-limiting enzyme in the biosynthesis of the polyamines spermidine and spermine. ODC activity, as well as S-adenosyl methionine decarboxylase activity (another polyamine biosynthetic enzyme), and the levels of their biosynthetic products have been observed to be elevated in various hyperproliferative systems. Epidermal ODC activity was found to be significantly induced by exposure to UVB, which is midwave or sunburn UV, 290 to 320 nm. Also, it has been proposed that the prolonged induction of ODC activity and the ensuing increase in polyamine levels are necessary events in the development of preneoplastic foci, hyperplastic nodules, and the eventual formation of malignant neoplasms in carcinogen exposed rat liver. Preliminary evidence in support of this hypothesis exists.

In many of the studies involving polycyclic aromatic hydrocarbon (PAH) metabolizing enzymes, the multiple forms of cytochromes P-450 and the other necessary components are purified before their use. Fundamental studies of the function of the mono-oxygenase system in carcinogen activation including the roles of protein-protein and

protein-lipid interactions are being pursued. The basic mechanisms of microsomal monooxygenation is hoped to be further understood. The observation that peroxide catalyzed monooxygenation at a single form of cytochrome P-450 is mechanistically very different from peroxidation catalyzed by peroxidases will be pursued further by extensive kinetic analyses to test the generality of the mechanism. Other studies involving PAH metabolizing are focused on the mechanism of induction of the enzyme system, aryl hydrocarbon hydroxylase (AHH) in liver cells. Mutants of the mouse hepatoma line, Hepa-1, are being isolated that are deficient in AHH activity. The technique of somatic cell hybridization is being used to determine the number of complementation groups into which these mutants fall. The biochemical defects of each class of mutants is also being determined. One class has been shown to be defective in the TCDD receptor protein that mediates PAH induction of AHH activity. Further characterization of the mutants will involve the attempted cloning of each of the AHH genes.

In one study, the role of PAH cytosolic binding proteins in the activation of AHH and carcinogenesis is to be clarified. A high affinity pool of binding sites in hepatic cytosol that specifically binds 3-methylcholanthrene or other potent PAH inducers of AHH has been recently discovered. The saturable binding protein(s) sedimented at 4.2S and promoted the translocation of 3-methylcholanthrene into nuclei in a time- and temperature-dependent fashion. A cloned epithelial rat hepatocyte culture, RL-PR-C, was used to study the noncovalent interaction of 3-methylcholanthrene with cytoplasmic components in whole cells (Heintz et al., 1981). Early passage cultures are resistant to the cytotoxic action of both aflatoxin B_1 and BaP due to a low or noninducible level of AHH activity, whereas late-passage RL-PR-C cells have both an elevated basal and 3-methylcholanthrene-inducible AHH activity. The number of high affinity (average K_d, 3.6nM) binding sites found was 20,000 to 80,000 per late-passage hepatocyte with a total capacity of about 2.2 pmoles of 3-methylcholanthrene bound per mg of cytosolic protein. The specificity of binding was tested by using other compounds to compete for 3-methylcholanthrene binding. Other PAHs, but not aflatoxin B_1, several steroids, phenobarbital, or Archlor 1254, inhibited the binding. When the temperature of the cultured cells was elevated to 37°C after the standard ligand-binding incubation at 4°C, a rapid decrease in cytoplasmic saturable binding with a concomitant increase in nuclear- and chromatin-associated ligand resulted. Adsorption of the 3-methylcholanthrene binding complex by nuclei in vitro suggested that the 4S binding protein facilitated the entry of 3-methylcholanthrene into the nucleus. The results from this study demonstrate that the presence of the 4S binding species correlated with the level of inducibility of AHH throughout its development in RL-PR-C cells and, therefore, may be involved in the induction of this enzyme. (CA 20711).

The presence and induction of AHH activity can have some important clinical implications. Previous work in this area has established that mitogen-stimulated cultured human lymphocytes have basal and PAH-inducible AHH activity. AHH inducibility has been shown in twin studies to have a strong genetic component. The role of genetics in acute leukemia, which ranks as the malignancy of the highest incidence in the pediatric population of the United States, has been shown primarily by epidemiologic studies. Since there is epidemiologic evidence suggesting that environmental chemicals may be responsible for a considerable portion of all human cancer, the importance of an interaction between the putative carcinogen and the enzyme system responsible for its metabolic activation is stressed. To study the interaction between the AHH enzyme system and leukemia, AHH activity and inducibility were examined in mitogen-stimulated cultured lymphocytes from children with acute leukemia in remission, with nonleukemic malignancies and with no family

or personal history of malignant disease (Blumer et al., 1981). Among the three sources of cells studied, no morphological differences or differences in mitogen responsiveness were observed. Levels of constitutive and dibenzanthracene-induced AHH activity were found to be similar among the three groups by analysis of variance. When results were analyzed in terms of inducibility ratios, however, cells from leukemic children were found to be significantly less inducible than cells from unaffected children or children with nonleukemic malignancies. When statistical criteria were employed for the separation of individuals who were highly aromatic hydrocarbon responsive from those who were minimally responsive, it became apparent that a significantly larger proportion of leukemic children than unaffected children or children with a nonleukemic malignancy were found to be minimally aromatic hydrocarbon responsive. In addition, in patients with acute lymphoblastic leukemia relapsing while on therapy, longer durations of the first remission were correlated with the highly inducible AHH phenotype. This relationship between AHH phenotype and the response to chemotherapy was unexpected. If substantiated, it may prove important in the evalution of present chemotherapeutic regimens and in the design of new approaches. (CA 30067).

Aromatic amines have been shown to be metabolically activated by at least two different pathways. Initially, the induction of liver tumors in rats by primary aromatic amines was associated with their conversion to reactive toxic sulfate conjugates of their hydroxamic acid derivatives. There is evidence that this pathway is restricted to rat liver and is ineffectually low in the liver of Sprague-Dawley-derived rats. An alternative mechanism for the metabolic activation of arylhydroxamic acids is by the formation of reactive N-acetoxyarylamines as a consequence of N,O-acyltransfer. It has been shown that cytosolic enzymes capable of activating N-hydroxy-AAF are present in a wide variety of tissues from a number of species that are susceptible to the carcinogenic effects of aromatic amines. In previous studies, it was shown that the lactating mammary glands of female Sprague-Dawley-derived rats, like rat liver, possess an N,O-acyltransferase and that RNA adducts formed in this tissue are compatible with an acyltransferase-mediated mechanism of activation. In order to examine more closely the relationship between the metabolic activation of arylhydroxamic acids and their tumor-inducing ability in the mammary gland of immature female Sprague-Dawley-derived CD rats, a study was undertaken which involved the intraperitoneal injection of N-formyl, N-acetyl, or N-propionyl derivatives of N-hydroxy-4-aminobiphenyl, N-hydroxy-AAF or N-(4-biphenyl)-glycolamide (Shirai et al., 1981). The results of these experiments show that both the liver and mammary gland possess enzymes that are relatively acyl specific for the activation of either N-formyl (one enzyme) or N-acetyl and N-propionyl (second enzyme) derivatives. These two enzyme activities were separable by DEAE-cellulose ion exchange chromatography and by gel filtration on Sephacryl. The liver microsomal activities and the formyl-preferring soluble enzymes were shown to be inhibited by diethyl-p-nitrophenylphosphate, a microsomal deacylase inhibitor, while the acetyl and propionyl preferring cytosolic enzymes were not inhibited. The results demonstrated that mammary gland tumor induction and the formation of both gamma-glutamyltranspeptidase-positive and cellular altered foci in liver were greatest with those compounds that were metabolized by the acetyl-preferring enzymes of these tissues to generate reactive derivatives. (CA 23386).

Role of DNA Repair in Carcinogenesis

Projects in this subject area deal with many aspects of the relationship between DNA repair and carcinogenesis. The specific aspects of DNA repair being addressed involve studies of gene regulation and mutagenesis as it refers to the repair process, the temporal events of the repair process, and an analysis of the mechanism

by which a cell's repair system handles DNA damage from exposure to chemical and physical agents. A wide range of experimental model systems are being used for this purpose. Human cells in culture and rodent cells and tissues are the predominent model systems used with bacteria, viruses, fungi, yeast, Drosophila, and haploid frog cells being used by others.

The mutagenic effects of DNA damage depend on how the replicational machinery of the cell responds to the damage and to what extent that damage can be removed by cellular repair mechanisms. The repair and replication of ultraviolet irradiation-damaged viral DNA (Simian virus 40) is being studied in permissive monkey cells at the University of California, L.A. (Stacks and Hercules, 1981). Viral DNA replication is markedly inhibited by UV damage which appears to affect the formation of supercoiled molecules (form I) more than total DNA. Inhibition increases as a function of time after irradiation and appears to be explained by a blockage of the progression of replication forks by the dimers in the DNA. No evidence was found for recovery of the replication rate due to DNA repair by the host cell even up to four hours after irradiation. Evidence to date, however, indicates that template molecules which contain 0 to 1 dimer appear to be the only ones completed. The fact that dimers can be bypassed during replication so that they appear in progeny molecules gives rise to significant opportunities for mutagenic events. (CA 28449).

In a similar vein, postreplication recovery systems are being studied in Neurospora to provide insights into possible similarities with mammalian, particularly human, recovery systems. There is considerable concern with understanding the post-replication recovery systems in eukaryotes because it seems that this recovery type is the process by which most heritable changes, mutations, may become fixed. Schroeder and coworkers have shown that Neurospora has a post-replication-recovery of DNA synthesis in response to UV-irradiation that is very similar to that of eukaryotes (Calza and Schroeder, 1982; Calza and Schroeder, in press). After UV, DNA synthesis is depressed and DNA is made in short segments for an abnormal length of time, due to blockage of synthesis by dimers. Eventually, the blockage to chain elongation is overcome, although dimers remain in the template, and normal length DNA is made. (CA 26314).

Working originally with viral genes and now with E. coli, Tessman and coworkers have established a new effect of ultraviolet irradiation which may explain why irradiated cells die. The new observation is that the effect of UV on E. coli is to mimic the effect of a deficiency in the rho protein, which is needed for transcriptional termination (Fassler and Tessman, 1981). These investigators have developed a theory, which is being tested with the construction of appropriate mutants, that irradiated cells that are normal in DNA repair functions die because of a deficiency in rho protein. (CA 22239).

At the Stanford University School of Medicine the molecular mechanism of the incision of damaged DNA is being investigated in the yeast, S. cerevisiae. Genes from this organism belonging to the so-called RAD 3 group that code for functions required for excision repair are being cloned from yeast gene pools and inserted into appropriate cloning vectors. Individual cloned genes are being subcloned and characterized, and attempts will be made to express them in E. coli maxicell preparations or in cell-free transcription-translation systems. The cloned genes will also be used as specific hybridization probes to detect homologous sequences in other eukaryotes, especially human DNA (Reynolds and Friedberg, 1981). (CA 12428).

At the New York Medical College, Wallace and colleagues are attempting to identify, purify and relate to known genetic markers the repair endonucleases found in the lower eukaryote, Drosophila melanogaster. Current work is underway to purify and characterize an apurinic endonuclease from embryos and ova (Wallace et al., 1981). (CA 24953).

A variety of radiation sensitive mutants derived from a haploid frog cell line (ICR2A) are being studied at the University of Rochester by Ohlsson-Wilhelm and coworkers. Since the parental cell line is deficient in excision repair but proficient in post-replication repair, it seems likely that these mutants may allow biochemical characterization of this mode of repair. The haploid genome should facilitate mutational analysis as well (Smith et al., 1980; Smith et al., 1981). (CA 25731).

Dr. D. S. Sarma's laboratory at the University of Toronto is assessing the role of carcinogen-induced DNA damage, repair and replication in the induction of preneoplastic and neoplastic hepatic lesions in the rat. Experiments to date have revealed that cell proliferation prior to the repair of carcinogen-induced critical lesions is an obligatory step for initiation as monitored by the appearance of enzyme altered hepatocytes or hepatocellular carcinoma (Columbano et al., 1981) The experimental reasoning suggested that the longer the interval between the administration of a carcinogen and a subsequent partial hepatectomy (to stimulate cell proliferation), the greater the likelihood of the critical lesions getting repaired and the fewer the number of initiated hepatocytes and consequently the lesser the incidence of hepatocellular carcinoma. This observation has been born out with 1,2-dimethylhydrazine, diethylnitrosamine and N-methyl-N-nitrosourea as the carcinogenic agent. (CA 23958).

At the Michigan State University, Dr. Goodman and coworkers have examined the intragenomic distribution of carcinogen-induced methylation of rat hepatic DNA following administration of either methylnitrosourea or dimethylnitrosamine. At the peak time of alkylation the transcriptionally active fraction of chromatin (euchromatin) was selectively alkylated as compared to the template in-active fraction (heterochromatin). By monitoring for four specific methylated purines commonly found in alkylated DNA following carcinogen treatment with MNU or DMN a qualitatively similar pattern of alkylation was observed in both heterchromatin and total chromatin. While the rat liver is composed of many cell types, parenchymal cells appear to be the target for AAF-induced hepatocarcinogenesis. Following a single intraperitoneal injection of N-2-acetylaminofluorene or its N-hydroxy metabolite (labeled in the ring position with tritium) hepatic parenchymal cells and nonparenchymal cell populations were isolated by centrifugal elutriation. Eighteen hours after injection (the time of peak alkylation) the ratio of carcinogen adducts/mg DNA of parenchymal cells as compared to non-parenchymal cells was 2.7 for AAF. Three days later 50-59% of the adduct initially present remained in the DNA of both cell types. The results suggest that AAF preferentially damages DNA of parenchymal cells due to an increased capacity to activate the carcinogen by N-hydroxylation (Baranyi et al., submitted).

Dr. Bresnick and coworkers at the University of Vermont College of Medicine are attempting to understand some of the factors which lead to removal of deoxyribonucleoside adducts from DNA. A cloned cell line derived from normal hamster tracheal epithelium has been characterized with respect to its response to benzo(a)pyrene. If cellular DNA is first labeled with tritiated thymidine and the cells then exposed to B(a)P, alkaline elution gradients of the DNA reveal only a

minimal increase in DNA strand breakage (except at very toxic doses). Therefore, although B(a)P causes considerable alkylation of the hamster cell DNA, it does not induce alkalai-sensitive lesions or DNA breaks per se. Removal of B(a)P adducts from hamster DNA appears to be biphasic. There is a rapid removal for the first four hour post-treatment incubation and then a slower repair so that about 50% of the hydrocarbon bound to DNA is still present after 48 hours. At high concentrations of B(a)P treatment, there is no qualitative difference in the adducts produced, however, there is a greater persistence of deoxyadenosine adducts (Eastman et al., 1981). One important technical improvement was made this year when it was realized that DNA purified by hydroxyapatite column chromatography gave a different HPLC profile of DNA adducts than did conventional phenol purified DNA. Apparently, RNase digestion had failed to completely remove contaminating RNA in the phenol procedure and therefore several peaks thought to be DNA adducts were not. Hydroxyapatite is now routinely used to purify DNA. (CA 23514).

Using human cells, Dr. Sirover and coworkers are asking if normal cells regulate DNA repair pathways in a defined temporal sequence of gene expression such that DNA repair is stimulated prior to DNA replication in the cell cycle. They have shown that WI-38 diploid fibroblasts, synchronized by serum depletion, have increased excision repair pathways for base and nucleotide removal prior to DNA replication. Both normal and XP complementation groups regulate base excision repair just prior to replication but nucleotide repair is not regulated during the cell cycle by XP cells (Gupta and Sirover, 1981). (CA 29414).

At the Washington University, Dr. Lieberman et al. have made substantial progress in perfecting a subcellular system in which to study molecular events associated with incision repair of carcinogen and ultraviolet radiation damage. With another assay, the investigators have been determining if excision repair is randomly distributed through the human genome; preliminary results suggest that it is not random. The method involves allowing cells to repair in the presence of bromodeoxyuridine and, following sonication, precipitation of the repaired regions with an antibody to bromodeoxyuridine. This laboratory has also been studying the repair of alkylated DNA in chromatin fractions to determine if repair occurs selectively in any particular class of nucleosomes. Preliminary results suggest that some classes of nucleosomes may be more repairable than others (Smerdon et al., 1979). (CA 20513).

Dr. Maher and coworkers at the Michigan State University have developed a sensitive assay that measures the kinetics of DNA repair following low doses of carcinogens. The assay is based on the loss of superhelical structure in the DNA of human cells during excision repair processes. Normal human fibroblasts (or XP) are exposed to UV irradiation or benzo(a)pyrene-diolepoxide, then extracted to prepare nucleoids (high-salt nuclei) and centrifuged on sucrose velocity gradients containing ethidium bromide. DNA which has lost its superhelical character, due to excision repair of the sugar-phosphate backbone, sediments more slowly than superhelical DNA. After UV doses to normal human cells that give 20% survival, the superhelical content of the DNA is the same as controls. However, after one hour the superhelical content is greatly reduced. Over a 36 hour repair incubation the superhelical content returns to control levels. A comparable dose of B(a)P (in terms of survival) requires 4 to 5 times as long for the DNA to return to control levels of super helicity. It would appear, therefore, that repair of chemical adducts is slower than repair of UV-induced photoproducts. The reasons for the slowness of the repair of chemical damage are not clear (McCormick and Maher, in press). (CA 21253).

Special Projects

Grants included in the Special Projects Program are those which emphasize multidisciplinary research, together with those in the Chemical and Physical Carcinogenesis Branch (CPCB) which are not assigned to other CPCB Programs. A principal thrust of this Program is to search the broad expanse of carcinogenesis research, with the object of identifying areas of emergent high promise relative to human cancer causation.

The Program includes 91 grants with FY 82 funding of $14.7 million. These consist of 81 R01 (Research Project) grants and 10 P01 (Research Program Projects) grants, with FY 82 funding of $9.1 million and $5.6 million, respectively, for a total of approximately $14.7 million. The R01 grants are principally concerned with the role of tumor promoters in carcinogenesis (28 grants; $2.7 million), endocrine-related biochemistry of cancer and cancerous hosts (20 grants; $2.4 million), interspecies comparisons in carcinogenesis (17 grants; $2.2 million), and the development of cell culture systems and of biological models for use in carcinogenesis research (8 grants; $0.8 million). All of the P01 grants (multidisciplinary research) assigned to the CPCB are included in this program (10 grants; $5.6 million). Twelve of the studies on tumor promoters, cited above, are the result of a recent Request for Applications (RFA) concerned with the role of non-phorbol tumor promoters, hormones, and other cofactors in human cancer causation. The intended emphasis of this RFA was on the characterization of tumor promoters and of other cofactors present in the human environment.

An analogous, major FY82 programmatic effort has consisted of study and consultations relative to carcinogenesis research on human cells, tissues, and subcellular fractions, with emphasis on studies on human cell transformation by chemical/physical carcinogens, in vitro versus in vivo cell transformation, the use of human tumor cells in carcinogenesis research, the metabolism of chemical carcinogens by human cells, and the availability and handling of human cells, tissues, and subcellular fractions intended for use in carcinogenesis studies.

Research conducted during the past year has provided further basic data on the proximate and ultimate carcinogenic metabolites of synthetic and naturally occurring carcinogens and the reactions of the ultimate forms with tissue macromolecules. Although ethyl carbamate, a fermentation product found in yeast, beer, bread, etc., has received extensive study, the nature of its proximate and ultimate carcinogenic metabolites and of adducts formed from it in vivo has remained elusive. Miller and coworkers have now demonstrated that injection of single doses of (ethyl-1,2-^{3}H)- or (ethyl-1-^{14}C)ethyl carbamate into 12-day-old male (C57BL/6 x C3H/He)F_1 or adult male A/Jax mice resulted in the formation of 1,N^6-ethenoadenosine and 3,N^4-ethenocytidine adducts in the hepatic rRNA. (Ribovich et al., in press). These adducts were characterized by comigration on HPLC of ^{3}H or ^{14}C in enzymatic hydrolysates of the rRNA with synthetic standards. Both of the etheno derivatives were further characterized by their conversion to acetylated products that comigrated with acetylated synthetic standards; ethenoadenosine was also converted by anhydrous trifluoroacetic acid to a product that comigrated with synthetic 1,N^6-etheno-adenine. These observations strongly suggest that ethyl carbamate is metabolized to an electrophilic metabolite via dehydrogenation to vinyl carbamate and epoxidation of the latter compound and that vinyl carbamate and its epoxide are proximate and ultimate carcinogenic metabolites of ethyl carbamate.

Alkenylbenzenes occur in mixtures with each other and with other low molecular weight organic substances in essential oils used as spice flavors. Studies on the

carcinogenicities of the alkenyl benzene derivatives have shown that the naturally occurring compounds safrole (1-allyl-3,4-methylenedioxybenzene) and estragole (1-allyl-4-methoxybenzene) have similar carcinogenic activities in the mouse; furthermore, both compounds are metabolized to 1'-hydroxy derivatives that appear to be proximate carcinogens. Stable 1'-safrolyl and 3'-isosafrolyl adducts, formed by reaction on the exocyclic amino groups of guanine and adenine residues, account for the major share of the adducts found in the hepatic DNA of mice to which these carcinogens were administered. (Phillips et al., 1981a; Phillips et al., 1981b).

Assay of a number of alkenylbenzenes by injection prior to weaning into (C57B1/6 x C3H/He)F_1 male mice showed that 1-allyl-3,4-dimethoxybenzene (methyl eugenol) and its 1'-hydroxy derivative have hepatocarcinogenic activities similar to those of estragole and its 1'-hydroxy metabolite. On the other hand, 1-allyl-3,4,5-trimethoxybenzene (elimicin) and its 1'-hydroxy derivative had little, if any, hepatocarcinogenic activity in this test. Likewise, myristicin (1-allyl-3-methoxy-4,5-methylenedioxybenzene) and dill and parsley apiol (1-allyl-2,3-dimethoxy-4,5-methylenedioxybenzene and 1-allyl-2,5-dimethoxy-3,4-methylenedioxybenzene) were inactive; thus, each of the four derivatives in which there are methoxy or substituted methoxy groups in the 3,4,5-positions was inactive. On the other hand, 1'-hydroxy-2',3'-dehydroestragole was a much more potent hepatocarcinogen than 1'-hydroxyestragole. (CA 22484).

To determine the structural aspects of polycyclic aromatic hydrocarbons that influence their activities and potencies as initiating carcinogens, a number of methylated benzo(a)pyrene (B(a)P) derivatives were studied. Substitution of various polycyclic aromatic hydrocarbons with methyl groups can dramatically alter the carcinogenicity of the parent hydrocarbon. The mechanism by which this occurs is not known, but it has been assumed that at least one of the effects of such substitutions would be to block epoxidation at the site of substitution. Studies on simple methylated derivatives of aromatic hydrocarbons, such as benzene and napththalene, suggest that this substitution does inhibit metabolism of the compound by the cytochrome P450 complex, which is necessary for the initial epoxidation reaction. The metabolism of 7-methyl, 10-methyl, and 7,10-dimethyl-B(a)P was studied using both microsomal preparations and whole cells. (Kinoshita et al., 1982.) The products formed were analyzed by high-performance liquid chromatography, fluorescence spectrophotometry and mass spectrometry. These studies revealed that many of the expected metabolites were formed by microsomes but in addition, 7-MeB(a)P yielded a compound which was isolated and identified as trans-7,6-dihydro-7,8-dihydroxyB(a)P. The metabolism of 7-MeB(a)P was also studied in 10T1/2 cells. The hydrocarbon was metabolized readily and became bound to the DNA of the cells, although the DNA binding was only about one-eighth that obtained with B(a)P. However, no 7-MeB(a)P 7,8-dihydrodiol could be detected in the culture medium. In the microsomal system, 10-methyl-B(a)P and 7,10-dimethyl-B(a)P yielded mainly oxidation products at the methyl groups, although some other metabolites were identified. (CA 21111).

Tannenbaum and coworkers have continued to explore the significance of the endogenous formation of nitrite and N-nitroso compounds in relation to the development of cancer in man. Nitrate metabolism was investigated in long-term metabolic balance studies on healthy young men. Under conditions of constant low ingestion of nitrate (<180 μmoles/subject/day), the amount of nitrate excreted in urine was an average of 4-fold greater than the amount ingested. Balance studies employing labeled nitrate ($^{15}NO_3^-$) showed that the source of the excess nitrate in urine was the endogenous biosynthesis of nitrate, rather than the emptying of a body

pool. Nitrate biosynthesis occurred when nitrate ingestion was high as well as low, and the amounts synthesized appeared to be independent of intake and comparable to the amounts ingested from normal diets. Analysis of the $^{15}NO_3^-$ data also revealed that less than half of ingested nitrate was recovered as urinary nitrate. Since nitrate in urine is the net result of intake, endogenous synthesis, and metabolic losses, the magnitude of the losses is such that, despite ongoing synthesis, the amount of nitrate in urine of people consuming most diets will be less than the amount ingested. (Green et al., 1981a; Green et al., 1981b). A physiological pharmacokinetic model has been formulated for nitrate-nitrite metabolism which describes blood, urinary, and salivary concentration as a function of time following a single oral dose. (Deen et al., in press).

Bacteria have been implicated in the formation of N-nitroso compounds under a wide variety of conditions representing both in vitro and in vivo situations. Mechanisms of participation and/or catalysis include: (a) decrease of the pH of the system, (b) reduction of nitrate to nitrite, (c) adsorption of amine onto the cell surface or cytoplasmic membrane, (d) actual enzymatic formation. From their studies on endogenous nitrosamine, Tannenbaum and coworkers concluded that the major role of bacteria in the nitrosation of dimethylamine is the reduction of nitrate to nitrite and the lowering of the pH of the medium. Furthermore, a complex medium itself catalyzes nitrosation. The nature of this catalysis is not known, although it could be due to the presence of carbonyl compounds, cysteine, or a variety of other compounds which are known to catalyze nitrosation. (Ralt and Tannenbaum, 1981).

Methylamines are likely substrates for nitrosamine formation; however, little is known of their origin, excretion, or pharmacology. Using a sensitive and accurate assay developed for methylamines, it was found that: (1) Humans fed choline chloride excrete significantly more methylamine (MA), dimethylamine (DMA) and trimethylamine (TMA) in their urine; (2) Humans fed choline stearate exhibit the same blood choline response to treatment but form less of the three methylamines; (3) Humans fed lecithin respond with higher blood choline concentrations but form less methylamines. A significant amount of TMA was excreted, however (3x control); (4) Analysis of currently available lecithin preparations used to treat humans showed that all contain significant amounts of TMA prior to use. Enough TMA was present to account for all the TMA excreted by humans in the above experiments; (5) Rats fed a high choline diet excrete more TMA in their urine, DMA excretion is unchanged; (6) DMA was found in the serum and saliva of humans in both control and choline-treated groups. (CA 26731).

In the area of tumor promotion, studies have focused on the mechanism of action of the phorbol ester, 12-O-tetradecanoyl-phorbol-13-acetate (TPA), and related compounds. It was found that a single topical application of 12-O-tetradecanoyl-phorbol-13-acetate (TPA) to mouse skin induced rapid and transient increases in the levels of both c-GMP and c-AMP, followed by the stimulation of ornithine decarboxylase (ODC) activity. (Perchellet et al., 1981a). Peak syntheses of c-GMP and c-AMP were achieved within 10 and 60 minutes, respectively, whereas ODC activity was maximally stimulated between 4.5 and 6 hours. The increased levels were dose-dependent and correlated with the tumor-promoting ability. A single topical application of 10 µmol of 3-isobutyl-1-methylxanthine (IBMX), which was able to raise the levels of cyclic nucleotides almost as much as did TPA, produced only a 9-fold increase in ODC activity. IBMX, as well as other phosphodiesterase inhibitors, enhanced the magnitude and the duration of the increases in cyclic nucleotide levels and ODC activity produced by TPA. Maximum stimulation of TPA-induced ODC activity was achieved when IBMX was applied within the 30-minute time interval preceding TPA

treatment. As a result of the IBMX pretreatment, the same or higher levels of cyclic nucleotides and ODC activity could be induced with about one-tenth of the TPA or with weak and moderate tumor promoters, as compared with the increased levels attributable to TPA alone. In addition, single or combined topical treatments with c-GMP and c-AMP were unable to mimic the effect of TPA on ODC activity and even depressed significantly basal and TPA-induced ODC activities in the presence of IBMX.

Extending these protocols for causing metabolic effects to tumor induction studies gave the following results. (Perchellet et al., 1981b). Topical application of IBMX prior to each promotion with TPA reduced by 78% the number of papillomas per mouse. The inhibition was dose-dependent. All phosphodiesterase inhibitors tested inhibited the development of skin papillomas. Topical applications of C-GMP or c-AMP before each treatment with TPA were also very effective in inhibiting the formation of skin papillomas. Combined treatments including IBMX reduced by 98% the incidence of skin papillomas promoted by TPA. However, IBMX treatment 24 hours after each promotion with TPA did not suppress the formation of skin papillomas. Furthermore, repeated applications of IBMX before or after initiation with 7,12-dimethylbenz(a)anthracene did not alter the development of skin tumors. These modifiers act on some of the biochemical events proposed to be necessary components of the promotion process. They inhibit dramatically both TPA-increased polyamine levels and TPA-stimulated RNA, protein, and DNA synthesis. Combined treatments including IBMX and cyclic nucleotides produced additive inhibitions of polyamine, RNA, protein, and DNA synthesis compatible with their greater reduction of the formation of skin papillomas. Since IBMX and c-AMP block the usual accumulation of putrescine and spermidine produced by TPA in mouse epidermis in vivo and inhibit the activity of ornithine decarboxylase when added in the assay mixture, these compounds may actually interact directly with the enzyme in situ, like other ornithine decarboxylase inhibitors. (CA 22484).

Weinstein and coworkers have found that TPA induces cell adhesion in a clone of Friend erythroleukemia cells and that this provides a simple and rapid assay for the phorbol esters and related macrocyclic diterpenes. Detailed studies by these investigators have shown a good structure-function relationship between these effects and tumor promoting activity on mouse skin. (Yamasaki et al., 1981).

Specific and saturable cell surface receptors for phorbol esters in intact cell monolayers of rat embryo fibroblast cultures have been demonstrated, and several properties of these receptors have been characterized. Since it was found that ^{3}H-phorbol dibutyrate (^{3}H-PDBu) binding to intact cells at 37°C rapidly reaches a plateau, and is also rapidly reversible, it appears that most if not all of the receptors are on the cell surface, presumably associated with the plasma membrane. Epidermal Growth Factor (EGF), platelet derived growth factor, fibroblast growth factor, vasopressin and a number of other polypeptide growth factors and hormones failed to inhibit ^{3}H-PDBu binding. The abilities of a series of TPA analogs to compete with ^{3}H-PDBu for binding to cell surface receptors correlated with their known potencies in cell culture and, with the exception of mezerein, with their activities as tumor promoters on mouse skin. The indole alkaloid teleocidin B was shown to be a potent inhibitor of ^{3}H-PDBu binding. This result is of particular interest since, although this compound is structurally unrelated to the phorbol esters, it shares with these compounds a number of biologic effects in cell culture, and is also as potent as TPA as a tumor promoter on mouse skin. In collaborative studies, it was found that, like TPA, nanamolar concentrations of teleocidin B and dihydroteleocidin induce a rapid increase in 2-deoxyglucose uptake, induce

arachidonic acid release and prostaglandin synthesis, and inhibit EGF receptor binding. The results obtained with the teleocidins greatly strengthen the conclusion that the ^{3}H-PDBu receptors play a role in mediating the action of these tumor promoters. (Laskin et al., 1980; Horowitz et al., 1981; Umezawa et al., 1981).

Using an intact cell assay, similar ^{3}H-PDBu receptors were detected in a variety of cell types including Friend erythroleukemia cells, the mouse embryo fibroblast cell line 10T1/2, the rat liver epithelial cell line K22, and an adenovirus transformed rat embryo cell line. The intact cell assay for phorbol ester receptors should facilitate further studies on the physiology of this unusual receptor-ligand system. It was also found that sera from several species caused a concentration dependent inhibition of ^{3}H-PDBu binding to intact cells. Significant activity was also detected in human amniotic fluid and extracts from rat embryos or rat liver. (Horowitz et al., 1981).

It seems likely that certain human cancers may be due to interactions between chemical agents and types of viruses which alone would have little or no oncogenic potential. To explore these aspects of chemical-viral interactions, an in vitro system was developed in which the transformation of rat embryo cells is markedly enhanced when, after infection with a mutant (H5ts125) of human adenovirus type 5, the cells are grown in the presence of TPA. EGF and melittin also enhanced adenovirus transformation. Although neither TPA or EGF enhance the growth in agar of normal rat embryo cells, both compounds do induce the growth in agar of morphologically altered adenovirus-transformed rat embryo cells. This effect appears to be inductive and not due to simple cell selection. Yet it is irreversible, since when the TPA is removed, the cells grow in agar with a higher efficiency than prior to exposure to TPA. This phenomenon may represent a useful in vitro model system for studying the process of tumor progression. It was found that TPA also accelerates the replication and cytopathic effects of adenovirus in human cells. (Fisher et al., 1980a; Fisher et al., 1980b; Fisher et al., 1981a; and Fisher et al., 1981b). (CA 26056).

Since glucocorticoids can inhibit polycyclic aromatic hydrocarbon carcinogenesis and specifically inhibit tumor promotion on mouse skin, their effects on EGF receptors have been examined. It was found that glucocorticoids alone induce an increase in EGF Receptor binding in C3H 10T1/2 cells and that pretreatment of the cells with a glucocorticoid opposes the inhibitory effect on EGF binding of BP. Thus it is suggested that glucocorticoids may inhibit the carcinogenic process by inducing membrane effects that are reciprocal to those induced by carcinogens and tumor promoters. (Ivanovic and Weinstein, 1981; Weinstein, 1981; Ivanovic and Weinstein, 1982). (CA 21111).

Boutwell and coworkers have performed additional experiments designed to determine whether protocols that show inhibition of TPA-induced metabolic changes also inhibit tumor formation. After optimal conditions for inhibition of metabolic responses to retinoic acid were established, indomethacin and dexamethasone were each tested alone (as well as the protease inhibitor, tosyl lysine chloromethylketone (TLCK)) and found to effectively inhibit tumor formation; when each of the three (indomethacin, dexamethasone, and TLCK) was tested in combination with retinoic acid, the combination was found to be more effective than either agent alone. (Boutwell et al., 1981). These results provide reason for optimism that cancer prevention in human beings is a realizable goal. (CA 22484).

Continuation of studies on the definition and characterization of the stages of initiation and promotion during hepatocarcinogenesis has led to several preliminary conclusions based on the quantitation of the number of enzyme-altered foci produced in animals initiated with a small dose of diethylnitrosamine following a 70% partial hepatectomy and with subsequent promotion by several agents. (1) The promoting agent phenobarbital was found to exhibit both a no-effect (threshold) level (approximately 0.005% in the diet) as well as a dose beyond which no further foci are induced (0.1% in the diet). (2) Following initiation there is an increase in the appearance of foci for two months when phenobarbital is fed. Following this time no further increase or decrease in the total number of foci occurs. After two months of phenobarbital feeding following initation, no change in the distribution of phenotypes in enzyme-altered foci using 3 histochemical markers, (Ɣ-glutamyl-transpeptidase, canalicular ATPase, and glucose-6-phosphatase) was observed.

Through the use of an automated system for the scoring and quantitative determination of the number of enzyme-altered foci, it has been demonstrated that the quantitation of altered foci by means of a two-dimensional analysis (simple enumeration of focal intersections/area of tissue section) is proportional to the quantitation of foci per volume of liver, provided that the mean diameter of the foci with each treatment is sufficiently uniform. When such mean diameters are unequal, as is usually the case in these experiments, quantitation from three-dimensional analysis gives significantly different results when compared with enumeration of focal intersections on two-dimensional areas. (Campbell et al., 1982).

When two additional markers, stainable iron deficiency and the presence of the "PN" antigen (epoxide hydrase), are employed, little or no increase in the total number of foci scored can be seen. The most efficient marker for scoring foci in animals subjected to both initiation and promotion is Ɣ-glutamyltranspeptidase. This marker scores between 70 and 80% of the total foci. (Pitot, 1980). (CA 22484).

During the past year progress was made in understanding the mechanism by which caffeine enhances the lethality of nitrogen mustard for hamster cells in culture. (Lau and Pardee, 1982). At low doses of nitrogen mustard, most of the DNA lesions are repaired while the cells are held in a late S/G2 state for several hours. Thereafter, these cells complete their cycle. Caffeine has the effect of preventing damaged cells from being arrested in S/G2 and so they proceed through mitosis and cell division while their DNA is still damaged. As a consequence, their chromosomes are shattered when they go through mitosis. The frequency of this nuclear damage correlates very closely with lethality. This role of caffeine apparently is not direct, but requires active protein synthesis, suggesting that some protein required for transit through G2 cannot be made after DNA is damaged; but it is made in spite of this damage when caffeine is present. A few other compounds tried in place of caffeine do not appear to be as effective.

These fundamental observations on the mode of lethal enhancement in hamster cells by caffeine were extended to human cells in culture. These cells respond quantitatively differently to caffeine after they are damaged than do hamster cells. The difference may be due to the very active excision system of human cells, a system not affected by caffeine which is functional in relation to "postreplication repair." Nevertheless, caffeine does have interesting effects of possible clinical relevance for human cells. (Lau and Pardee, 1982). (CA 22427).

Progress has been made towards the elucidation of the role of hormones in cancer causation. The effect of steroid hormones on RNA synthesis was studied in isolated

cell suspensions and tissue minces prepared from high yield serially transplanted estrogen-dependent hamster renal carcinoma. In these studies, minced tumor or viable isolated tumor cells were preincubated in the presence or absence of hormone. Following this preincubation period, a pulse of (^{3}H)-uridine was introduced. Results of these studies indicate that 17β-estradiol stimulates the uptake and incorporation of uridine into RNA at concentrations 10^{-8} to 10^{-6}M. Other estrogens as well as androgens at similar concentrations, but not 17α-estradiol, markedly enhanced the uptake of uridine into RNA compared to control incubations with no hormone present. In contrast, both progesterone and triamcinolone did not appear to stimulate the incorporation of labeled precursor into RNA. These results represent the first demonstration for a direct effect of steroid hormones on RNA synthesis in the hamster renal carcinoma. (Li et al., 1980).

The effect of certain compounds which differently affect mixed-function oxygenases on the inducibility of renal tumors by estrogens was assessed. Neither butylated hydroxyanisole (BHA), 0.5% in diet, β- nor α-naphthoflavone, 0.2% in diet, appreciably competed for the estrogen, androgen, progesterone or glucocorticoid receptor in the hamster kidney at 1000-fold excess concentration. In addition, the presence of these inhibitors did not affect the ability of either diethylstilbestrol (DES) or 17β-estradiol to induce progesterone receptor in the hamster kidney. These data indicate that hormonally active estrogens are reaching the kidney in sufficient amounts to cause a biologic effect in the presence of these inhibitors. Concomitant administration of either BHA or β-naphthoflavone and DES only slightly reduced the incidence of estrogen-induced renal tumor foci in the hamster whereas a dramatic decline (>85%) in the number of renal tumor foci was observed in animals treated with DES and α-naphthoflavone after 8.5-months of treatment compared to similar animals treated with DES alone. This finding represents the first nonhormonal intervention of estrogen carcinogenesis in the hamster kidney. (CA 22008).

It has previously been shown that chronic restriction of food intake inhibits the development of mammary tumors in mice. Meites and coworkers have found that reduced food intake during the "critical period" for induction of mammary tumors in rats by 7,12-dimethyl benz(a)anthracene (DMBA) can produce inhibition of mammary tumor development and that administration of estradiol benzoate, haloperidol, and growth factor can counteract the inhibition by underfeeding. (Sylvester et al., 1981). The data indicate that the inhibitory effect of underfeeding on the formation of DMBA-induced mammary tumors in rats was largely the result of a hormonal deficiency state at the time of tumor initiation. (CA 10771).

Although dehydroepiandrosterone (DHEA) and DHEA-sulfate are major secretory products in men and women, their biological function is still unknown. Studies by several investigators have suggested that women with subnormal levels of these steroids may be predisposed to develop breast cancer. Schwartz and coworkers have shown that DHEA inhibits Epstein-Barr virus (EBV) induced morphologic transformation and stimulation of DNA synthesis in human lymphocytes. (Henderson et al., 1981). Since it was also found that 16 α-Br-epiandrosterone, a more potent inhibitor of mammalian glucose-6-phosphate dehydrogenase than DHEA, was also much more effective as an inhibitor of EBV-induced transformation, it is suggested that the inhibition of transformation may result from the inhibition of glucose-6-phosphate dehydrogenase. (CA 14661).

5-Fluoro-12-methylbenzanthryl-7-acetic acid (5-FMBAAA) is a noncarcinogenic analog of 7,12-dimethylbenz(a)anthracene (DMBA). When conjugated to the protein carrier BSA, 5-FMBAAA elicited broadly reactive antibodies in mice. It was found that

repeated cutaneous applications of ng quantities of DMBA in dodecane, alternating with application of the potent tumor promoter TPA, constitutes a satisfactory low-dose carcinogenesis model. (Moolten et al., 1981). Mice immunized with the 5-FMBAAA:BSA conjugate and exposed to this regimen developed significantly fewer skin tumors than did unimmunized mice, mice immunized with BSA or mice immunized with an unconjugated mixture of BSA and 5-FMBAAA. Immunization did not significantly reduce the incidence of tumors in mice treated with TPA alone. These studies indicate that, when mice are exposed to low levels of carcinogen, immunization against the carcinogen would appear to provide specific protection. (CA 23534).

Biological and Chemical Prevention

Selenium supplementation to the diet or drinking water has been shown to inhibit chemically-induced carcinogenesis in colon, skin, mammary gland and liver; to suppress mouse mammary tumor virus-induced tumorigenesis; to inhibit DMBA- and MNU-induced rat mammary gland carcinogenesis even more effectively when combined with retinyl acetate; and under conditions of selenium depletion and high polyunsaturated fat levels in the diet to increase DMBA-induced mammary cancer. Epidemiologically, observations have shown an inverse relationship between selenium levels in grains and forage crops and human breast cancer. Recent studies (Medina and Shephard, 1981) now show that selenium inhibits DMBA-induced mammary carcinogenesis in the mouse and that such inhibition is general among several mouse strains. Furthermore, selenium inhibits the appearance of DMBA-induced ductal hyperplasias in several mouse strains as well as mammary tumor virus-induced alveolar hyperplasias. On the other hand, selenium has little effect on early transplant generation mammary tumors, tumors known to have similar growth properties to the in situ tumor. These results indicate that selenium inhibits the occurrence of early stages in mammary tumorigenesis. Since selenium in these studies was provided two weeks prior to carcinogen for the duration of the studies, it is not known whether selenium-mediated inhibition is more significant as an anti-initiator or an anti-promoter (CA 11944). Results of others do demonstrate, however, that selenium can inhibit post-initiation events.

The glutathione S-transferases (GST) constitute a major detoxification system catalyzing the binding of numerous electrophiles to glutathione, including carcinogenic electrophiles. Enhancement of this detoxification system is potentially an important approach for inhibiting the neoplastic effects of chemical carcinogens. Recent studies have shown that a variety of substances, many of them edible foods or compounds isolated from dietary material, have this capacity to induce increased GST activity (Sparnins et al., 1982). Many of these inducers are, in fact, inhibitors of carcinogenesis. For example, benzylisothiocyanate, coumarin, indole-3-carbinol, indole-3-acetonitrile, α-angelicalactone, disulfiram and β-naphthoflavone all induce increased liver and small intestinal GST in the mouse, and all are inhibitors of chemical carcinogenesis. The first four of these compounds exist in natural sources of food. Brussels sprouts, cabbage, coffee beans and tea leaves have similarly significantly induced the GST system (CA 14146).

Since the glutathione-S-transferase system is importantly involved in xenobiotic detoxification to form glutathione (GSH) thiol ethers, which are subsequently converted to mercapturic acids by sequential reactions catalyzed by γ-glutamyl transpeptidase, dipeptidase and N-acetylase, studies proceed to define the components of this complex system in many organs and tissues under a variety of conditions. Seven GSTs have been isolated from rat liver and at least five have

been reported to exist in human liver. All of the human liver transferases are cationic in nature and immunologically similar. Recent work has now identified two new human liver transferases (Awasthi et al., 1980). These additional GST, however, are anionic in nature. The major anionic transferase differs significantly in catalytic properties from the cationic transferases. Antibodies raised against the cationic GST cross-react with the major anionic transferase (CA 27967).

Dr. David E. Ong of the Vanderbilt University School of Medicine (CA 20850) has shown from autoradiographs of rat liver nuclei that retinol bound specifically to retinol binding protein is distributed throughout the nucleus. Thus, it was concluded that cellular retinol binding protein delivers retinol to the interior of the nucleus to specific binding sites which are primarily, if not solely, on the chromatin. Binding to these sites may affect gene expression (Liau, et al, 1981).

Dr. Rajendra G. Mehta of the IIT Research Institute (CA 26030) has found that hormone independent mammary tumors contain elevated levels of retinoic acid binding protein (RABP), and that the glands of pregnant animals contain significantly higher levels of cytosolic RABP than that of lactating glands. It was concluded that an inverse relationship may exist between retinoid and estrogen receptors (Moon and Mehta, 1982).

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SUMMARY REPORT
BIOLOGICAL AND CHEMICAL PREVENTION PROGRAM

Strategies for cancer prevention involving reduction or elimination of human exposure to environmental carcinogens may not always be possible. Further, significant portions of the human cancer burden may be due to endogenous carcinogens, cocarcinogens and promoters. The biological and chemical approach to cancer prevention seeks to inhibit, reverse, arrest or delay the carcinogenic process by administration of selected chemical compounds or combinations of chemical compounds, biological agents or combinations of biological agents, or by combined use of both biological and chemical agents. A large number of studies on experimental animals have already demonstrated inhibition of tumorigenesis at many organ sites such as liver and lung, large and small intestine, breast, skin, bladder, and forestomach. Inhibition has been shown for both chemically-induced and radiation-induced cancers, as well as inhibition of the so-called spontaneously-arising tumors. Furthermore, chemoprevention agents have suppressed malignant and phenotypic transformation in culture and inhibited both UV-and carcinogen-induced bacterial mutagenesis.

Retinoid Program

A number of efforts investigate the chemoprevention of carcinogenesis by retinoids. These natural and synthetic analogs of vitamin A have already been shown to inhibit or delay the development of invasive malignancy in animal models of epithelial carcinogenesis in skin, lung, breast, bladder, and pancreas, and to suppress malignant and phenotypic transformation in culture whether caused by chemical carcinogens, ionizing radiation, or polypeptide transforming factors. Effective chemoprevention of epithelial cancer by retinoids will require compounds capable of arresting, reversing, and otherwise inhibiting the carcinogenic process in the desired target organ and tissue, while at the same time, providing sufficiently non-toxic effects upon the host.

For this purpose, several contracts are devoted to the synthesis of new retinoid structures, to assay in vitro for their possible chemopreventive efficacy, to determine their toxicologic properties, and to evaluate their anticarcinogenic activity in animal systems in vivo. These efforts are providing increased knowledge of retinoid pharmacokinetics, structure-activity relationships and chemopreventive capacity.

In the area of retinoid synthesis, three contracts are devoted to investigations on structure/activity relationships and the development of compounds with improved pharmacological properties. At the University of California (NO1-CP-05715) emphasis is placed upon synthesis of allenic retinoids at various positions along the side chain of the molecule (9,10-; 11,12-; 7,8-; 6,7-; and 10-11- allenes are potential target molecules). In many cases, the geometric isomers and functional group derivatives such as the vinylallene alcohols, aldehydes, acids and esters will be synthesized. Synthesis of acetylenic retinoids will also be pursued. Efforts have been devoted initially to the synthesis of 9,10-allenes, their thermal rearrangement products and acetylenic retinoids. One of these compounds has significant activity in reversal of keratinization in the hamster tracheal organ culture bioassay system. Two acetylenic retinoids appear to be inactive. An important result is the determination that (7Z)-9,10-allenes undergo spontaneous electrocyclic ring closure across carbons 5 and 10 to afford drimatriene derivatives. This fleeting existence appears to make this class of allenic retinoids impossible to synthesize.

At Cornell University (NO1-CP-05716) efforts are directed at synthesis of bicyclic and tricyclic analogues of retinoic acid. The contract investigates the hypothesis that the precise conformation of the flexible retinoid side chain importantly determines biological activity. To this end a series of cyclic retinoid analogues have been designed containing aromatic rings as integral parts of the polyene side chain and all therefore have restricted side chain rotation. With few exceptions these compounds have been found to be much more stable than normal retinoids. A few have activities for reversal of keratinization in the hamster tracheal organ assay equivalent to all-trans-retinoic acid. These compounds have structures containing a dimethyltetrahydronaphthalene ring system in place of the trimethylcyclohexene ring of retinoic acid. On this basis, the tentative conclusion has been made that the 1,7 single bond must maintain an s-cis conformation and that coplanarity of the 1,2 and 7,8 double bonds is a requirement for activity.

In the contract at SRI International (NO1-CP-05600), studies this past year have been aimed at the synthesis of analogs having more desirable pharmacological properties. These analogs have both steric and electronic modifications in the side chain, polar terminus and ring of the retinoid skeleton. They can be grouped into the following categories:

1. Compounds in which the (13E)-13-CH_3-15-CO-$_2$H moiety of (E)-, (7E,9E,11Z)-and (7E, 9Z,11E)-retinoic acid has been replaced by a 3,4-diacetoxyphenyl ring.

2. Compounds in which the (11E,13E)-13-CH_3-15-CO_2H moiety of retinoic acid has been replaced by a meta-substituted-4-carboxyphenyl or a meta-substituted-4-carboethoxyphenyl group.

3. A compound in which the side chain and polar terminus of retinoic acid has been replaced by a 4'-carboxybiphen-4-yl group.

4. Compounds in which the β-cyclogeranylidene ring has been replaced by an acyclic species.

A wide range of activities has been displayed by these compounds in the hamster TOC bioassay system, even within a single class of derivative, with a number of compounds having quite reasonable activities. A review of compounds from all three of these synthesis efforts is underway for potential use in studies of inhibition of carcinogenesis and toxicity.

A major undertaking in the program has been the study of the efficacy of retinoids to inhibit or delay the development of cancer in well-defined animal models of carcinogenesis. Retinoids are known to inhibit or delay carcinogenesis in skin, breast, bladder, respiratory tract and, as reported tentatively last year, in pancreas (Dartmouth Medical School, NO1-CP-85675). This new development on retinoid inhibition of azaserine-induced pancreatic carcinogenesis in Lewis rats has been further extended in experiments with a second group of four retinoids. All retinoids were fed in the diet following a course of azaserine injections. Although there has been some concern for toxicity in these experiments, significant inhibition of the progression of carcinogen-induced lesions has again been shown. The most effective retinoids appear to be N-(pivaloyloxyphenyl)retinamide, N-(2,3-dihydroxypropyl)retinamide and N-(2-hydroxypropyl)retinamide. In another study, these same retinoids were employed in experiments on the inhibition of N-nitrosobis(2-oxopropyl)amine-induced pancreatic carcinogenesis in Syrian golden hamsters. Retinoids were again fed after carcinogen administration (2x20 mg/kg body

weight). In this case, statistically significant inhibition was not shown. However, in female hamsters fed all four retinoids and in males fed two of the retinoids (six out of eight retinoid groups), a trend is demonstrated by the Spearman rank correlation coefficient toward reduced numbers of carcinomas when compared with the non-retinoid-treated control groups (Dartmouth Medical School, NO1-CP-85675). Interesting results with four different retinoids have been found in the hamster-BOP model of pancreatic carcinogenesis at the University of Nebraska (NO1-CP-85674). Here in male hamsters fed the four different retinoids and in females fed two of these retinoids (six out of eight retinoid groups), apparent enhancement of carcinogenesis is seen when BOP is given once at the dose 40 mg/kg body weight. Two to four-fold higher levels of retinoid were employed in these experiments than those cited above. If, however, a lower retinoid level was employed, comparable to the Dartmouth studies, no influence on pancreatic carcinogenesis was seen.

In the contract at IIT Research Institute (NO1-CP-05718) studies continue on retinoid inhibition of mammary carcinogenesis. Previous work has shown that five different retinoids significantly prolong the latency of mammary cancer appearance and reduce the number of mammary cancers induced by N-methyl-N-nitrosourea (MNU) in Sprague-Dawley rats. Five other retinoids were ineffective in this system. In a recently completed study, β-carotene was found to be ineffective in inhibiting MNU-induced mammary cancer. Relatively poor absorption of carotene may play a role in this result. Emphasis in this contract includes combination chemoprevention studies of retinoid plus tamoxifen (an antiestrogen) and retinoid plus flurbiprofen (an inhibitor of prostaglandin biosynthesis), as well as studies on the efficacy of the retinoid N-(4-pivaloyloxyphenyl)retinamide against MNU-induced carcinogenesis. The combination chemoprevention studies are important since this group has previously shown that dietary 4-hydroxyphenylretinamide combined with ovariectomy almost completely prevents MNU-induced mammary cancer.This result along with other data has led to the significant though tentative conclusion that ovarian hormone-independent cancer appears to be highly sensitive to inhibition by retinoids.

A project at the Brookhaven National Laboratory (YO1-CP-00202) investigates the effect of retinyl acetate on x-ray-induced mammary gland carcinogenesis. This project has special significance since radiation is the only known carcinogen for human breast cancer on the one hand, while investigations on retinoid suppression of experimental radiation carcinogenesis have not been done on the other. Other studies include the effect of retinyl acetate on dimethylbenzanthracene (DMBA)-induced mammary cancer and on the occurrence of spontaneously-arising mammary cancers. All studies employ the female Sprague-Dawley rat. A specific focus in these studies is on the capacity of the retinoid for inhibition of carcinogenesis over the lifespan of the animal. In both x-ray and DMBA studies, retinoids were provided in the diet one week following carcinogen. At ten months into the studies both incidence of carcinogen-induced mammary adenocarcinomas and total numbers of cancers are decreased in the retinyl acetate-treated animals. In the x-irradiated and DMBA-exposed animals the incidences of adenocarcinomas are decreased by 56% and 32% respectively.

At the Middlesex Hospital Medical School (NO1-CP-05602) a new series of experiments has begun on retinoid suppression of N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN)-induced urinary bladder carcinogenesis. In these experiments B6D2F1 mice are being employed in which BBN induces highly invasive transitional cell carcinomas similar to those frequently seen in man. At this time, after a relatively high dose of BBN, an experiment has been terminated after five months in which N-(4-hydroxyphenyl)-

retinamide (HPR) has produced a marked reduction in tumor incidence and in grade and stage of the developing tumors. On the other hand, N-(tetrazol-5-yl)retinamide (TR) has neither inhibited incidence nor improved stage or grade of the developing cancers. An experiment in which the mice have received just half the BBN dose is continuing in which the effects of three retinoids on carcinogenic response after a longer period of time can be evaluated. In other efforts, emphasizing the development of an anti-promotion model of bladder carcinogenesis, preliminary range-finding experiments have been completed in the F344 rat determining an appropriate low dose of bladder carcinogen (BBN) for use in a study of saccharin promotion, and then potential anti-promotion by retinoids. These studies should start in the near future.

In the contract at Michigan State University (NO1-CP-05717), inhibition of mammary carcinogenesis by retinoids in several different rat and mouse models is being investigated. In addition, combination chemoprevention studies have been conducted employing various combinations of retinyl acetate (RA), immune stimulation (IS) and hormone inhibition (HI) against DMBA-induced mammary carcinogenesis in female Sprague-Dawley rats. Both non-specific immune stimulation (either Norcardia rubra subcutaneous weekly injections or methanol-extracted residue of Bacillus Calmette-Guerin injected twice after DMBA administration) and specific immune stimulation have been studied. Specific IS was provided by injecting i.p. mammary carcinoma membrane preparations mixed with complete Freunds adjuvant at weeks 1, 3 and 5 after DMBA treatment. Hormone inhibition (HI) was provided by daily injections of the non-steroidal estrogen antagonist tamoxifen and the dopaminergic agonist and prolactin secretion suppressor 2-bromo-α-ergocryptine. Results in these combination prevention experiments are very exciting. It has been found that mammary tumorigenesis is significantly reduced in rats treated with HI or RA, and that tumor incidence is further reduced by combining these two treatments, (HI + RA). Specific IS significantly reduced mammary tumor incidence in retinyl acetate-fed rats (Specific IS + RA). Furthermore, rats which were fed RA and concurrently received Specific IS and HI treatments never developed mammary tumors at all for the duration of the 20 week experiment. By this time, 100% of the controls had developed mammary carcinomas. It is noteworthy that Specific IS reduced mammary tumor incidence in retinoid-fed rats but not in placebo-fed animals. Non-specific immune stimulation was not effective, either alone or in combination with RA, HI or (RA + HI).

Investigations on the efficacy of retinyl acetate in three different mouse models of mammary carcinogenesis have not been hopeful up to this time. Thus, no inhibition of DMBA-induced mammary cancer in the BDF/J mouse has been seen nor has inhibition of spontaneous mammary tumorigenesis in the mammary tumor virus-carrying C3H/He mouse been found (neither nulliparous nor multiparous). In the third mouse model, hormone-induced mammary tumorigenesis in female GR/A mice, enhancement has, in fact, been seen. Profound differences between the carcinogen-induced rat mammary model and this steroid hormone-induced mouse mammary model are known, and will need to be considered in assessing this result (Michigan State University, NO1-CP-05717).

Investigations on the toxicology of retinoids are carried out under two contracts. Recent emphasis at Battelle Columbus Laboratories (NO1-CP-85650) has been placed upon short term toxicologic studies of synthetic retinoids of the "stilbenoid" class. These compounds have very high activities in reversal of keratinization in the hamster tracheal organ culture system. Unfortunately, these compounds are also more toxic than all-trans-retinoic acid. Endpoints for toxicological evaluation included clinical appearance, growth, mortality and radiographic analysis of the long bones.

At the Southern Research Institute (NO1-CP-85615) an interesting series of structure/activity studies have been performed on the toxic effects of various substituted all-trans and 13-cis retinamides, in comparison with the toxic effects of the retinoic acids. Lethality data obtained after intraperitoneal administration to mice as well as clinical observations and histopathological evaluation gives the following order of toxicity: all-trans-retinoic acid was most toxic followed by 2-hydroxypropylretinamide >13-cis-retinoic acid >2-hydroxyethyl-retinamide >2,3-dihydroxypropylretinamide >3-hydroxypropylretinamide >4-hydroxybutylretinamide >4-hydroxyphenylretinamide >propylretinamide >ethylretinamide >butylretinamide. The LD50 values ranged from 32.6 for all-trans-retinoic acid to 5780 mg/kg body weight for butylretinamide. In general, lethality data obtained after oral administration gave the same relative rankings. It is clear from these data that modification of the polar terminal group of retinoic acid substantially alters toxicity. Endpoints indicative of toxicity in these studies included peripheral anemia, increases in alkaline phosphatase, decreases in albumin concentration, hepatocellular degeneration and necrosis, suppurative or granulomatous peritonitis, mesenteric and thoracic lymph node atrophy, and spermatogenic arrest.

Phenolic Antioxidants

The phenolic antioxidants butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are perhaps the most broadly studied of all presently known chemopreventive agents. They are known to effectively inhibit neoplasia induced by many classes of chemical carcinogen at many organ sites. For example, these two compounds are inhibitors of tumorigenesis induced by nitrosamines; polycyclic aromatic hydrocarbons; 4-nitroquinoline-N-oxide; 1,2-dimethylhydrazine; azoxymethane; methylazoxymethanol acetate; 4-dimethylaminoazobenzene; N-2-fluorenylacetamide; N-hydroxy-N-2-fluorenylacetamide; uracil mustard; and urethane. Inhibition of neoplasia has been demonstrated for mouse lung, forestomach, skin and colon as well as for liver, breast and colon of the rat. Moreover, in recent work BHA has been shown to inhibit neoplasia of the forestomach, lung, and lymphoid tissues of the mouse induced by (+)-trans-7,8-dihydroxy-7,8-dihydrobenzo(a)pyrene, which is considered a proximate carcinogenic metabolite of the parent hydrocarbon, and to inhibit mutagenesis induced by known carcinogens and a number of antischistosomacidal compounds.

However, much of this information on inhibition of carcinogenesis by these compounds is available only at high concentrations. For this reason, basic dose-response studies have been initiated to investigate BHA and BHT inhibition of neoplasia at four organ sites: mammary gland, liver, colon and lung. At the American Health Foundation (NO1-CP-05721) studies are proceeding on inhibition of carcinogen-induced intestinal cancer by four levels of BHT in NIH-07 diet. These studies employ male F344 rats and two levels of the intestinal carcinogen azoxymethane (AOM), which was injected once subcutaneously. Dietary antioxidant was provided for a four week period only, two weeks before through two weeks after AOM administration. Endoscopic examination at 44 weeks after the lower AOM dose indicates a clear dose-response relationship for inhibition of incidence of colonic tumorigenesis (0 ppm/58%; 300 ppm/42%; 1000 and 3000 ppm/17%; and 6000 ppm/8%). A second experiment is proceeding on the dose-response relationship for inhibition of colon carcinogenesis by the related phenolic antioxidant, butylated hydroxyanisole (BHA). These studies employ female CF1 mice, methylazoxymethanol (MAM)-acetate as the carcinogen, the same levels of BHA in the diet and similar time relations for carcinogen and inhibitor (BHA two weeks before through two weeks after carcinogen). Results are not yet known in this study though an interesting decrease in toxicity and mortality has been noted with BHA increase in MAM-treated mice.

A second project at the American Health Foundation (NO1-CP-05722) has investigated inhibition of DMBA-induced mammary carcinogenesis by BHT in female Sprague-Dawley rats. Two doses of DMBA and 4 levels of BHT in the diet were employed. BHT was provided in the diet for two weeks before through two weeks after DMBA. At the lower DMBA dose, a clear-cut dose-response effect was seen for mammary tumor inhibition. On the other hand, at the higher DMBA dose only the highest level (6000 ppm) of BHT in the diet was capable of inhibiting tumor development. Benign tumors of the adrenal cortex and preneoplastic liver foci induced by the higher DMBA dose were also inhibited by BHT in a dose-response manner.

In a third project at the American Health Foundation (NO1-CP-05723), the chemopreventive activity of BHT in inhibition of liver carcinogenesis is being explored. In this case, BHT at the same four levels in the diet as indicated previously is provided concurrently and continuously along with carcinogen (2-acetylaminofluorene). Results available at 18 weeks into the study demonstrate that BHT produces a dose-dependent inhibition of preneoplastic altered foci in the liver. Further, the development of tumors was also inhibited, with no tumors seen at all at this time with 6000 ppm BHT. In summary, these three studies at American Health Foundation demonstrate a dose related inhibition of carcinogenesis in three target organs, colon, breast and liver. The observations that inhibition was more pronounced at relatively low dose levels of carcinogen and was apparent even with the low dose level of antioxidant employed are important, since it seems likely in many instances that humans may be exposed to relatively low dose rates of carcinogen. Furthermore, it was found that there was a relationship between the appearance of early lesions and the eventual later development of cancer. Therefore, in studies on chemoprevention, it may be indicated to utilize the early lesion as an indicator of chemoprevention. This may allow screening for chemoprevention effects more efficiently, yet reliably.

A fourth contract on phenolic antioxidant inhibition of neoplasia at the University of Minnesota (NO1-CP-05605) concentrates on the efficacy of 2(3)-butylated hydroxyanisole (2(3)-BHA) in inhibiting carcinogen-induced pulmonary adenoma formation in the mouse. These experiments employ the female ICR/Ha mouse, two dose levels of the carcinogen benzo(a)pyrene (BP) and four levels of 2(3)-BHA in the diet, 5 mg/gm, 3 mg/gm, 1 mg/gm and 0.33 mg/gm. Experiments were terminated 52 weeks after the first BP administration. Results indicate that all levels, including the lowest level of 330 ppm antioxidant, suppress pulmonary adenoma formation, with a greater inhibitory effect against the lower dose of BP. Thus, these results again suggest the important conclusions that the efficacy of antioxidant inhibition of neoplasia increases as carcinogen exposure decreases, and that significantly lower doses of antioxidant are effective than heretofore known.

Natural Inhibitors

The objectives of efforts on natural inhibitors of carcinogenesis are to determine if naturally-occurring materials have the capacity to inhibit the occurrence of neoplasia, to determine the range of tumors inhibited and the conditions under which inhibition is seen, and to identify the specific chemical compounds in the naturally-occurring materials responsible for inhibiting activity. In the project at the American Health Foundation (NO1-CP-85659), efforts are made to assess the protective role of various sources of dietary fiber on chemically-induced digestive tract cancer. Previous results have shown that wheat bran at a level of 15% in the diet is capable of producing a significant reduction in both azoxymethane (AOM)-induced and 3,2'-dimethyl-4-aminobiphenyl (DMAB)-induced rat colonic tumorigenesis, as well as a significant reduction in tumorigenesis in the small intestine induced

by these two carcinogens. Dehydrated citrus pulp has also been shown to inhibit AOM-induced tumorigenesis in both colon and small intestine and DMAB-induced intestinal tumorigenesis. Citrus pulp has had no effect on colonic tumorigenesis induced by DMAB under the conditions of these experiments. A number of hypotheses on the mechanism(s) of fiber inhibition of digestive tract tumorigenesis have been proposed including dilution of carcinogens, promoters, and cocarcinogens in the digestive tract due to increased stool bulk; specific and/or enhanced carcinogen binding by fiber components; diet-dependent induction of hepatic enzymes or intestinal mucosal enzymes of activation and detoxification; a complex balance modulated by diet on the bioavailability of micronutrients such as various vitamins and minerals; and effects on the composition and/or metabolic capacity of the fecal flora.

New experiments on the efficacy of corn bran to inhibit AOM-induced digestive tract tumorigenesis have been completed recently. This source of dietary fiber differs from wheat bran with respect to its content of certain fiber components. The hemicellulose content of corn bran, for example, is higher while the lignin content is lower. Corn bran at the 5% and 10% level in the diet does not appear to inhibit AOM-induced tumorigenesis in either the colon or the small intestine. Further studies are in progress on the potential of 15% corn bran, and on 7.5% lignin-containing diets to inhibit DMAB-induced tumorigenesis. Results in this investigation are not yet available. However, colonic endoscopy of 10 control and 10 lignin-fed animals shows 4 tumor-bearing rats in each group at this time.

BIOLOGICAL AND CHEMICAL PREVENTION OF CARCINOGENESIS

CONTRACT INDEX

AMERICAN HEALTH FOUNDATION (NO1-CP-05721)

Title: Dose Response Studies on Phenolic Antioxidants (Intestinal Tract)

Contractor's Project Director: Dr. Bandaru S. Reddy

Project Officer (NCI): Dr. Carl E. Smith

Objectives: The overall objective of the program is to evaluate the efficacy of phenolic antioxidants, namely butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT), as chemopreventive agents in intestinal carcinogenesis. Specifically, the program will assess the efficacy of dietary 300, 1000, 3000 and 6000 ppm BHT or BHA on azoxymethane (AOM)- or methylazoxymethanol (MAM) acetate-induced intestinal carcinogenesis in male F344 rats or female CF_1 mice, respectively.

Major Findings: In order to determine the protective effect of BHT during the initiating phase of carcinogenesis, different levels of BHT (NIH-07 diet with 0, 300, 1000, 3000 and 6000 ppm; AIN-76 semipurified diet with 0 and 6000 ppm) were fed to 5-week-old male F344 rats 2 weeks before, during, and 2 weeks after azoxymethane (AOM) administration. The animals receiving BHT diets were then transferred to their control diets without BHT. AOM treatment started at 7 weeks of age (two dose levels; 29.6 or 14.8 mg/kg body wt; once subcutaneously). The experiment is in its 44th week post-AOM treatment and will be terminated in 2 weeks. Endoscopic examination of the colons of rats treated with low level of AOM indicate a dose-response inhibition of colonic tumors with 58% incidence in rats fed 0 ppm BHT, 42% incidence in 300 ppm BHT-fed animals, 17% incidence in 1000 and 3000 ppm BHT groups, and 8% incidence in rats fed 6000 ppm BHT diet.

A second experiment has been initiated to investigate inhibitory effect of 4 levels of BHA (300, 1000, 3000 and 6000 ppm) in NIH-07 diet and 6000 ppm of BHA in AIN-76 semipurified diet on MAM acetate-induced colon carcinogenesis in female CF_1 mice. Toxicity studies were conducted to determine the optimum dose levels of MAM acetate. The 72-hour survival rate of mice fed the BHA diets and treated with 15 mg MAM acetate/kg body wt. was 100%, whereas in mice treated with 20 mg MAM acetate/kg body wt., the survival rate was dependent on the level of BHA in the diet. The dietary BHA protected against MAM acetate-induced toxicity and mortality in female CF_1 mice. Based on the toxicity study, two dose levels of MAM acetate were selected: 15 mg/kg body wt., 4 times in 11 days or 8 times in 22 days with a total dose of 60 or 120 mg/kg body wt., respectively. Dietary and carcinogen treatment were as described for the rat study. The experiment is in its 32nd week and will be terminated during the 46th week. During the course of the experiment, mortality was observed in mice treated with high dose of MAM acetate (total dose of 120 mg/kg body wt.). Interestingly, none of the animals died in groups fed 6000 ppm BHA, whereas the mortality rate in animals fed 0, 300, 1000 and 3000 ppm BHA was 33, 23, 6 and 8%, respectively.

Significance to Biomedical Research and the Program of the Institute:
Chemoprevention which focuses on the inhibition of carcinogenesis by chemical agents is a concept that exists for preventing cancer not only because these chemical agents prevent carcinogens from reaching or reacting with critical target sites, but they also can inhibit the promotional phase of neoplasia. These chemical agents include, besides other compounds, phenolic antioxidants, such as BHA or BHT. However, in terms of human consumption, there should be detailed dose-response studies on BHA and BHT under defined protocols in animal models. Thus, a data base on the potency of these inhibitors obtained from this program could provide convincing evidence on the inhibitory effect of these antioxidants in colon carcinogenesis. An understanding of the mechanism of the effect of these antioxidants in relation to colon cancer inhibition may provide a sound basis for a reduction of the risk of developing cancer of the colon.

Proposed Course:
1. Termination of the experiment and histopathologic evaluation of intestinal tumors in rats fed different levels of BHT and mice fed different levels of BHA.

2. Investigate the effect of various levels of dietary BHT in rats and BHA in mice on AOM- or MAM acetate-induced intestinal tumors. In these experiments, antioxidants will be fed beginning 2 weeks prior to carcinogen administration and continuing for the entire course of the experiments.

Date Contract Initiated: September 30, 1980

Current Annual Level: $128,452

AMERICAN HEALTH FOUNDATION (NO1-CP-05722)

Title: Dose Response Studies on Phenolic Antioxidants (Mammary Gland)

Contractor's Project Director: Dr. Leonard A. Cohen

Project Officer (NCI): Dr. Carl E. Smith

Objectives: The overall objective of the program is to evaluate, in animal model systems, the efficacy of two phenolic antioxidants, namely butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), as chemoprophylactic agents in breast carcinogenesis.

Major Findings: The first segment of this study, namely assessment of the inhibitory effect of BHT added to the diet at 6000, 3000, 1000 and 300 ppm for a period of 2 weeks before and 2 weeks after administration of high (15 mg/rat) and low (5 mg/rat) concentrations of dimethylbenz(a)anthracene (DMBA), has been completed. The key research findings are as follows: A clear-cut dose-response effect in terms of palpable mammary tumor incidence and/or tumor multiplicity was found only in animals treated with a low dose of DMBA (5 mg/rat). In contrast, at the high dose of DMBA (15 mg/rat) although BHT at 6000 ppm inhibited tumor development, tumor incidences at 3000, 1000 and 300 ppm BHT were indistinguishable from controls. These results suggest in the latter 3 test groups that the strength of the carcinogenic stimulus overcame the modulating effects of BHT. In addition, benign tumors of the adrenal cortex, induced by a high dose of DMBA in 60-70% of treated animals, were inhibited in a dose-response manner by BHT; whereas similar

tumors induced by a low dose of DMBA though inhibited, were not inhibited by BHT in a dose-response manner. Pre-neoplastic foci were also found in the livers of almost all animals treated with a high dose of DMBA. These foci (#/cm^2) decreased in number proportionate to the dose of BHT administered. Hence, BHT appears to antagonize the carcinogenic effect of DMBA at three different organ sites in this model system.

In an attempt to gain further insight into the mechanism by which BHT inhibits DMBA-induced mammary tumor development, an established neoplastic cell line, derived from a DMBA tumor, was tested for its in vitro growth responses to BHT. Preliminary tests indicate that BHT, added to the culture medium at 10^{-5}M, inhibits tumor cell growth up to 40% of untreated controls, while at 10^{-6}M - 10^{-8}M the inhibitory effect of BHT was minimal. These results suggest that BHT may act by a direct mechanism to inhibit the growth of developing mammary tumors, as well as by interfering with the metabolism of DMBA by microsomal enzymes from its inactive to its active form.

Significance to Biomedical Research and the Program of the Institute:
Phenolic antioxidants are extensively used as food additives to protect against lipid oxidation. Moreover, it has been estimated that in the U.S., consumption of phenolic antioxidants averages 2 mg/day. The fact that mammary tumorigenesis is inhibited in an experimental model system at low doses of both carcinogen and antioxidant is significant, therefore, because such low-level exposures are the most likely to occur in human populations. As a consequence, dietary antioxidants may prove useful in the prevention of breast cancer, which is, at present, the most common cause of cancer death in U.S. women.

Proposed Course: The second segment of this study is presently underway. In this segment, BHT will be included in the diet at 6000, 3000, 1000 and 300 ppm starting 2 weeks before DMBA administration (day 50 of age) and continuing until the termination of the experiment (25 weeks). Using this protocol, the effect of phenolic antioxidants on both the early (initiation) and later (promotion) stages of mammary carcinogenesis can be assessed. In addition, in an attempt to determine whether, and to what extent, prostaglandin metabolism is altered by dietary antioxidants, several different prostaglandins will be assayed in both tumors and normal mammary glands, in collaboration with Dr. R. Karmali (Memorial Sloan-Kettering Inst., N.Y.). Studies on the in vitro effects of both BHT and BHA on cultured mammary tumor cells will be continued.

Date Contract Initiated: September 30, 1980

Current Annual Level: $95,905

AMERICAN HEALTH FOUNDATION (NO1-CP-05723)

Title: Dose Response Studies on Phenolic Antioxidants (Liver)

Contractor's Project Director: Dr. Gary M. Williams

Project Officer (NCI): Dr. Carl E. Smith

Objectives: The overall objective of this program is to evaluate butylated hydroxytoluene as a chemopreventive agent in liver carcinogenesis.

Major Findings: To study inhibition of liver carcinogenesis in rats by butylated hydroxytoluene (BHT), it was fed in the diet concurrently with 200 ppm of 2-acetylaminofluorene (2-AAF). Four dose levels of BHT were used ranging from 300 to 6000 ppm. Rats were killed at 6, 12, 18 and 24 weeks of feeding and their livers were examined in detail for preneoplastic altered foci identified by histochemical abnormalities and neoplasms.

At the time of this report, data are available for the first 18 weeks of the study. AAF alone produced a significant incidence of liver altered foci by 6 weeks of feeding, whereas no foci were produced by any dose of BHT. BHT produced a dose-dependent inhibition of AAF-induced foci, with an 83% inhibition at the high dose of BHT.

The numbers of liver foci produced by AAF increased at 12 and 18 weeks of administration. Similar to the findings at 6 weeks, BHT produced a dose-dependent inhibition of the induction of foci by AAF. In addition to foci, at 12 and 18 weeks liver neoplasms were present in rats exposed to AAF. The development of tumors was also inhibited by BHT, such that at 6000 ppm no tumors occurred in rats also given AAF. Thus, BHT clearly produced an inhibition of AAF-induced liver carcinogenesis. In addition, the parallel inhibition of foci and neoplasms by BHT support the relationship of foci to neoplasms and indicates that quantification of the effects on early cellular lesions can be used as a rapid means of assessment of chemopreventive efficacy.

Significance to Biomedical Research and the Program of the Institute: These studies are delineating for the first time the dose response characteristics of inhibition of liver carcinogenesis by BHT. This information will provide insight into the underlying mechanisms of chemoprevention by BHT and therefore serve as a basis for future research on this subject. The information that is being developed in these studies will assist the National Cancer Institute in formulating its research objectives and policies in an important area of cancer prevention. The demonstration that inhibition of early preneoplastic lesions predicts inhibition of tumor development provides a rapid means for quantitatively assessing the chemopreventive efficacy of agents that could facilitate the screening of potentially useful anticancer substances.

Proposed Course: In a second study on the chemopreventive action of AAF, a lower dose will be fed for a longer duration to determine the inhibitory effects of BHT under these conditions. Further studies will examine the effect of BHT administered after completion of AAF exposure to determine whether BHT under these circumstances inhibits, promotes, or has no effect on carcinogenesis.

Date Contract Initiated: September 30, 1980

Current Annual Level: $89,878

AMERICAN HEALTH FOUNDATION (NO1-CP-85659)

Title: Studies of Natural Inhibitors of Chemical Carcinogens

Contractor's Project Director: Dr. Bandaru S. Reddy

Project Officer (NCI): Dr. Carl E. Smith

Objectives: The overall objective of this program is to identify naturally occurring fibers suitable for addition to normal diets in order to inhibit the development of colon cancer. Specifically, the program will assess the protective role of various dietary fibers, namely wheat bran (cereal-based) and citrus pulp (fruit-based), on chemically-induced colon carcinogenesis in animal model and identify the individual components of fibers that exhibit a protective effect on colon carcinogenesis.

Major Findings: Studies were initiated to determine the protective effect of dietary corn bran and lignin on colon carcinogenesis in rats. Weanling male F344 rats were obtained commercially. At 5 weeks of age, all animals were alloted at random to experimental groups and fed ad libitum one of the semipurified diets containing 5% alphacel, 5% alphacel + 7.5% lignin, or 5% alphacel + 15% corn bran. At 7 weeks of age, all animals, except vehicle-treated animals, received weekly subcutaneous injections of 3,2'-dimethyl-4-amino-biphenyl (DMAB) at a dose level of 50 mg/kg body wt. for 20 weeks. Carcinogen treatment has just been completed. The experiment will be terminated in another 15 weeks. Weekly body weights were recorded and found to be similar in all dietary treatments. At week 15, daily individual fecal samples were collected for 4 days from rats fed the experimental diets and will be subjected to bile acid analysis.

Significance to Biomedical Research and the Program of the Institute:
This program is of special significance since it is designed to provide important information on the relationship between various dietary fibers and their components such as pectin, lignin, cellulose, etc., and colon carcinogenesis.

It is hoped that the data base thus generated in animal models can significantly enhance our knowledge of the controllable etiological factors which play a role in cancer of the large bowel. The long term goal is to provide a basis for rational prevention by dietary means of a disease affecting over 100,000 individuals per year in the United States.

Proposed Course:
Contract terminated May 31, 1982.

Date Contract Initiated: September 30, 1978

Current Annual Level: 0

BATTELLE COLUMBUS LABORATORIES (NO1-CP-85650)

Title: Studies on the Toxicology of Retinoids

Contractor's Project Director: Dr. Perry J. Kurtz

Project Officer (NCI): Dr. Carl E. Smith

Objectives: Conduct short-term toxicity studies in laboratory rats and mice in order to evaluate the relative toxicity of synthetic retinoid compounds. The results are expected to assist in the selection of retinoid compounds for use in human cancer chemoprevention.

Major Findings: During the past year, preliminary toxicological studies with five synthetic retinoids of the stilbenoid class have been performed. BASF compound Nos. 38607, 39145, 38846, 39146 and 39209 were evaluated for relative toxicity. These studies in rats and mice consisted of a 21-day period of dosing with a radiographic analysis of skeletal integrity at the conclusion of each study. In these studies, clinical appearance, growth, mortality and radiographic analysis of the long-bones served as the primary toxicological endpoints. All five stilbenoids were found to be substantially more toxic than all-trans-retinoic acid. Additionally, rats were found to be more susceptible to long-bone fracture and lethality than mice, based on the incidence of these toxicological manifestations at equivalent dose/body weight ratios. There were clear toxicity differences among the compounds, and these differences could be related to molecular structure.

Significance to Biomedical Research and the Program of the Institute: Analysis of the molecular structure of the stilbenoids and the other retinoid analogs previously studied is providing useful information with regard to the relationship between specific regions of the retinoid molecule and the toxicity associated with substitutions and/or additions in these areas.

Proposed Course: A thorough in vivo toxicologic comparison of BASF compound Nos. 39557 and 38607 is currently underway. This study will include animals exposed to all-trans retinoic acid for further comparison. An investigation of the toxicity all-trans N-4-pivaloyloxyphenyl retinamide is also in its early stages.

Date Contract Initiated: September 30, 1978

Current Annual Level: 0

CALIFORNIA, UNIVERSITY OF (NO1-CP-05715)

Title: Synthesis of New Retinoids for the Chemoprevention of Epithelial Cancer

Contractor's Project Director: Dr. William H. Okamura

Project Officer (NCI): Dr. Carl E. Smith

Objectives: The objectives of the four-year program which began on August 30, 1980, are outlined below. As proposed in the contract, Phase One of the research is projected as a two-year program in which we plan to complete Items 1 and 2 and begin Item 3 listed below.

1. Synthesize 9,10-allenic retinoids including the (7E,12Z), (7E,12E), (7Z,12Z) and (7Z,12E) geometric isomers; the vinylallene alcohols, aldehydes, carboxylic acids, esters, etc., of these allenic retinoids are target compounds of interest.

2. Similarly, synthesize 11,12-allenic retinoids including the (7E,9E), (7E,9Z), (7Z,9E) and (7Z,9Z) geometric isomers. As in Item 1, synthesis of the carboxylic acids and other derivatives are target compounds of interest.

3. Perform studies on thermal isomerization and base catalyzed rearrangements of the compounds in Items 1 and 2 as routes to addtional retinoids for bioassay. As parts of these studies, synthesis will be pursued of alkylated and ring-fused analogues.

4. Similar to the efforts in items 1, 2 and 3, above, perform synthesis of 7,8-allenic retinoids and their rearrangement reactions.

5. Synthesize acetylenic retinoids in which the double bonds of the natural molecules are replaced by triple bonds.

6. Synthesize 6,7- and 10,11-allenes after the biological potential of the allenic retinoids and isomerization products above have been determined.

Major Findings: The synthesis and thermal rearrangement studies of (7E,12Z)-10,14-retro-retinol (1) (the first 9,10-allenic retinoid) was completed. Its thermal rearrangement products include 9,11,13-tricis-retinol (2), 11,13-dicis-retinol (3), 11-cis-retinol (4) and what appears to be a further rearrangement product of 9,11-dicis-retinol (5). The acetate of the tricis isomer 2 exhibited an ED_{50} of $2x10^{-10}$M where this value represents the dose for reversal of keratinization in epithelium of 50% of retinoid deficient hamster tracheas in organ culture (bioassay by Dr. L. Schiff, I.I.T. Research Institute; the ED_{50} of all-trans-retinoic acid 6 was $1x10^{-11}$M). The other isomers 1, 3, 4 and the rearrangement product of 5 have not yet been submitted for assay.

The instability of 1 has thus far precluded its submission for bioassay or for further chemical transformations to its aldehyde, ester or carboxylic acid. In order to stabilize 1, the alkylated analogue (7E)-12,20-trimethylene-10,14-retro-retinol (7) has been synthesized and its thermal rearrangement products have been identified and characterized. They include: the 9,11,13-tricis, 11,13-dicis and the 11-cis isomers of 12,20-trimethylene-retinols (8, 9 and 10, respectively); in addition, the further rearrangement product 11 of the 9,11-dicis isomer, similar to 5, was obtained. The ED_{50} of 7 was $>10^{-7}$M, but 8-11 as well as the aldehydes 12-14 derived from 8-10, respectively, have not yet been submitted for bioassay.

Two acetylenic retinoids, (6Z,8E,12E)- and (6Z,8E,12Z)-10,11-didehydro-18,14-retro-retinol, 15 and 16, respectively, were also synthesized but they appeared to be inactive in the hamster tracheal organ culture assay. Their carboxylic acid forms, however, should be a better test for their potential activity.

The readily available (Z)-1-bromo-2-methyl-4-butene (17), when coupled with (e)-9-ethynyl-β-ionol benzoate (18), was successfully used in synthesizing the (7E,12Z)-9,10-allene 1. The corresponding (E)-1-bromo-2-methyl-4-butene (19) failed to react with 18 to produce the (7E,12E)-9,10-allene 20, a geometric isomer of 1. This is extremely puzzling and we are attempting to prepare the corresponding iodide of 19 as a coupling reagent. The geometric isomer of 18, namely (Z)-9-ethynyl-β-ionol benzoate (21), has been prepared and coupled with various organocuprates of the type $R_2Cu(CN)Li_2$ as a model for preparing the (7Z)-isomers of 1 and 20. However, we have determined that such (7Z)-9,10-allenes (analogous to 1 and 20) undergo spontaneous electrocyclic ring closure across carbons 5 and 10 to afford drimatriene derivatives. Thus, it has been established that such (7Z)-9,10-allenic retro-retinoids will have fleeting existence at best. However, these electrocyclic ring closure products appear promising as starting materials for preparing 6-s-cis, 8-s-cis-locked retinoid analogues.

A key intermediate used for preparing the acetylenic retinoids 15 and 16 was (1,1'-Z,2E)-1-(6',6'-dimethyl-2'-methylenecyclohexylidene)-3-methylpent-2-en-4-yne (22). We are also attempting to transform this intermediate into (18,14)-retro-retinol (23), the parent system corresponding to 15/16.

Significance to Biomedical Research and the Program of the Institute: A significant relationship between the role of vitamin A in controlling differentiation of epithelial cells and the development of malignancy in epithelial tissues has been established. The natural all-trans form of vitamin A has been reported to have beneficial effects in the prophylaxis of various types of carcinomas, but suffers from excessive localization in the liver leading to toxic liver damage. By contrast, the synthetic analogs, 13-cis-retinoic acid and retinyl methyl ether, are far less toxic but possess similar biological effectiveness in controlling cell differentiation. Thus, the search for an even more effective cancer prophylactic drug in the domain of retinoidal synthetic unnatural products would appear to be both conceptually and practically a worthwhile goal, especially in view of the importance of the cancer problem. It is the purpose of this proposal to systematically investigate the incorporation of reactive allenic and acetylenic functional groups into the retinoid skeleton by analogy with similar useful and interesting studies of other inhibitory biological molecules. Furthermore, the same allenic retinoids will be used in a synthetic sense for rearrangement to new hindered geometric isomers of the normal retinoid skeleton and analogs which otherwise would be difficult to synthesize.

Proposed Course: The proposed course during the next 12 months includes:

1. Further studies of 9,10-allenic retinoids under Item 1 of Contract Objectives. Using new reverse phase LC columns, we will make further attempts to purify the (7E,12Z)-isomer for bioassay. Using the corresponding iodide of 19, we will make another attempt at preparing the (7E,12E)-isomer. As mentioned in the progress report section, while the syntheses of (7Z)-9,10-allenes now appear impossible, we will divert our efforts toward the 6-s-cis, 8-s-cis-locked retinoids.

2. The preparation of the 11,12-allenic retinoids will be continued (Item 2 of Contract Objectives). This area has been slow in developing because of our efforts in the 9,10-allene area, but we hope to accelerate our efforts here.

3. The thermal studies of the above retinoids as well as the preparation and rearrangement of alkylated and ring-fused analogues will continue (Item 3 of Contract Objectives). The 12-s-cis-locked retinols and retinals will be submitted for bioassay. The 12-s-cis-locked carboxylic acids should also become available during the next year.

4. Further studies of the acetylenic retinoids (15 and 16) and the related (18,14)-retro-retinoids (e.g., 23) will continue. The acetylenic retinoids are the first examples in our efforts related to Item 5 of the Contract Objectives.

Date Contract Initiated: August 30, 1980

Current Annual Level: $66,228

CORNELL UNIVERSITY (NO1-CP-05716)

Title: Synthesis of New Retinoids for the Chemoprevention of Epithelial Cancer

Contractor's Project Director: Dr. John E. McMurry

Project Officer (NCI): Dr. Carl E. Smith

Objectives: Although a large number of synthetic retinoid analogues have been prepared, clearcut structure-activity relationships are still not apparent. Thus, various analogues with greatly modified polar heads and others with greatly modified non-polar tails still retain substantial activity. The contractor has hypothesized that the precise conformation of the flexible retinoid side chain may play a major role in biological activity, and has designed a series of cyclic retinoid analogues which "freeze out" various side chain rotational isomers. Thereby, it might be possible to learn which conformations are active and which are inactive.

Major Findings: In the past year, some 32 new retinoid analogues have been prepared and submitted for testing. These 32 analogues are of several structural types but, without exception, all have aromatic rings inserted as integral parts of the polyene side chain and all therefore have restricted side chain rotation.

Thus far, two general attributes of all of the compounds prepared have been noted: first, all are much more stable than normal retinoids by virtue of their having aromatic rings. With few exceptions, the interactors synthetic materials have been air-stable crystalline compounds, factors which greatly facilitate their preparation and manipulation. Second, only two of the compounds thus far tested have failed to show substantial activity in the hamster tracheal organ culture assays carried out by Dr. Leonard Schiff at IIT Research Institute.

The ED_{50}'s for the others have ranged from 10^{-8} to 10^{-11}M and two analogues have scored as highly as all-trans-retinoic acid.

Both of the compounds with ED_{50}'s of 10^{-11}M have structures based on the presence of a dimethyl tetrahydronaphthalene ring system in place of the trimethylcyclohexene ring of retinoic acid. The tentative conclusion arrived at is that the 1,7 single bond must maintain an s-cis conformation and that coplanarity of the 1,2 and 7,8 double bonds is a requirement for activity. Other conclusions await further test results.

Significance to Biomedical Research and the Program of the Institute: This project is aimed both at preparing new retinoids which are more stable, more potent, more site specific, and less toxic than presently known compounds, and at probing structure-activity relationships involving the retinoid side chain. Attainment of these goals will increase our understanding of the biology of retinoid action and may have a favorable impact on cancer chemotherapy.

Proposed Course: This project is still in the data gathering phase, and more information is needed. In the coming year, further cyclic analogues of retinoic acid will be synthesized and the leads which are beginning to emerge will be followed closely.

Date Contract Initiated: August 30, 1980

Current Annual Level: $81,582

DARTMOUTH MEDICAL SCHOOL (NO1-CP-85675)

Title: Prevention of Pancreatic Cancer in Experimental Animals by Retinoids

Contractor's Project Director: Dr. Daniel S. Longnecker

Project Officer (NCI): Dr. Carl E. Smith

Objectives: To induce an incidence of carcinomas or preneoplastic epithelial lesions in pancreas of azaserine-treated rats and N-nitrosobis(2-oxopropyl)amine-treated hamsters, to see if there is a decrease in the incidence, size, or number of such cancers or preneoplastic lesions among the rats and hamsters given retinoid-supplemented diets during the latent period of carcinogenesis.

Major Findings: During the past 12 months, the contractor has continued with a second group of retinoids and control diets being fed to Lewis rats that previously had been injected with azaserine (5 x 30 mg/kg) and Syrian golden hamsters that had been injected with BOP (2 x 20 mg/kg). The retinoids assigned to us this round are as follows:

N-4-pivaloyloxyphenylretinamide
N-2-hydroxypropylretinamide
N-3-hydroxypropylretinamide
N-2,3-dihydroxypropylretinamide

The levels of retinoid in the diets were initially 1.0 or 2.0 mmole for rats and 0.25 or 0.50 mmole for hamsters. Due to signs of toxicity in some of the groups, the levels in the diets were reduced to maintain a satisfactory state of health. All animals were sacrificed after 52 weeks on the retinoid-containing or control diets. Pancreases were removed, weighed, grossly evaluated, and fixed whole. Representative normal-appearing tissues were sampled from each diet group of each species, and sections of all grossly abnormal tissues were sampled. After fixation, all tissues were embedded in paraffin, cut, stained with hemotoxylin and eosin, and evaluated histologically.

Results in rats: Using the Spearman rank correlation coefficient, we conclude that retinoids inhibited the progression of carcinogen-induced lesions. The effectiveness of the retinoids is as follows:

Most Effective	N-4-pivaloyloxyphenylretinamide
	N-2,3-dihydroxypropylretinamide
	N-2-hydroxypropylretinamide
Least Effective	N-3-hydroxypropylretinamide

Results in hamsters: Using the Spearman rank correlation coefficient, the contractor concludes that the female groups fed all four retinoid-supplemented diets and the male groups fed diets supplemented with N-4-pivaloyloxyphenylretinamide and N-2-hydroxypropylretinamide show a trend toward reduced numbers of carcinomas when compared with the non-retinoid-treated control groups, but there is no significant correlation.

Significance to Biomedical Research and the Program of the Institute: The results provide additional evidence that supplementation of the diet with retinoids can inhibit the progression of experimentally induced pancreatic cancer. This suggests that a similar result might be achieved in humans at risk for development of carcinoma of the pancreas.

Proposed Course: This concludes the objectives of this contract, and the final report and manuscripts are in preparation.

Date Contract Initiated: September 30, 1978

Current Annual Level: 0

DEPARTMENT OF ENERGY, BROOKHAVEN NATIONAL LABORATORY (Y01-CP-00202)

Title: Chemoprevention of Epithelial Cancer by Retinoids (Mammary Gland)

Contractor's Project Director: Dr. Claire J. Shellabarger

Project Officer (NCI): Dr. Carl E. Smith

Objectives: To determine the extent to which retinyl acetate can arrest, delay, or reverse the development of mammary cancer in an established model system, the female Sprague-Dawley rat. Specifically, to determine:

1. If retinyl acetate can inhibit x-ray induced mammary carcinogenesis.

2. If the retinyl acetate inhibition of mammary carcinogenesis, either x-ray or chemically-induced, will continue for the lifespan of the animal.

3. If retinyl acetate can inhibit spontaneously occurring mammary carcinogenesis.

Major Findings: Carcinogen treatment, either whole-body irradiation (200 R of 250 kVp x-rays) or dimethylbenz(a)anthracene (3.3 mg/100 g body weight) intragastrically, were given to 55-day-old female Sprague-Dawley rats. Seven days later, one half of the animals receiving either carcinogen and one half of the appropriate controls began receiving standard diet supplemented with retinyl acetate at 0.45 m M/Kg diet.

Two-hundred and ninety-six days after the carcinogen treatments, retinyl acetate added to the diet of x-irradiated rats has produced a 56 precent reduction (12/110 vs 27/110) in the number of rats with a mammary adenocarcinoma, and a 63 percent reduction (13 vs 35) in the total number of adenocarcinomas produced compared to irradiated rats fed the standard diet. Similarly, retinyl acetate added to the diet of dimethylbenz(a)anthracene treated rats produced a 32 percent reduction (19/70 vs 28/70) in the number of rats with a mammary adenocarcinoma and a 34 percent reduction (35 vs 53) in the total number of mammary adenocarcinomas produced, compared to dimethylbenz(a)anthracene treated rats fed the standard diet. Thus far, too few spontaneous mammary adenocarcinomas have appeared in the non-carcinogen treated animals to evaluate the effect of retinyl acetate on these tumors.

Rats fed the retinyl acetate supplemented diet had a mean body weight 11 percent lower (283 vs 317) than rats fed the standard diet. This may indicate some retinoid toxicity, but at this time it appears not to be a significant problem.

Significance to Biomedical Research and the Program of the Institute:
Before retinoids can be used for the chemoprevention of human breast cancer, more information is needed about their inhibitory action on animal mammary carcinogenesis and their toxicity. In the present contract, mammary carcinogenesis induced by a chemical carcinogen, by x-rays, and occurring spontaneously in female Sprague-Dawley rats, is used as a model system. This model system is used because the hormonal control and histopathology of human and rat mammary adenocarcinomas are similar.

Previous studies on the inhibitory effects of retinoids on chemially induced carcinogenesis in rats were short-term studies which could not determine if the inhibitory action was more than just a delay in the onset of carcinogenesis. The present lifespan study is designed to determine the extent to which retinyl acetate can arrest, delay, or reverse mammary carcinogenesis produced by x-rays, a chemical carcinogen, and unknown factors (spontaneously occurring tumors).

Data from the present study should be helpful in determining if retinoids can be used for practical chemoprevention; that is, can they be given during the entire lifespan of an animal at a dose that will give both significant inhibition of carcinogenesis and minimal toxicity or side effects.

Proposed Course: All rats will be maintained on the present diets and will be studied as stated in the contract protocol for the lifespan of the animals. Ten animals within each group are being used to monitor toxicological, physiological, and endocrinological changes produced by the long-term retinoid treatment. Blood and tissue samples from these animals are being analyzed.

Date Contract Initiated: September 19, 1980

Current Annual Level: $187,756

IIT RESEARCH INSTITUTE (NO1-CP-05718)

Title: Chemoprevention of Epithelial Cancer by Retinoids (Mammary Gland)

Contractor's Project Director: Dr. Richard C. Moon

Project Officer (NCI): Dr. Carl E. Smith

Objectives: The major goal of this project is to assess the chemopreventive activity of retinoids (natural and synthetic analogues of vitamin A) in long-term studies conducted in rodent models for breast cancer.

Major Findings: Previous work has indicated that retinyl acetate, retinyl methyl ether, 4-hydroxyphenylretinamide, 13-cis-4-hydroxyphenylretinamide and axerophthene significantly prolong the latency of mammary cancer appearance and reduce the number of mammary cancers induced by N-methyl-N-nitrosourea (MNU). On the other hand, retinyl butyl ether, retinylidene dimedone, retinylidene-2,4-pentadione, 2-hydroxyethylretinamide, and ethylretinamide were ineffective against MNU-induced mammary cancer in Sprague-Dawley female rats. In these studies, 4-hydroxyphenyl-retinamide was well tolerated. In a life time mammary carcinogenesis study (low dose carcinogen), the chemopreventitive activity of 4-hydroxyphenyl retinamide has been evaluated; tumor tissues from this study are presently undergoing histological processing prior to pathological classification.

During the present contract period, the chemopreventive efficacy of β-carotene was tested against MNU-induced mammary cancers; no anticancer activity of β-carotene was noted. A study to test the activity of (4-pivaloyloxy)phenylretinamide on MNU-induced mammary carcinogenesis is in progress.

We have previously noted that the efficacy of retinoid-induced inhibition of mammary carcinogenesis can be significantly enhanced when the retinoid is administered with another inhibitor of carcinogenesis (combination chemoprevention).

In these combination studies, the combined effect of 4-hydroxyphenylretinamide and ovariectomy almost completely prevented MNU-induced mammary cancer; ovarian hormone independent cancers appear to be highly sensitive to inhibition by retinoids. The retinoids also delay the rate of second tumor appearance following the surgical excision of the first tumor. In the present contract period, we have extended these combination studies to include combination regimens of retinoid plus tamoxifen (an antiestrogen) and retinoid plus flurbiprofen (an inhibitor of prostaglandin biosynthesis). These studies are ongoing.

Significance to Biomedical Research and the Program of the Institute:
Studies performed under this contract indicate that efforts to synthesize organotrophic retinoids with increased chemopreventive activity and diminished toxicity, in comparison to previously tested compounds, are meeting with success. The data obtained from long-term evaluation of retinoids will hopefully lead not only to the establishment of the concept of cancer chemoprevention, but will also provide evidence for the use of retinoids in suppressing progression of early neoplastic lesions in women who are at high risk for breast cancer.

Proposed Course: As they become available, newly synthesized retinoids will be evaluated for chemopreventive activity in rodent models for breast cancer, both alone and in combination with other chemopreventive agents.

Date Contract Initiated: September 30, 1980

Current Annual Level: $110,249

MICHIGAN STATE UNIVERSITY (NO1-CP-05717)

Title: Chemoprevention of Epithelial Cancer by Retinoids (Mammary Gland)

Contractor's Project Director: Dr. Clifford W. Welsch

Project Officer (NCI): Dr. Carl E. Smith

Objectives:
1. To enhance the mammary tumorigenic suppressive activities of retinoids in the carcinogen treated Sprague-Dawley rat by concurrent hormone suppression and/or immune stimulation.

2. To determine whether or not retinoids possess mammary tumorigenic suppressive activities in 3 distinctly different types of mouse mammary carcinoma models, i.e., the carcinogen treated BDF/J mouse, the hormone treated GR mouse, and the spontaneous (overt MTV) C3H/He mouse.

Major Findings:
1. Effect of retinyl acetate feeding, hormone inhibition and/or non-specific immune stimulation on the genesis of DMBA-induced mammary carcinomas in female Sprague-Dawley rats:

Two groups of 320 female rats were treated i.g. with 20 mg (series 1) or 10 mg (series 11) of 7,12-dimethylbenzanthracene (DMBA) per rat at 50 days of age. At 53 days of age each series was divided into 8 groups of rats (40 rats/group), i.e., (1) controls (C), (2) immune-stimulated (IS), (3) hormone inhibited (HI), (4) HI+IS, (5) retinoid fed (RA), (6) RA+IS, (7) RA+HI, (8) RA+HI+IS. HI = Tamoxifen (25 μg/100 g B.Wt.) and 2-Br-α-ergocryptine (CB-154) (400 μg/100 g B.Wt.) injected s.c. once daily. IS = Norcardia rubra (1 mg/rat) injected s.c. once weekly (series 1) or methanol extracted residue (MER) of Bacillus Calmette-Guerin (BCG) (0.5 mg/rat) injected i.p. at 3 and 5 weeks after DMBA treatment (series 11). RA = retinyl acetate, fed daily, 0.6 mM/kg ration (series 11, moderate dose) and 0.2 mM/kg ration (series 1, low dose). Mammary tumor incidence was significantly ($P<0.005$) reduced in rats treated with HI or fed a moderate dose of RA. Tumor incidence was further reduced ($P<0.005$) in HI treated rats fed either a moderate or low dose of RA; low dose of RA alone was ineffective. IS alone or in combination did not influence this neoplastic process.

2. Effect of retinyl acetate feeding, hormone inhibition and/or specific immune stimulation on the genesis of DMBA-induced mammary carcinomas in female Sprague-Dawley rats:

A group of 320 female rats were treated i.g. with 5 mg of DMBA per rat at 50 days of age. At 53 days of age, the rats were divided into 8 groups of rats (40 rats/group) as indicated in section 1 above. Retinyl acetate (RA) was fed daily, 1.0 mM/kg ration. Immune stimulation (IS) was specific, i.e., mammary carcinoma membrane preparations from 20 DMBA induced rat mammary tumors were mixed with complete Freunds adjuvant and this combination was injected i.p. (1 mg/rat) at weeks 1, 3, & 5 after DMBA treatment. Mammary tumor incidence was significantly ($P<0.005$) reduced in rats treated with HI or fed RA. Tumor incidence was further reduced ($P<0.005$) in rats fed RA and treated with HI. IS significantly ($P<0.05$) reduced mammary tumor incidence in RA fed rats but not in placebo fed rats.

3. Effect of retinyl acetate feeding on mammary tumorigenesis in carcinogen (DMBA) treated female BDF/J mice:

Feeding of retinyl acetate (0.2 mM/kg ration) to DMBA treated BDF/J mice has not produced any discernable effects on the incidence of mammary tumors. Retinyl acetate feeding was begun one week after the last of 6 carcinogen treatments. The total number of mammary tumors 27 weeks after the onset of retinoid feeding in control and retinoid-fed mice is nearly identical. 75 mice are in each group. The histopathology of these tumors has not yet been completed; what proportion of these tumors are adenocarcinomas and acanthomas has not yet been determined.

4. Effect of retinyl acetate feeding on mammary tumorigenesis in nulliparous and multiparous C3H mice:

The feeding of retinyl acetate to nulliparous (.04 mM/kg ration) or multiparous (0.2 mM/kg ration) C3H mice did not produce any significant effect on the incidence of mammary tumors. Number of mammary carcinomas that developed in control and retinoid fed nulliparous mice were 185 and 208, respectively, 39 weeks after onset of feeding. 120 mice were in each group. The number of mammary carcinomas that developed in control and retinoid-fed multiparous mice were 92 and 80, respectively, 13 weeks after onset of feeding. 85 mice were in each group.

5. Effect of retinyl acetate feeding on hormone-induced mammary tumorigenesis in female GR/A mice:

Feeding of retinyl acetate (0.25 mM/kg ration) for 13-16 weeks to estrone/progesterone-treated nulliparous and multiparous GR mice resulted in a substantial increase in the incidence of mammary carcinomas. Mammary carcinoma incidence in nulliparous control and retinoid-fed mice in experiment #1 was 22/65 (34%) and 37/65 (57%) ($P<0.05$), respectively; in experiment #2, 27/48 (56%) and 37/48 (77%) ($P<0.05$), respectively. Mammary carcinoma incidence in multiparous control and retinoid-fed mice in experiment #1 was 13/30 (43%) and 23/30 (77%) ($P<0.05$), respectively; in experiment #2, 19/19 (100%) and 19/19 (100%), respectively.

Significance to Biomedical Research and the Program of the Institute:
There is no question that moderate to high dietary consumption of retinyl acetate can sharply reduce mammary carcinoma incidence in carcinogen-treated female rats. This inhibition of mammary tumorigenesis by retinyl acetate can be further enhanced by concurrent drug induced hormone suppression or by specific immune stimulation. Non-specific immune stimulation is not effective. Enhancement of the mammary tumor chemopreventive activities of retinoids by specific immune stimulation and/or hormone inhibition is an important observation, one that has tremendous applicable potential. Indeed, the rats which were fed retinyl acetate, and concurrently received specific immune stimulation and hormone inhibition treatments never developed mammary tumors for the duration of treatments, i.e., 20 weeks after DMBA treatment. By this time, 100% of the controls developed mammary carcinomas. Needless to say, these results are most striking.

The failure to achieve chemoprevention of mammary tumorigenesis with retinyl acetate in the 3 mouse models is admittedly disappointing. However, it should be pointed out that these mice were fed doses of retinyl acetate considerably below the levels which were fed to the carcinogen-treated rats. With but one exception (nulliparous C3H mice), maximally tolerated doses of retinyl acetate were fed to these mice. Mice simply cannot tolerate the doses of retinyl acetate which consistently inhibit mammary tumorigenesis in carcinogen-treated rats.

Proposed Course:
1. To test other retinoids (e.g., N-4-hydroxyphenylretinamide) that have reduced toxicity yet retain effective chemoprevention activities in the 3 mouse models (i.e., C3H, GRA and BDF/J).

2. To further explore the enhancement of retinyl acetate activity by specific immune stimulation in the DMBA induced rat mammary tumor model. We will alter dose and timing of immune stimulation, for the propose of improving the efficacy of this chemopreventive adjunct to retinoid feeding.

3. To test the chemopreventive activities of retinoid feeding, hormone inhibition and/or immune stimulation in the MNU-induced rat mammary tumor model.

Date Contract Initiated: September 19, 1980

Current Annual Level: $68,201

MIDDLESEX HOSPITAL MEDICAL SCHOOL (NO1-CP-05602)

Title: Chemoprevention of Epithelial Cancer by Retinoids (Bladder)

Contractor's Project Director: Dr. R. Marian Hicks

Project Officer (NCI): Dr. Carl E. Smith

Objectives:
1. The long-term study of inhibition by retinoids of urinary bladder cancer in the mouse.

2. The effect of retinoids on the morphology of tumor development in the mouse.

3. Investigation of the anti-promoting capacity of retinoids in the rat.

4. The effect of retinoids on the rate of cell proliferation in bladder tumors.

Major Findings: For objective 1, 414 female B6D2F1 hybrid mice were dosed weekly for 10 weeks with the selective bladder carcinogen N-butyl-N-(4-hydroxybutyl)nitrosamine (BBN) to give a total dose of either 15 or 30 mg. They were then placed on a placebo- or retinoid-containing diet for the duration of the experiment. It was intended to kill half the animals 40 weeks after the final dose of carcinogen and the remainder after 80 weeks. However, the 30 mg dose level of BBN unexpectedly caused an unacceptable number of deaths from bladder cancer in the first 5 months following carcinogen dosing, and therefore surviving animals were killed after maintaining for only 19 weeks on a placebo- or retinoid-containing diet to avoid loss of data through cannibalisation. This did not apply to the animals receiving 15 mg BBN and they are still on test.

Of the animals killed so far, in the group which had received the placebo diet for 19 weeks, 21% had hyperplastic urothelia and 66% had bladder cancer ranging from carcinoma in situ to solid, infiltrating, poorly differentiated transitional cell carcinomas which had invaded the full thickness of the bladder wall. Animals maintained on a diet containing 1 mM N-(tetrazol-5-yl)retinamide (TR) were similar to the placebo-fed groups in their response to 30 mg BBN, with a 20% incidence of urothelial hyperplasia and a 62% incidence of bladder cancer. Comparison of the histology has shown there to be no improvement in the stage or grade of the cancers in these TR-treated animals. By contrast, those animals fed 1 mM N-(4-hydroxyphenyl)-retinamide (HPR) had a 49% incidence of hyperplasia but only a 25% incidence of bladder cancer. Furthermore, the urothelia in these aimals were strikingly better differentiated than those in the placebo-fed or TR-treated groups; for example, only one animal with carcinoma in situ was found in this group, by comparison with 13 in the placebo-fed and 17 in the TR-fed groups.

These results following a fully carcinogenic treatment with 30 mg BBN suggest that feeding HPR is effective over a 5 month period in reducing the incidence and severity of neoplastic disease in the mouse bladder. By contrast, TR was apparently without effect on the neoplastic response of the urothelium.

The effect of the third retinoid, ie, N-(n-butyl)retinamide on the neoplastic response of the mouse to 30 mg BBN still has to be assessed.

Animals treated with the lower dose of carcinogen (15 mg BBN) are still on trial, and the effect of the three retinoids on the carcinogenic response after a longer period of time will be available by the beginning of 1983.

Project 2 has just been started. A dose of 10 mg BBN was used; and the retinoids being further tested are HPR and TR. The time-related effect of these retinoids on the morphology of the carcinogen-treated urothelium will be studied.

In our previous studies, the effect of two other retinoids, 13-cis-retinoic acid and N-ethylretinamide, on the development of slow growing, well-differentiated, exophytic rat bladder tumors was assessed. The results were relatively disappointing, although the retinoids delayed by a few weeks the start of tumor growth so that at any point in time tumors in the retinoid-fed animals were less advanced than those in the placebo-fed controls. They did not prevent tumors from developing, nor did they improve the histology of the tumors or slow the growth of tumors once exponential growth was established. Nor did these two retinoids prevent death of the animals from bladder cancer. In the current experiments, a different animal model has been used and the effect of three retinoids on the development of fast growing, flat infiltrating bladder cancer in a mouse hybrid is being assessed.

As yet, only preliminary results are available following 5 months' feeding of retinoids after a completely carcinogenic course of BBN; but already there is a clear difference in the effect produced by two retinoids. N-tetrazol-5-ylretinamide is without effect on the grade or stage of tumors produced, whereas the hydroxyphenyl-retinamide produced a marked reduction in tumor incidence and also in both grade and stage of the developing tumors.

Significance to Biomedical Research and the Program of the Institite:
Providing these observations are confirmed by the results of the remainder of this trial with the lower dose of carcinogen, and by trial 2 which has just been started, they will have considerable significance for the chemoprevention program:

1. They confirm the usefulness of this mouse model, originally developed by Becci and Moon, as a rodent model of carcinoma in situ and invasive bladder cancer.

2. They show that HPR is far more effective than TR in preventing the development of bladder cancer following previous exposure of the animal to a carcinogenic dose of a proven bladder carcinogen.

3. This highlights the importance of choosing the right retinoid for any particular organ. Like the nitrosamine carcinogens, retinoids may prove to be organ site specific and effective only in their particular target organ. The "bad press" which would follow if the "wrong" retinoid for a particular organ site were to be tested in clinical trails could have a disproportionately damaging effect on the whole concept of chemoprevention with retinoids.

4. The results obtained so far with HPR suggest that, unlike most of the currently available modalities for treating bladder cancer which are successful only with slow growing, exophytic, relatively benign tumors, ingestion of an appropriate organ-specific retinoid may help to control carcinoma in situ and invasive cancer of the bladder. If our subsequent studies confirm this, it could form the basis of a major advance in bladder cancer therapy.

Proposed Course:
1. The effect of N-(n-butyl)retinamide on the carcinogenic response of the mouse bladder to 30 mg BBN will be assessed in those animals already killed after 5 months' feeding of this retinoid.

2. The animals which received the lower (15 mg) dose of BBN, which are still on trial, will be used to judge the longer-term anticarcinogenic effects of the three retinoids on test.

3. The time-related effect of TR and HPR on tumor development will be assessed in trial 2 both by light microscopy and electron microscopy.

4. In trial 3, we will be investigating the point of action of one of these retinoids in the multi-stage process of carcinogenesis in the bladder. In view of the promise shown in the trial so far by 4HPR, this would seem to be the retinoid of choice to use here. For this trial we shall be using the rat and will aim to determine whether HPR acts as a specific antipromoting agent or whether it acts later in the process of carcinogenesis as an anti-propagative agent.

5. Trial 4 will be set up at the end of the year after consultation with the project officer to determine the effect of a retinoid, probably HPR, on the rate of cell proliferation in preexisting tumors.

Date Contract Initiated: September 30, 1980

Current Annual Level: $151,804

MINNESOTA, UNIVERSITY OF (NO1-CP-05605)

Title: Dose Response Studies on Phenolic Antioxidants

Contractor's Project Director: Dr. Lee W. Wattenberg

Project Officer (NCI): Dr. Carl E. Smith

Objectives: The basic objective of this contract is to determine the efficacy of 2(3)-butylated hydroxyanisole (2(3)-BHA) in inhibiting carcinogen-induced pulmonary adenoma formation in the mouse. An important component of this objective is to determine whether low doses of 2(3)-BHA, of the magnitude likely to be consumed by man, inhibit chemical carcinogens and the conditions under which inhibition would occur.

Major Findings: A series of experiments have been initiated to determine the effects of varying concentrations of 2(3)-BHA on benzo(a)pyrene (BP)-induced pulmonary adenoma formation. Thus far two experiments have been completed. In both, diets containing 2(3)-BHA were fed to female ICR/Ha mice four weeks prior to the initial dose of BP by oral intubation and were continued throughout the entire course of carcinogen administrations. The amount of 2(3)-BHA added to the diets was 5 mg/Gm, 3 mg/Gm, 1 mg/Gm, 0.33 mg/Gm and a control group with no 2(3)-BHA added. In one experiment the dose of BP administered was 0.7 mg; and in the other, the dose was 2.0 mg. In both, 12 doses of BP were administered by oral intubation, two times a week for six weeks. The animals were sacrificed 52 weeks after the initial dose of BP. In the experiment employing 0.7 mg of BP, the average number of adenomas per

mouse in the control group was 9. All dose levels of 2(3)-BHA produced an inhibitory effect as determined by the number of pulmonary adenomas formed. The results were as follows (average number of adenomas in the test group/average number of adenomas in the control group): 2(3)-BHA-5 mg, 0.47; 2(3)-BHA-3 mg, 0.41; 2(3)-BHA-1 mg, 0.57; and 2(3)-BHA-0.33 mg, 0.69. If all four groups receiving 2(3)-BHA are averaged and compared to the controls, the ratio is 0.54.

In the experiment with the 2 mg dose of BP, the average number of pulmonary adenomas in the control group was 24. The results were as follows (average number of adenomas in test group/average number of adenomas in the control group): 2(3)-BHA-5 mg, 0.54; BHA-3 mg, 0.70; BHA-1 mg, 0.62; 2(3)-BHA-0.33 mg, 0.84. If all four groups receiving 2(3)-BHA are averaged and compared to the controls, the ratio test/control is 0.68. A comparison of the two experiments shows that 2(3)-BHA has a greater inhibitory effect against the lower dose of BP than the higher. The data suggest that as the dose level of BP is reduced, smaller amounts of 2(3)-BHA become more effective in bringing about inhibition. Further studies with lower doses of BP are in progress.

Significance to Biomedical Research and the Program of the Institute: 2(3)-BHA is an antioxidant widely used as a food additive. 2(3)-BHA has been found to inhibit a diverse group of chemical carcinogens. An elucidation of its capacity to inhibit at low concentrations would give information as to its potential impact as an inhibitor of environmental carcinogens to which humans are exposed and also will provide information applicable to other inhibitors having similar properties.

Proposed Course: The course as outlined in the original contract will be followed.

Date Contract Initiated: September 30, 1980

Current Annual Level: $120,518

NEBRASKA, UNIVERSITY OF (EPPLEY INSTITUTE FOR RESEARCH IN CANCER AND ALLIED DISEASES) (NO1-CP-85674)

Title: Prevention of Pancreatic Cancer in Experimental Animals by Retinoids

Contractor's Project Directors: Dr. Parvis Pour
Dr. Diane Birt

Project Officer (NCI): Dr. Carl E. Smith

Objectives: The present study evaluates the ability of several retinoids to inhibit the development of experimental pancreatic cancer. Tumors will be induced in Syrian hamsters with the pancreatic carcinogen N-nitrosobis-(2-oxopropyl)amine (BOP), and retinoids will be fed in the diet beginning one week after carcinogen treatment. BOP will be administered in a single dose at a low (10 mg/kg BW) or a high (40 mg/kg BW) level, each of four retinoids (13-cis-retinoic acid, N-ethylretinamide, 2-hydroxyethylretinamide, 4-hydroxyphenylretinamide) will be fed at several levels. Body weights, food consumption, clinical chemistry and circulating retinoids will be measured. Histopathology will be evaluated in the pancreas, lung, liver, and kidney.

Major Findings: Studies with intermediate (0.4-0.5 mM/kg diet) and high (0.8-1.0 mM/kg diet) doses of retinoids following the low (10 mg/kg BW) or high (40 mg/kg BW) dose of BOP indicated an enhancement of BOP induced pancreatic cancer when retinoids were fed after the high BOP dose. This was observed in male hamsters fed the highest levels of all four retinoids and in females fed these levels of 13-cis-retinoic acid or N-ethylretinamide. Feeding the lower retinoid levels or the lower BOP dose did not influence pancreatic carcinogenesis. Survival was consistently decreased by feeding the highest levels of retinoids (0.8-1.0 mM/kg diet), and levels as low as 0.2 mM/kg diet reduced male survival. Retinoid feeding was also determined to reduce circulating and hepatic retinol in hamsters.

Significance to Biomedical Research and the Program of the Institute: Inhibition of cancer by retinoids has proven successful in several experimental models, in particular, in the breast and bladder. This contract studies the effect of these compounds on experimental pancreatic cancer. The inhibition and prevention of cancer is the most logical future course for cancer research.

Proposed Course: Publication of results will be completed.

Date Contract Initiated: September 30, 1978

Current Annual Level: 0

SOUTHERN RESEARCH INSTITUTE (NO1-CP-85615)

Title: Studies on Toxicology of Retinoids

Contractor's Project Directors: Dr. R. G. Meeks
Dr. B. P. Sani

Project Officer (NCI): Dr. Carl E. Smith

Objectives: The objective of this contract is to evaluate the toxicity of retinoids. These studies will aid in assessing the potential use of retinoids as chemopreventive agents.

Major Findings: Retinoids display profound prophylatic and inhibitory effects in experimental carcinogenesis. It is also known that vitamin A deficiency enhances susceptibility to chemical carcinogenesis. Epidemiological studies show that human cancer risks are correlated with blood retinol levels and dietary β-carotene; however, chronic administration of all-trans-retinoic acid results in dose-limiting toxicity in experimental animals and in man. The major side effects include headache, dry skin, and desquamation, alterations in mucous membranes, and bone abnormalities such as joint pain and thinning. Early studies conducted in our laboratory on the toxicity of retinoids indicate that 13-cis-retinoic acid is less toxic than the naturally occurring all-trans isomer. It is possible that new synthetic retinoids can be synthesized that are less toxic than the all-trans retinoic acid and are effective in the prevention of cancer. We have conducted comparative simultaneous toxicity studies in Swiss mice and Sprague-Dawley rats of various N-substituted all-trans and 13-cis isomers of retinamides. Groups of animals were treated orally for 21 days or 12 weeks as well as intraperitoneally for 21 days. Surviving animals were x-rayed prior to sacrifice. At the time of

sacrifice animals were bled, and effects on hematology and clinical chemistry parameters were measured. Selected tissues were collected and processed for histopathological evaluation.

On the basis of the lethality data obtained after intraperitoneal administration to mice as well as the clinical and histopathological findings, the following order of toxicity was determined: all-trans retinoic acid was most toxic followed by 2-hydoxypropylretinamide >3-cis-retinoic acid >2-hydroxyethylretinamide >2,3-dihydroxypropylretinamide >3-hydroxypropylretinamide >4-hydroxybutylretinamide >N-4-hydroxyphenylretinamide >propylretinamide >ethylretinamide >butylretinamide. Lethality data obtained after oral administration of the retinamides followed the same general order as that listed above. In general, the all-trans isomers exhibited greater toxicity than the corresponding 13-cis isomers. The estimated LD90, LD50 and LD10 of all-trans-retinoic acid following intraperitoneal administration to mice were 39.4, 32.6 and 27.0 mg/kg, respectively. For 13-cis-retinoic acid following intraperitoneal administration, these lethal doses were 246.0, 145.2 and 85.7 mg/kg, respectively. When the polar terminal end group of all-trans-retinoic acid is modified as in N-ethyl-, N-propyl- and N-butylretinamide, the LD 50 values increased to 1801.1, 1647.4 and 5782.2 mg/kg; respectively. However, hydroxylated N-substituted retinamides such as 2-hydroxyethylretinamide, 3-hydroxypropylretinamide, 4-hydroxybutylretinamide and 2,3-dihydroxypropylretinamide gave LD 50 values in mice upon intraperitoneal administration in the range of 150 to 250 mg/kg. Thus, modification of the terminal polar group of retinoic acid substantially alters the toxicity of the retinoid derivatives. Based on our studies, the all-trans-N-butylretinamide is the least toxic retinoid. Changes in hematology parameters in retinoid intoxication include peripheral anemia as evidenced by erythrocytopenia, decreased hemoglobin concentration and packed cell volume. Acute administration of retinoids caused an increase in alkaline phosphatase activity and a decrease in the albumin concentration. Histopathologic evaluations of tissues taken from mice at the time of sacrifice of the N-substituted retinamides indicate hepatocellular degeneration and necrosis, hepatic inflammation, suppurative or granulomatous peritonitis, mesenteric and thoracic lymph node atrophy and spermatogenic arrest. Additional changes possibly related to high doses of retinamides include a mild focal chronic or granulomatous inflammation within the ventricular myocardium or subendocardium.

Significance to Biomedical Research and the Program of the Institute: Chemopreventive agents such as vitamins and micronutrients are presently contemplated to decrease the risk of cancer and other major diseases. A thorough study on the toxicity of such agents is extremely important to their potential for human use.

Proposed Course: Most of the compounds we have evaluated so far for toxicity are all-trans-retinoids. Preliminary work in our laboratory indicates that the 13-cis isomers exhibit lower toxicity than the corresponding all-trans isomers. It would be worthwhile therefore to study more 13-cis isomers of retinoids as well as other chemopreventive agents for their toxicity. These additional studies may provide a means for better selection of the least toxic agents that may also possess high biological potency.

Date Contract Initiated: September 30, 1978

Current Annual Level: O

SRI INTERNATIONAL (NO1-CP-05600)

Title: Synthesis of New Retinoids for the Chemoprevention of Epithelial Cancer

Contractor's Project Director: Dr. Marcia I. Dawson

Project Officer (NCI): Dr. Carl E. Smith

Objectives: To synthesize retinoids that may have better pharmacological properties than those that have already been tested. These analogs have both steric and electronic modifications in the side chain, polar terminus and ring of the retinoid skeleton.

Major Findings: During the past year the following retinoid analogs have been prepared:

1. Compounds in which the (13E)-13-CH_3-15-CO_2H moiety of (E)-, (7E,9E,11Z)- and (7E,9Z,11E)-retinoic acid has been replaced by a 3,4-diacetoxyphenyl ring.

2. Compounds in which the (11E,13E)-13-CH_3-15-CO_2H moiety of retinoic acid has been replaced by a meta-substituted-4-carboxyphenyl or a meta-substituted-4-carboethoxyphenyl group.

3. A compound in which the side chain and polar terminus of retinoic acid has been replaced by a 4'-carboxybiphen-4-yl group.

4. Compounds in which the β-cyclogeranylidene ring has been replaced by an acyclic species.

The biological testing of some of these compounds is in progress. The preliminary results for these compounds and for the retinoids that we had previously prepared but whose biological results were not reported in the last report, are presented below. The latter compounds are marked by an asterisk. The compound name, given first, is followed by the result of the hamster-tracheal-organ culture assay for reversal of keratinization, which is expressed as the percentage of cultures lacking keratin and keratohyaline granules at retinoid concentrations of 10^{-10} M and 10^{-9} M (a). This assay was conducted by Dr. L. J. Schiff at IIT Research Institute, Chicago, Illinois. Next is listed the effect of the retinoid on the inhibition of the induction of ornithine decarboxylase by 12-O-tetradecanoylphorbol-13-acetate in mouse skin, which is expressed as the percentage of enzyme inhibition at two dose levels, 1.7 and 17 nmoles (b). This assay was conducted at SRI.

(E)-1-(3,4-Diacetoxyphenyl)-4-methyl-6-(2,6,6-trimethyl-1-cyclohexen-1-yl)-1,3,5-hexatriene: a) 18%, 21%.

(1Z,3E,5E)-1-(3,4-Diacetoxyphenyl)-4-methyl-6-(2,6,6-trimethyl-1-cyclohexen-1-yl)-1,3,5-hexatriene: a) 29%, 54%; b) 19%, 28%.

(1E,3Z,5E)-1-(3,4-Diacetoxyphenyl)-4-methyl-6-(2,6,6-trimethyl-1-cyclohexen-1-yl)-1,3,5-hexatriene: a) 15%, 33%.

(1Z,3E)-1-(4-Carboethoxyphenyl)-1-fluoro-2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-1,3-butadiene: a) 46%, 83%; b) 10%, 65%.

(1Z,3E)-1-(4-Carboxyphenyl)-1-fluoro-2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-1,3-butadiene: a) 38%, 92%; b) 25%, 85%.

(E)-1-(4-Carboethoxyphenyl)-1-fluoro-2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-1,3-butadiene: a) 0%, 8%; b) 0%, 35%.

Ethyl (2E,4E,6E,8E)-10-(1,1-dimethyl-1-propyl)-3,7,11-trimethyl-2,4,6,8,10-dodecapentaenoate: a) 27%, 83%; b) 40%, 81%.

Ethyl (2Z,4E,6E,8E)-10-(1,1-dimethyl-1-propyl)-3,7,11-trimethyl-2,4,6,8,10-dodecapentaenoate: a) 30%, 80%; b) 14%, 20%.

Ethyl (2E,4E,6E,8E)-10-(1,1-dimethyl-1-propyl)-3,7,11-trimethyl-2,4,6,8,11-dodecapentaenoate: a) 36%, 77%; b) 4%, 54%.

Pentaerythritol monoretinoate*: a) 43% (10^{-11}M), 72%, 100%.

2$^{\alpha}$-Phenyl-5$^{\beta}$-hydroxymethyl-5$^{\alpha}$-retinoyloxymethyl-1,3-dioxane*: a) 33% (10^{-11}M), 37%, 100%.

2$^{\alpha}$-Phenyl-5$^{\alpha}$-hydroxymethyl-5$^{\beta}$-retinoyloxymethyl-1,3-dioxane*: a) 33% (10^{-11}M), 50%, 94%.

(E)-1-(5-Carboxythiophen-2-yl)-2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-1,3-butadiene*: a) 47%, 93%; b) 37%, 73%.

(E)-Retinoic acid: a) 65%, 93%; b) 87-91% (1.7 nmoles).

(1Z,3E)-1-(5-Carboxythiophen-2-yl)-2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-1,3-butadiene*: b) 35%, 74%.

(E)-6-(2-(2-6,6-Trimethyl-1-cyclohexen-1-yl)ethen-1-yl)-2-naphth-aldehyde*: a) 28%, 50%; b) 0%, 43%.

The following compounds have also been submitted for testing.

(E)-1-(4-Carboethoxy-2-methoxyphenyl)-2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-1,3-butadiene.

(E)-1-(4-Carboethoxy-2-fluorophenyl)-2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-1,3-butadiene.

(E)-1-(4-Carboxy-2-fluorophenyl)-2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-1,3-butadiene.

Significance to Biomedical Research and the Program of the Institute: Retinoid deficiency enhances the susceptibility of the epithelial tissue of the colon, bladder and lung of experimental animals to chemical carcinogenesis. Synthetic retinoids can inhibit the development of epithelial cancer of the skin, respiratory tract, mammary gland and bladder in experimental animals and can reverse the hyperplasia induced by chemical carcinogens in prostatic and tracheal organ cultures. Drug development in this area is required because the prophylactic usefulness of the natural retinoids is limited by their toxicity, tissue distribution pattern and metabolic deactivation. Synthetic efforts must be aimed at developing nontoxic drugs that could be administered regularly to augment normally operative body mechanisms that arrest or reverse preneoplastic processes during the progression to invasive maligancy.

Proposed Course: To continue the preparation of retinoid analogs, using all available structure-activity data for the design of future compounds.

Date Contract Initiated: August 30, 1980

Current Annual Level: 0

SUMMARY REPORT
CARCINOGENESIS MECHANISMS

Included in the Carcinogenesis Mechanisms Program are studies relating to metabolism, toxicity and physiological disposition of carcinogens and their metabolites; isolation, identification and synthesis of known and suspect carcinogens and their metabolites, mechanisms of action, molecular structure activity relationships and carcinogen-mutagen relationships. This Program supports basic research only. Thus, in accordance with the National Cancer Institute's policy to phase out basic research contracts, all of the contracts in this Program were brought to an orderly conclusion at their termination date.

Only one contract was active for one month during FY '82. This contract, located at Southern Research Institute (NO1-CP-55721) completed studies on the metabolism of six carcinogens. These studied elucidation of metabolic pathways, identification of metabolites and macromolecular binding. The contract terminated October 31, 1981.

CONTRACT INDEX

CONTRACT NARRATIVE

CARCINOGENESIS MECHANISMS

SOUTHERN RESEARCH INSTITUTE (NO1-CP-55721)

Title: Metabolism of Carcinogenic Compounds

Contractor's Project Director: Dr. Donald L. Hill

Project Officer (NCI): Dr. Marcia D. Litwack

Objectives: To synthesize or otherwise acquire six designated carcinogens labeled with carbon-14, and to study the metabolic pathways of these compounds in rats in attempts to determine their mechanism of action.

Major Findings: The major rat urinary metabolite of reduced Michler's ketone (RMK) was isolated by thin-layer chromatography, and its structure was determined by a combination of mass spectral and proton magnetic resonance analysis. The metabolite was identified as 4,4'-(hydroxymethylene)bisacetanilide, and structural confirmation was achieved by chemical synthesis.

Tentative identification of several minor rat urinary metabolites was accomblished. Possible structures include a dimethyl analog of the major urinary metabolite, a didemethylated, acetylated RMK derivative, and a ring-hydroxylated isomer of the major urinary metabolite. No evidence was found that any of the urinary metabolites were present as glucuronic acid conjugates.

Chemical synthesis of symmetrical and unsymmetrical didemethyl RMK and tridemethyl RMK, microsomal metabolites of RMK, was achieved for evaluation as mutagens.

RMK but not Michler's ketone was found to be mutagenic in the Ames test after activation with mouse liver microsomes but not with rat liver microsomes. In addition, the mutagenicity of RMK and its partially and totally demethylated derivatives, upon incubation with the S9 fraction of mouse liver microsomes, was observed to be inversely proportional to the degree of methylation. This interesting observation demonstrates that the methyl groups of RMK are not necessary for mutagenic activity.

Significance to Biomedical Research and the Program of the Institute: Elucidation of the enzymatic processes of activation and mechanisms of action of carcinogens present in the human environment will allow steps to be taken to inhibit formation of active metabolites, to induce detoxifying enzymes, or to provide receptor substances that prevent binding of the active species to cellular macromolecules. Such inhibitors and inducers would be candidate anticarcinogens.

Proposed Course: The contract has terminated.

Date Contract Initiated: June 30, 1975

Current Annual Level: 0

SUMMARY REPORT
MOLECULAR CARCINOGENESIS

Research in the Molecular Carcinogenesis Program area focuses on the characterization of carcinogen-macromolecule interactions; changes in biological macromolecules, cell structure, ultrastructure, and functions as a result of carcinogen or cocarcinogen exposure; the identification of biochemical and molecular markers and properties of cells transformed by carcinogens; the genetic and other mechanisms of cell transformation; the development of carcinogenicity/mutagenicity testing procedures; the mechanisms of carcinogen-induced mutagenesis and genetic damage; enzymes associated with carcinogenesis induced by chemical and physical carcinogens; and the role of DNA repair in carcinogenesis. The contract mechanism for support of research in the Molecular Carcinogenesis Program area is rapidly being phased out. Contracts currently remaining in this category fall into five subject areas: (1) the development of carcinogenicity testing procedures; (2) markers and properties of transformed cells; (3) carcinogen metabolizing enzymes; (4) changes in cell membrane structure and function; and (5) the role of DNA repair in carcinogenesis. The major accomplishments of each of these areas are highlighted below:

Development of Carcinogenicity Testing Procedures

Several contractors are conducting research aimed at the development of animal and cell culture test systems for use in detecting potential carcinogens and/or tumor promoters. A hairless mouse model system is being developed for UV radiation and chemical carcinogenesis studies under another interagency agreement with DOE/Oak Ridge National Laboratory (YO1-CP-90201). Four different, dominant, autosomal mutations that influence the phenotype of the skin of the mouse have been identified. These mutants result in differences in the presence of sebaceous glands, skin thickness, and hair follicles from that seen in haired or the hr/hr hairless mouse. The structural, cytological, and cytokinetic characteristics appear to mark these mutants as potentially useful model systems for investigating certain aspects of UVR and chemical carcinogenesis. The mutants are being backcrossed onto the BALB/c genetic background. The eighth generation is now being produced.

The development of cancer has been shown to be a multistage process beginning with initiation followed by promotion. Few cell culture systems exist which allow the analysis of initiation and promotion events in vitro. Since the majority of human cancers arise from epithelial tissue, the development of in vitro transformation systems using epithelial cells is of importance. To parallel the established mouse skin two-stage tumorigenesis model, the investigators at DOE/Oak Ridge National Laboratory (YO1-CP-70227) have continued studies on the development of a two-stage transformation system using epidermal cells from skin tumor sensitive mice. Transformation of cells from newborn mice by carcinogens was successfully demonstrated although there was a problem in that a high rate of spontaneous transformation was observed. Because of this problem, more emphasis has been placed on in vitro transformation studies using epidermal cells from adult mice. The development of techniques and markers which will allow the early determination of transformation of epidermal cells is being continued. Carcinomas have been found to lack high molecular weight keratins, glucocorticoid receptors, and filaggrin and to become positive for gamma-glutamyltranspeptidase activity. These may become useful markers for identifying carcinogen transformed cells.

Other systems for identifying carcinogens and promoters are being developed at DOE/Argonne National Laboratory (YO1-CP-70222). Chinese hamster V79 cell mutants

resistant to the cytotoxic effect of mycophenolic acid are being selected. This compound is a potent inhibitor of IMP dehydrogenase, one of the nucleic acid biosynthetic enzymes whose activity is enhanced in malignant cells. The studies suggest that the mutants may be possible regulatory mutants which can affect the control of cell growth. Thus, mycophenolic acid resistance is suggested as another useful marker in short-term assays for the identification of potential chemical carcinogens. Tumor-promoting phorbol diesters have been shown to induce human HL-60 promyelocytic leukemia cells to differentiate into macrophage-like cells. HL-60 cells resistant to the action of phorbol diesters have been isolated. A nonphorbol diester tumor promoter, teleocidin, has been shown to produce effects similar to PMA in susceptible and resistant HL-60 cells. This system may thus be useful to detect other chemicals that may be potential tumor promoters.

Markers and Properties of Transformed Cells

It is a generally accepted view that the development of cancer is a multistep process in which new cell populations arise representing stages in the cellular evolution from normal, through initiated, preneoplastic and premalignant to frank neoplasia. The acquisition of various biochemical, molecular, and functional markers as a function of time after carcinogen exposure is being investigated. This will serve as a means of identifying cells at a particular stage in carcinogenesis. Two contractors at DOE/Oak Ridge National Laboratory are developing in vivo and in vitro techniques for studying the development of neoplasia in respiratory tract epithelium. One contractor (YO1-CP-90207) using a tracheal implant-organ culture-cell culture model has demonstrated that the cytopathology of cells exfoliated into the culture medium can be used to identify lesions on carcinogen-exposed tracheal explants with a 92% accuracy. A tentative relationship was also determined between the number of morphologically-determined lesions and the number of carcinogen-altered cell populations, identified as primary cell cultures having the ability to survive a selective growth medium in which untreated tracheal epithelial cells do not survive. Tumorigenic cell lines were shown to exhibit a high level of multi-nucleation in response to cytochalasin B. Non-tumorigenic, carcinogen-exposed cells show a decrease in toxicity to cytochalasin B and an increase in multinucleation during subculture suggesting that this property may be a good marker for transformation. The second contractor (YO1-CP-90211) has used the epithelial focus (EF) assay, which they developed, to quantitate emerging carcinogen altered and neoplastic cells following carcinogen exposure. The principal effect of the tumor promoter TPA on cells exposed to DMBA was to increase the rate of progression to a potentially neoplastic state and/or inhibit reversal of the potentially neoplastic state in intact trachea. The tumor latency period was shown to be unaffected by TPA but was decreased by exposure to X-rays.

Basic mechanisms and properties of cells that may be characteristic of neoplastic transformation are being investigated at the Johns Hopkins University (NO1-CP-55713). Statistical evaluation of data on the quantitation of tumorigenicity and in vitro growth properties of Syrian hamster tumor cell lines shows that the properties of anchorage-independence and enhanced fibrinolysis correlates well with tumorigenesis. Other studies have shown that the loss of post-confluence inhibition of cell division marks an early stage in neoplastic progression in vitro. This phenotype occurs at a high frequency in tumorigenic cell lines, appears to correlate with anchorage-independent growth, and appears to precede anchorage-independent growth by about 15 population doublings. Neoplastic transformation induced by carcinogens was shown to be ploidy dependent. Also, anchorage-independence is suggested to be a ploidy-dependent recessive phenotype. These studies implicate chromosome variability and random allelic assortment as providing a potential pathway for neoplastic progression.

Carcinogen Metabolizing Enzymes

There are currently relatively few studies which allow us to understand potential similarities and differences in the response of experimental animals and humans to chemical carcinogen exposure. Data on the comparative metabolism of carcinogens suggest that it is, in general, qualitatively similar. In recognition of the perceived need for more comparative studies, a program was recently initiated on interspecies comparisons in carcinogenesis. To gain more information on the carcinogen metabolizing enzymes of human origin, a contractor at the University of Texas (NO1-CP-85671) has been studying three enzymes involved in the detoxification pathway of PAH metabolism. These three enzymes, glutathione-S-transferase (GST), phenolsulfotransferase (PST), and UDP-glucuronyl-transferase (UDPGT) have been isolated from human liver. A single form of PST, having a molecular weight of 58,000 daltons and consisting of two 31,000 dalton subunits, has been purified to apparent homogeneity. A single form of UDPGT, having a subunit molecular weight of 58,000 daltons, has also been purified. Six isozymes of GST, five having alkaline isoelectric points and one acidic, have now been purified. These isozymes have been characterized as to size, immunologic similarity, and substrate specificity.

Changes in Cell Membrane Structure and Function

Alterations of cell membrane structure, properties and function are known to occur in cells exposed to phorbol ester tumor promoters or transformed by chemical or viral agents. The ability of phorbol ester tumor promoters to induce or inhibit cell differentiation, depending on the cell types being examined, appears to be mediated by the nature of its binding to cell surface membranes. A contractor at DOE/Oak Ridge National Laboratory (YO1-CP-90205) is examining the physiological mechanisms by which cells regulate their membrane transport activities. The enzyme Na,K-ATPase, which maintains intracellular K at high levels that are necessary for many cell functions, normally turns over rapidly. In response to physiological stress, the rate of removal of Na,K-ATPase from the cell membrane was shown to be slowed. Surface membranes were also shown to be recycled by the cells. An epithelial cell line from pig kidney was shown to grow and differentiate in culture with the acquisition of Na^+-hexose concentrating capacity. Studies have shown that individual cells, independently and rapidly with respect to the time of differentiation of the whole culture, can acquire their maximum number of transporters and transport capacity.

Role of DNA Repair in Carcinogenesis

Three contractors have been working on the development of methods for the detection of heterozygous carriers of certain genetic disorders known to involve DNA repair deficiencies. It is well established that individuals with homozygous genotypes for the recessively inherited genetic disorders such as xeroderma pigmentosum (XP), ataxia telangiectasia (A-T), Fanconi's anemia (FA), and Bloom's syndrome (BS) have a significantly higher predisposition to cancer. Cancer incidence in A-T heterozygotes is also reported to be increased. At the Sloan-Kettering Institute for Cancer Research (NO1-CP-85665) the contractor has been developing a test which uses chromosome instability (breakage and sister chromatid exchange) as the endpoint for the detection of heterozygous genotypes for FA and A-T. For FA, the contractor has developed a rapid method for the detection of affected individuals pre-and postnatally and can identify the carrier state in affected families by the use of diepoxybutane as a clastogenic agent. Work on A-T is now in the early developmental stage. A great deal of phenotypic and cellular heterogeneity, presumably based on genetic heterogeneity, has become apparent and will likely hamper the attempt to develop tests for heterozygote detection applicable to the general population. In another approach to the same problem, contractors at The Johns Hopkins University

(NO1-CP-85670) have been purifying seven enzymes from human placenta which repair and restore the integrity of damaged DNA. In the process of purification to homogeneity, significant information has been obtained on the specificity and characteristics of these enzymes. A great deal of effort has been expended in detecting very low levels of antibody against these enzymes, for the purposes of characterizing their presence, distribution and nature in cells derived from patients with repair deficiencies. Although antibodies have been obtained against polynucleotide ligase, it has been extremely difficult to obtain antibodies against the other six enzymes. However, our knowledge of the properties of these as enzymes and proteins will permit examination of the level of these enzymes in normal and repair deficient cell lines. At the University of Chicago (NO1-CP-85669), it was found that some human lymphoblastoid cell lines are deficient in the ability to remove O^6-methylguanine adducts after reaction with mutagenic methylating and ethylating agents. It was hoped that a major polymorphism would be found which would permit division of populations into two classes on the basis of their ability to remove O^6-methylguanine, a lesion commonly considered as critical for the induction of tumors. This expectation now seems less likely since both removal competent and incompetent lines can be obtained from the same male individual, implying the difference is developmental or related to the transformation event in the production of lymphoblastoid lines. Characterization of the kinetics and capacity of competent, incompetent and hybrid cells yielded some valuable insight into the repair of this critical lesion and raised a number of important questions for new work.

In 1980, Lindahl reported the partial purification of a protein from E. coli which catalyzes the removal of O^6-methylguanine (O^6-MeG) from (^{3}H) methylated DNA and to which the methyl group becomes covalently bound through a sulfhydryl group of cysteine in the methyltransferase. Since each molecule of repair "enzyme" can repair only one O^6-methylguanine residue, the protein has come to be known as the kamikase protein. At the Oak Ridge National Laboratory (YO1-CP-00200), investigators have now obtained the first unequivocal evidence in a mammalian system that O^6-MeG repair involves in situ demethylation without alteration or removal of the guanine residue and thus is similar to the repair pathway in E. coli. By the use of single-stranded DNA substrates, containing high specific activity ring-labeled O^6-MeG, they showed that incubation with extracts of rat liver nuclei resulted in the appearance of (^{3}H) guanine concomitant with the loss of (^{3}H) O^6-MeG in the recovered DNA. The kinetics of the demethylation reaction in vitro with rat liver extracts are similar to those seen at the University of Chicago using whole human lymphoblastoid cells. The levels of demethylase activity found in different organs of the rat may help to explain the differential susceptibility to carcinogenesis in different organs exposed to alkylating agents.

Other studies at ORNL are being conducted on the characteristics and expression of DNA repair in man. One laboratory (YO1-CP-90203) has been investigating the molecular events which occur in human cells when damaged by radiation or chemical agents. The ability of mammalian cells to excise ultraviolet light induced pyrimidine dimers from their DNA has been characterized through the use of a battery of assays for determining the number and size of repaired sites and the rate of removal. After biological doses of UV 254 nm irradiation, about one-half of the dimers are excised within 24 hours. With the use of arabinofuranosyl (ara-C) for quantitation of excision repair, the contactor has shown that excision repair levels are the same in normal and XP variant cells. However, the XP-variant is less sensitive to ara-C arrest suggesting the intracellular deoxynucleoside pools are considerably higher in the XP-variant.

In another study at ORNL (YO1-CP-90208), investigators are attempting to genetically dissect the DNA repair systems in man and to identify and map the number and kinds of genes required for DNA repair. Utilizing man x mouse somatic cell hybrids as the experimental system, the contractor has isolated sixty-five hybrid clones, characterized their type of repair of UV-induced DNA damage (human-like, mouse-like, or intermediate), and correlated DNA repair capacity with specific human chromosomes. The data suggest that a gene or a series of genes required for DNA repair are located on human chromosome #3. There was also a correlation between chromosome #14 and excision repair capacity. Hybrid clones from human XP cells and mouse spleen cells have shown that mouse cells will complement the defective repair for XP groups A, B, C and D, but not group E. This defect is apparently qualitatively different. Work is now underway with inhibitors of the DNA repair process to determine if it is possible to assign genes coding for specific steps in DNA repair to specific chromosomes.

CONTRACT INDEX

CHICAGO, UNIVERSITY OF (NO1-CP-85669)

Title: Identification of Heterozygous Carriers of DNA Repair Defects

Contractor's Project Director: Dr. Bernard S. Strauss

Project Officer (NCI): Dr. David G. Longfellow

Objectives: To develop a test for the identification of heterozygotes of human DNA repair deficiency syndromes in order to permit the unequivocal recognition of such individuals. The differential ability of cultured lymphoblastoid cell lines to remove O^6-methylguanine lesions from their DNA was to form the basis of this test.

Major Findings: The contractor has completed a study of the removal of the O^6-alkylguanine adduct produced in the DNA of human lymphoblastoid cells by reaction with mutagenic methylating and ethylating agents. The O^6-methylguanine adduct is rapidly removed from cellular DNA with about 40 percent of the reaction complete in ten minutes. The capacity of cells is limited and is easily "used up." Regeneration of removal capacity takes over 24 hours. The acceptor will apparently react with ethyl as well as with methyl groups (Sklar, Brady and Strauss, 1981). In an effort to understand the control of the removal characteristic, we have made hybrids between lines proficient in their ability to remove O^6-methylguanine and lines deficient in this ability. Hybrid cells have an O^6-methylguanine removal capacity per mole of guanine, about one third to one half that of the removal competent parents, i.e., about the same per cell. Cell hybrids removed the same amount of the alkylation adduct 3-methyladenine as did their parents per mole of guanine, i.e., about twice as much per cell. Although the cell hybrids had intermediate resistance to the cytotoxic action of N-methyadenine, there is evidence that the ability to remove O^6-methylguanine and resistance to the cytotoxic effect of N-methyl-N'-nitro-N-nitrosoguanidine are dissociable characteristics. We interpret these data to mean that some structural change at the chromosomal level results in the difference between removal competent and incompetent cells. Since both removal competent and incompetent lines can be obtained from the same male individual, the difference cannot be genetic but must be either developmental or related to the transformation event involved in the production of lymphoblastoid lines.

Significance to Biomedical Research and the Program of the Institute: The O^6-methylguanine adduct is a major source of mutagenesis and carcinogenesis, and a large literature is devoted to the study of its action and removal. Many of these studies involve analyses only after relatively long times. Awareness of the rapidity of the repair reactions is important to adequately study the biological consequence of treatment with carcinogenic alkylating agents. At the same time, the realization that cells may have their O^6-methylguanine removal capacity "turned off" by a biological mechanism which is not yet understood, is important in understanding the susceptibility of cell and of organisms to alkylating agents. Cells unable to remove O^6-methylguanine should more easily mutate and undergo malignant transformation after treatment with methylating agents. At the same time, tumors which are deficient in this removal reaction may be more sensitive to the lethal effects

of alkylation treatment, thereby providing a clue to the chemotherapy of a significant fraction of malignancies.

Proposed Course: The contract expired December 30, 1981.

Date Contract Initiated: September 30, 1978

Current Annual Level: 0

DEPARTMENT OF ENERGY/OAK RIDGE NATIONAL LABORATORY (YO1-CP-00200)

Title: A Novel Approach to the Investigation of the Role of DNA Repair in Chemical Carcinogenesis

Contractor's Project Director: Dr. Sankar Mitra

Project Officer (NCI): Dr. David G. Longfellow

Objectives:
1. To elucidate the mechanism(s) of removal of O^6-alkylguanine from DNA in rat liver.

2. To purify and characterize the repair enzyme(s) involved.

3. To investigate the inducibility of the repair enzyme(s) in rat liver.

Major Findings: O^6-Methyl(8-^{3}H)dGTP of high specific activity (4.5 Ci/mmol) was synthesized and used to prepare a single-stranded DNA polymer, poly(dC,dG,(8-^{3}H)m^6dG). Incubation of this polymer with extracts of rat liver nuclei resulted in the appearance of (^{3}H)guanine concomitant with the loss of (^{3}H)O^6-methylguanine in the recovered DNA. This is the first unequivocal demonstration in a mammalian system that O^6-MeG repair involves in situ demethylation without alteration or removal of the guanine residue, and thus is similar to the repair pathway in E. coli. These findings are complementary to those of others who have shown, using DNA substrates containing (alkyl-^{3}H)O^6-alkylguanine, that the O^6-alkyl group is transferred to a protein cysteine residue. O^6-MeG demethylase (methyltransferase) activity also has been found in porcine and bovine liver and in HeLa cells by the contractor.

Kinetic data for demethylation of O^6-MeG by both E. coli and mammalian cell extracts are consistent with a stoichiometric mechanism, first proposed by Robins and Cairns for O^6-MeG repair in E. coli in which each molecule of repair "enzyme" can repair only one O^6-MeG residue. Thus the rate of demethylation levels off after approximately 30 minutes, and the extent of demethylation (up to 40% of the O^6-MeG) is linearly dependent on the amount of extract added to incubation mixtures. The stoichiometric nature of the reaction allows the quantitation of demethylase activity in terms of molecules of "enzyme" per cell, and such values were determined for the following mammalian cells and nuclei:

	Demethylase (molecules/cell)
Nuclei	
Rat liver	~3,000
Porcine liver	~8,000
Bovine liver	28,000
Cells	
HeLa (CCL2)	96,000
HeLa (S3)	<50 (None detected)
CHO	<1,000 (None detected)

Of particular interest are the two HeLa cell strains which were obtained from Dr. Rufus Day of NCI. HeLa CCL2 contained the highest level of methyltransferase in any cell yet tested by the contractor, whereas S3, which Dr. Day had previously shown to be defective in O^6-MeG repair contained no detectable activity. Mixing experiments involving CCL2 and S3 extracts showed no evidence of an inhibitor in S3.

Levels of O^6-MeG demethylase activity in whole extracts of rat liver, kidney, and brain were also compared:

Tissue	fmol (^{3}H)G/mg protein/hr
Liver	54
Kidney	10
Brain	<8

The relative order of activities in these in vitro assays is the same as for the known tissue-specific rates of O^6-MeG removal in vivo: liver>kidney>brain.

In an attempt to induce O^6-MeG repair activity to a higher level in rat liver, Fisher-344 rats were given dimethylnitrosamine (2 mg/kg/day) by stomach tube for 20 days, according to Montesano et al. In contrast to the 2- to 3-fold induction reported by these authors, the contractor found a 35% decrease in the activity of nuclear extract relative to the untreated control. It is possible that this difference may have been due to the difference in animal strains (Montesano et al. used BDIV and Sprague-Dawley rats) or to differences in extract preparation and assay methods.

Significance to Biomedical Research and the Program of the Institute:
Lindahl has partially purified a protein from E. coli which catalyzes the disappearance of O^6-methylguanine from (^{3}H)methylated DNA and to which the methyl group becomes covalently bound through a sulfhydryl group of cysteine in the methyltransferase. Similar activities have been found in rodent liver. The contractor's experiments with DNA substrates containing ring-labeled O^6-methylguanine are complementary to these findings by demonstrating unequivocally that demethylation occurs in situ and that the resulting guanine residue remains intact in the DNA. These results are, therefore, of major significance in understanding the exact mechanism of repair of this promutagenic base.

The (8-^{3}H)O^6-MeG-DNA substrates also appear to be excellent substrates for quantitation of demethylase activity. Assays based on the use of (^{3}H)methylated DNA require the measurement of the loss of (^{3}H)O^6-MeG from the substrate relative to

some other base, generally (^{3}H)7-MeG. However, 7-methylguanine is also subject to spontaneous and enzymatic release from DNA so that this method may not always be reliable. In contrast, the formation of (8-^{3}H)guanine, even at low levels relative to (8-^{3}H)O^6-methylguanine, can be accurately quantitated in the contractor's synthetic DNA substrates. Although the rate of demethylation of O^6-MeG in his synthetic single-stranded substrate by partially purified rat liver O^6-MeG demethylase was slightly slower than in a double-stranded (3H)methylated DNA, the extent of demethylation was essentially the same. Because the ultimate biological effects of O^6-MeG depend on both its miscoding properties and its rate of repair, these quantitative assays of repair activity are important for understanding the differential mutagenic/carcinogenic effects of alkylating agents in various cells and tissues.

Proposed Course: The contract ended December 31, 1981.

Date Contract Initiated: July 1, 1980

Current Annual Level: 0

DEPARTMENT OF ENERGY/OAK RIDGE NATIONAL LABORATORY (YO1-CP-70222)

Title: Malignant Cell Transformation and Mutagenesis Induced by Carcinogenic Chemicals

Contractor's Project Director: Dr. Eliezer Huberman

Project Officer (NCI): Dr. Paul Okano

Objectives:
1. To isolate mutagen-resistant cells for mutagenesis and DNA repair studies.

2. To study the induction of different markers of differentiation in human malignant cells by tumor promoting agents.

3. To study the alterations in lipid biosynthesis in differentiating human tumor cells.

Major Findings: Human HL-60 promyelocytic leukemia cells can be induced to differentiate into macrophage-like cells by tumor-promoting phorbol diesters, including phorbol-12-myristate-13-acetate (PMA). Teleocidin, a nonphorbol diester tumor promoter, causes effects in the HL-60 cells similar to that of PMA. These include similar changes in cell morphology, as well as increases in lysozyme and nonspecific esterase activity--characteristics associated with macrophage cell differentiation. In addition, both compounds cause HL-60 cells to accumulate in G_1-phase at the expense of S-phase cells. The contractor has been able to select an HL-60 cell variant (HL-60-R) that is resistant to PMA-induced differentiation. None of the above-described effects was observed in this resistant variant. These PMA resistant cells are, however, as susceptible as the parent cells to induction of granulocyte-like cell differentiation by agents such as dimethyl sulfoxide and retinoic acid. These results indicate that PMA and teleocidin produce similar, if not identical changes in the HL-60 cells.

In other studies, the contractor has shown that the PMA-resistant cells are defective in the "down regulation" of specific binding of phorbol diesters (i.e., a

loss of cellular bound phorbol dibutyrate following its maximal specific binding). This may indicate that down regulation of specific receptors common to both the phorbol diesters and teleocidin is important in the induction of macrophage-like cell differentiation in the HL-60 cells.

Cell variants resistant to the cytotoxic effect of mycophenolic acid, an inhibitor of IMP dehydrogenase (IMP:NAD^+ oxidoreductase EC 1.2.1.14), were selected by a one-step procedure from Chinese hamster V79 cells. The frequency of these variants was increased in a dose-dependent manner after treatment with the mutagen N-methyl-N'-nitro-N-nitrosoguanidine and after an expression time of 8 days. The degree of resistance in five of six isolated cell variants was associated with a comparable increase in the specific activity of IMP dehydrogenase, which was 3- to 6-fold higher than that of the parent V79 cells. The enzyme activity from both the variants and the parent cells had a similar affinity for the substrate IMP and a similar response to mycophenolic acid. These results show that there are no significant differences between the wild-type and variant cell enzymes. The studies to date suggest that the regulation of genes of IMP dehydrogenase are altered in the cell variants.

Significance to Biomedical Research and the Program of the Institute:
Our current state of knowledge suggests that carcinogenesis is a multistage process which begins with an initial mutational event and requires further steps of a nonmutational nature,i.e., promotion. Phorbol diesters such as PMA have been shown to be active tumor promoters in a two-stage (initiation-promotion) mouse skin carcinogenesis model. PMA has also been found to alter cell differentiation in some avian, murine, and human cells. The induction or inhibition of terminal differentiation in these cells is related to the tumor promoting activity on mouse skin. The mechanisms by which phorbol diesters control cell growth and cell differentiation may be relevant to our understanding of processes involved in tumor promotion by chemicals. Since teleocidin, a nonphorbol diester tumor promoter, has been shown to produce effects similar to PMA, the susceptible and resistant HL-60 cell system employed may be useful to detect other chemicals that may be potential tumor promoters.

Mutations in regulatory genes, including those that control cell growth, may be the initial step involved in malignant transformation. Nucleic acid biosynthetic enzymes may, in part, regulate cell replication. IMP dehydrogenase is one of these enzymes whose activity is enhanced in malignant cells. Cell variants, resistant to the cytotoxic effect of mycophenolic acid (a potent inhibitor of IMP dehydrogenase), can be induced by carcinogens and mutagens. These mutations are suggested to occur in regulatory genes. Thus, mycophenolic acid resistance may be another useful marker in short term assays for the identification of potential chemical carcinogens.

Proposed Course:
1. To study the induction of cell differentiation in human T lymphoid leukemia cells.

2. To study the control of phospholipid methylation by phorbol diesters in differentiating human tumor cells.

Date Contract Initiated: September 30, 1977

Current Annual Level: 0

DEPARTMENT OF ENERGY/OAK RIDGE NATIONAL LABORATORY (YO1-CP-70227)

Title: In Vitro Transformation of Tumor Sensitive Epidermal Cells: A Bioassy and a Model for the Study of the Mechanism of Action of Tumor Initiators and Promoters

Contractor's Project Director: Dr. Thomas J. Slaga

Project Officer (NCI): Dr. Paul Okano

Objectives: To develop a reliable and quantitative in vitro transformation system using epidermal cells from skin tumor sensitive mice which will be a relevant one in which two-stage transformation is operational using phorbol ester tumor promoters. Specifically, the contractor plans:

1. To compare transformation of primary or secondary cultures of newborn and adult epidermal cells using much improved culture conditions which allow the epidermal cells to grow and differentiate for a long period in culture much as they do in vivo.

2. To perform similar transformation studies with one of the isolated non-tumorigenic epidermal cell lines which has a faster growth rate.

3. To determine the effects of inhibitors of tumor initiation and promotion in the cell culture systems.

Major Findings: The contractor has previously reported that there was a high rate of spontaneous transformation using either primary newborn epidermal cells or the contractor's SECA cell line derived from newborn epidermal cells. However, in all cases, it was found that B(a)P, MNNG and B(a)P-diol-epoxide decreased the time to obtain transformation. This data is currently being prepared for publication. Because of the above results using newborn epidermal cells in culture, more emphasis has been placed on in vitro transformation studies using adult epidermal cells in culture. Preliminary results in the contractor's laboratory as well as in Dr. Yuspa's laboratory suggest that spontaneous transformation is very low in adult mouse epidermal cell cultures. Using similar culture conditions as with the newborn epidermal cells, the adult epidermal cells in culture can grow and differentiate similar to that in vivo. However, the growth rate of adult epidermal cells in culture is slower than newborn epidermal cells in culture. In addition, they can only be subcultured four or five times before they die out. The contractor started a large transformation study in September of 1981 using adult epidermal cells isolated similar to the method used for newborn epidermal cells. Three dose levels of B(a)P, B(a)P-diol-epoxide and MNNG were used. Four days after exposure of the carcinogen, some of the cultures were treated with TPA. Although the experiments have not progressed far enough to determine if a dose-dependent transformation has occurred or if a two-stage transformation has occurred, a number of cell lines have been obtained from the carcinogen and/or promoter treatments. All the control cultures from adult epidermal cells died out after the fifth subculture. These results once again suggest that there is a very low level or no spontaneous transformation using adult epidermal cells.

Also, the contractor has previously reported that a technique to separate mouse epidermal cells by density centrifugation through Percoll, a colloidal silica gradient, was developed. Using an improved Percoll gradient, adult epidermal cells were separated into eight groups. Three of the most dense bands contain basal cells

with a high plating efficiency. The contractor is currently determining their growth potential and cloning efficiency in culture. In the near future, the contractor plans to do some transformation studies using these subpopulations of adult epidermal basal cells. It is felt that one of the subpopulations of basal cells represents a stem cell population which may enable the contractor to obtain a high frequency of transformation.

The contractor will also continue to develop techniques which may allow for early determination of epidermal cell transformation in culture. The contractor previously found that carcinomas lack high molecular weight keratins. Recently, it was found that carcinomas also lack glucocorticoid receptors and filaggrin and are positive for gamma-glutamyl-transpeptidase. These markers should be very useful in determining transformed cells in the in vitro transformation studies.

Significance to Biomedical Research and the Progam of the Institute: Since the majority (>85%) of human cancers arise from epithelial tissue, it is important to have a quantitative in vitro transformation system using epithelial cells. The question must be asked whether the results obtained from the in vitro transformation of fibroblasts are significant in terms of all forms of cancer and if the results can be extended to transformation studies using epithelial cells. These studies, therefore, to develop a reliable and quantitative in vitro transformation system using mouse epidermal cells from tumor sensitive mice are highly significant. In addition, it is important that the transformed cells give rise to keratinizing squamous cell carcinomas when injected into a syngeneic host.

Proposed Course: This has not changed since the contractor's renewal proposal, except that more emphasis will be put on transformation studies using adult epidermal cells in culture. In addition, more emphasis will be put on separating adult epidermal cells by our Percoll method in order to obtain subpopulations of epidermal basal cells for transformation studies. This Interagency Agreement is scheduled to terminate on August 31, 1982.

Date Contract Initiated: September 30, 1977

Current Annual Level: $222,402

DEPARTMENT OF ENERGY/OAK RIDGE NATIONAL LABORATORY (YO1-CP-90201)

Title: Ultraviolet Radiation Carcinogenesis

Contractor's Project Director: Dr. R. J. Michael Fry

Project Officer (NCI): Dr. David G. Longfellow

Objectives: This project consists of two parts:

1. The development of mouse model systems for the study of ultraviolet radiation (UVR) and chemical carcinogenesis and the factors that influence carcinogenesis of the skin in particular, and cancer in general.

2. Investigation of the factors that determine susceptibility for UVR-induced carcinogenesis using two hairless (hr/hr) stocks - HRS/J/Anl and SKH:hairless-1 mice.

Major Findings:
1. Development of Mouse Model Systems--Four dominant autosomal mutations that influence the phenotype of the skin of the mouse have been identified. These mutants are of interest because of the differences in the skin from other hairless mutants. Three of the genes which result in a hairless phenotype in the heterozygotes are allelic or very closely linked and have been designated Frl^a Frl^b and Frl^c. The series is non-allelic to hairless hr, and there is no evidence of linkage between the Frl and hr loci. The fourth mutation shows no evidence of linkage to the Furloss (Frl) series but is an allele of hr and is now designated by the symbol hr^N.

In the Frl series, mutant heterozygotes become hairless at 12-14 weeks of age. The epidermis is several cells thick unlike haired or hr/hr mice and does not have the many incomplete follicles seen in hr/hr mice. The proliferation in the basal cells is greater than in hr/hr which reflects the greater total epidermal cell population. Langerhans cells are markedly more numerous in the skin of the mutants compared to haired, nude, or hr/hr mice. The structural, cytological, and cytokinetic characteristics appear to mark these mutants as potentailly useful model systems for investigating certain aspects of UVR and chemical carcinogenesis. The eighth backcross generation on the BALB/c background is now being produced.

2. Susceptibility for Skin Cancer.--The importance of immune surveillance in cancer of many tissues has been doubted, but the evidence that the immune system plays a role in skin cancer remains strong. The contractor's studies of the comparative immune competence of HRS/J/Anl and SKH:hairless mice have revealed some differences but not any clear consistency that might correlate with differences in susceptibility. For example, the number of natural killer (NK) cells is significantly higher in the more resistant HRS/J/Anl mice compared to SKH:hairless-1, but delayed hypersensitivity is significantly greater in the more susceptible SKH:hairless-1 stock. The responses of the various elements of the immune systems of the two stocks of mice to UV radiation show little difference. The results suggest that either some unidentified factor or very small and subtle difference in some humoral and cellular components of the immune system are responsible. It is clear that the differences in susceptibility between these stocks of mice lies in the factors that influence expression rather than those that influence initiation.

Significance to Biomedical Research and the Program of the Institute:
Cancer of the skin is by far the most common neoplasia in the U.S. white population, and melanoma is one of the few tumors for which the incidence is increasing. It is clear that UVR is a major etiological factor and that UVR-induced lesions in DNA and their repair are becoming increasingly understood. Thus, UVR-induced skin cancer in mice is an appropriate model for investigating the role of specific DNA lesions and their repair in carcinogenesis. As most of the events following exposures to UVR that result in cancer are similar in man and mouse, with the exception of excision repair of pyrimidine dimers, these studies will provide data that will help to elucidate the factors that determine species-dependent differences and possibly suggest methods of extrapolation.

Proposed Course: The Interagency Agreement will end September 29, 1982.

Date Contract Initiated: September 30, 1979

Current Annual Level: 0

DEPARTMENT OF ENERGY/OAK RIDGE NATIONAL LABORATORY (Y01-CP-90203) (Formerly Y01-CP-50200)

Title: DNA Repair Mechanisms in Carcinogenesis

Contractor's Project Director: Dr. James D. Regan

Project Officer (NCI): Dr. David G. Longfellow

Objectives: The primary objectives are to elucidate the molecular events in human cells when cellular macromolecules such as DNA are damaged by radiation or chemical agents. The contractor will study and characterize:

1. The sequence of DNA repair events.

2. The various modalities of repair.

3. The genetic inhibition of repair due to mutation.

4. The physiological inhibition of repair due to biochemical inhibitors.

Major Findings: The ability of mammalian cells to excise ultraviolet light induced pyrimidine dimers from their DNA was measured by:

1. The amount of dimers remaining in acid hydrolysates of large pieces of DNA labeled with radioactive thymidine.

2. The number of sites in DNA susceptible to a UV-endonuclease.

3. The analysis of the single-strand breaks in DNA induced by the photolysis of bromodeoxyuridine incorporated into dimer excised regions.

4. The rise of arabinofuranosyl (ara-C) which is inserted into the DNA strand undergoing repair resulting in the prevention of strand rejoining.

The data obtained from these assays indicate that normal human cells in tissue culture are able to excise dimers from their DNA at an initial rate of about 10^5 dimers per hour. After biological doses (5-20 J/m^2) of 254 nm irradiation, about one-half of the dimers are excised within 24 hours. Dimers are removed from cells at the same rate when irradiated with either 254 nm light or the dimer producing wavelengths of sunlight. Ara-C is useful in the quantitation of excision repair during short periods of time. However, ara-C eventually results in the cessation of the enzymatic steps involved in the repair process. The mode of action of ara-C, aphidicolin, and other compounds which interfere with the steps of DNA excision repair are under investigation.

Using the ara-C arrest-pulse assay, DNA excision repair has been compared in normal and XP variant human fibroblasts following 254 nm irradiation. In addition, blockage of ara-C arrest by deoxycytidine (dC) antagonism has been studied in these cell lines. The results indicate that excision repair levels are the same in normal and XP variant cells. However, the XP-variant is less sensitive to ara-C arrest, and the effect of ara-C is more easily reversed by dC in these cells, suggesting that intracellular deoxynucleoside pools are considerably higher in the XP-variant.

The effect of C-type baboon virus infection on UV-induced post-replication repair in human fibroblasts has been measured. These studies indicate a significant deficiency in the amount of post-replication repair in infected cells as compared to uninfected ones. Specifically, it was found that molecular weight of DNA synthesized at 0, 2, and 4 hours after 10 J/m^2 of 254 nm radiation is reduced to greater extent in the infected cells than it is in the uninfected controls. The difference in sensitivity to UV radiation is such that the infected cells accumulate 5 times as many breaks in nascent DNA as do the infected cells at 2 hours after irradiation and 2 times as many breaks at 4 hours. The ability of DNA synthesis to recover (as measured by the molecular weight of the nascent DNA) from UV-irradiation is known as post-replication repair, and the excess of breaks is evidence of a deficiency in this process in the infected cells.

Significance to Biomedical Research and the Program of the Institute:
The significance of these studies lies in:

1. The ubiquitousness of repair (most organisms, including man, have several complex repair systems).

2. The belief that mutagens and carcinogenic events may arise only from residual (nonrepaired) lesions, or that error-prone repair systems may be the major induction mechanisms of the mutagenic or carcinogenic event.

3. The clear association of repair defects and highly carcinogenic disease states in man (xeroderma pigmentosum).

Proposed Course: The Interagency Agreement ended February 28, 1982

Date Contract Initated: April 1, 1977

Current Annual Level: 0

DEPARTMENT OF ENERGY/OAK RIDGE NATIONAL LABORATORY (YO1-CP-90205) (Formerly YO1-CP-50200)

Title: Regulation of Membrane Transport Systems and Membrane Turnover in Carcinogenesis

Contractor's Project Directors: Dr. R. J. M. Fry
Dr. J. S. Cook

Project Officer (NCI): Dr. David G. Longfellow

Objectives: To explore the physiological mechanisms by which cells regulate their membrane transport activities as a function of growth state, differentiation, and transformation. Special emphasis is placed on the role and mechanisms of action of tumor promoters on these transport systems.

Major Findings:
1. Regulation of Na,K-ATPase in HeLa Cells--HeLa cells are used as a model system of cloned human cells in which we study the molecular dynamics of the Na-K transporter. The enzyme maintains intracellular K at high levels that are essential to many cell functions, including protein synthesis. The contractor has shown that

this enzyme turns over with a half-time of about 5 hours, whereas most proteins of the cell surface turn over with half-times in excess of 100 hours. If the activity of the transporter is acutely inhibited more than 50% by toxic or even therapeutic (e.g., cardiac glycosides, like digitalis) influences, cell K would fall to levels too low to support protein synthesis if it were not for rapid turnover and replacement of inhibited enzyme with new enzyme. The rapid turnover thus acts as an important parameter in cellular repair. The discrepancy in turnover rates between this essential enzyme and the majority of the surface proteins, points to heterogeneity in the regulation of surface molecules, with adaptive consequences of obvious physiological importance.

In conditions of long-term stress (hypokalemia, or chronic drug intoxication), HeLa cells respond with an increase in numbers of enzymes at the cell surface and a correspondingly increased ability to extract K from the environment. When the stress is relieved, the number of enzymes returns promptly to control levels. This regulation of surface activity does not reflect changing rates of enzyme synthesis, which in fact remains constant, but is due to changing rates of removal of Na,K-ATPase from the cell surface in turnover. Regulation by turnover has special importance for membrane enzymes or receptors. From the onset of synthesis (presumably in the endoplasmic reticulum) to the final insertion in the cell surface, there is a transit time of about 4 hours. If surface concentrations were regulated by synthesis, there would be a lag of at least 4 hours between the environmental signal to the cell and the functional response. Since the enzyme is capable of exchanging all of the cell's cations in about 1 hour, this lag between signal and response has the potential of inducing undesirable, even uncontrollable, oscillations in cell cation contents. Such oscillations may be avoided by the prompt, virtually lag-free regulation at the opposite end of the pathway, i.e., regulation by turnover.

2. Membrane Recycling--Evidence is accumulating that surface membrances undergo recycling in the sense that they are internalized into cells by endocytosis but, for the most part, are not degraded in lysosomes. By a cycling vesicular transport mechanism, the internalized membranes may be reinserted in a still functional state in the cell surface. The contractor has used a chemical probe that modifies and simultaneously labels cell-surface sialic acids on both glycoproteins and glycolipids; the modified compound is not reutilizable for synthesis of new surface molecules. If a substantial fraction of such labeled surface molecules is allowed to be internalized and the remainder stripped off the surface, the surface is repopulated with label from an internal pool that is the equivalent of about 2 cell surfaces. Internal and surface membranes are in constant exchange with a half-time of less than 8 hours in HeLa and rat hepatoma HTC cells.

3. Effects of the Tumor Promoter TPA on Amino Acid Transport in Erythroleukemic Cells--Treatment of Friend erythroleukemic cells with TPA, with or without prior treatment with erythroblastogenic inducers, leads to a rapid cycloheximide-sensitive augmentation of both Na^+-dependent amino acid transport as well as of ouabain-sensitive K^+ uptake. The response is clearly evident in 30 minutes and reaches a maximum in 2-6 hours. Ouabain-insensitive uptakes are unaffected. The results suggest that an early effect of the tumor promoter is a protein-synthesis dependent modification of Na,K-ATPase leading to an enhanced electrogenicity, possible by increasing the Na:K stoichiometry in this coupled transporter. The mechanism is under investigation.

4. A Model for Epithelial Differentiation in Cultured Kidney Cells--The cell line LLC-PK_1, isolated from pig kidney, differentiates in culture by the criterion of

acquiring Na^+-hexose concentrating capacity, a function of kidney proximal tubules. Last year the contractor showed that differentiation could be accelerated by compounds that increased, even transiently, the cyclicAMP content of the cells. Conversely, differentiation (and cAMP content) could be suppressed by the tumor promoter TPA. This cell system, which has been cloned, has great promise for the study of differentiation of membrane functions as well as for exploring the effects of tumor promoters. A problem has been the lack of demonstrable cellular correlates, as opposed to the hexose-concentrating capacity of the entire population of the differentiated function. A model has been developed based on population transport kinetics suggesting that the following potential explanations for differentiation in the population are inadequate to explain the observations:

a. An increased Na^+-electrochemical driving force at the cells' apical surface during differentiation.

b. A reduced "leak" efflux from the cells' basolateral surfaces.

c. Coupling between transporting and non-transporting cells.

d. A slowly (in parallel with the accumulation capacity of the population as a whole) increasing number of transporters per cell.

Most of these mechanisms have been directly tested and ruled out. Consistent with all the data is that individual cells independently and rapidly, with respect to the time of differentiation of the whole culture, acquire their maximum number of transporters and transport capacity. The conclusion is important in further study of differentiation mechanisms and their modification by tumor promoters and is being tested by independent means.

Significance to Biomedical Research and the Program of the Institute:
The plasticity of transport systems carrying essential metabolites into mammalian cells has been recognized for a number of years, and the increase in activity of these systems with malignant transformation, or more generally, with many stimuli to cell growth, has been phenomenologically documented in a very large number of cases. The activity of these systems is also a function of the differentiated state, and tumor promoters have a major effect in inhibiting differentiation while maintaining active growth. Despite the extensive literature, little is known with respect to mechanisms. The work described here has as its goal the stepwise dissection of mechanisms of transport regulation, especially as they are influenced by tumor promoters.

Proposed Course: This Interagency Agreement terminated on February 28, 1982.

Date Contract Initiated: April 1, 1979

Current Annual Level: 0

DEPARTMENT OF ENERGY/OAK RIDGE NATIONAL LABORATORY (YO1-CP-90207) (Formerly YO1-CP-50200)

Title: Respiratory Carcinogenesis - Markers of Neoplastic Development in the Respiratory System

Contractor's Project Director: Dr. Ann C. Marchok

Project Officer (NCI): Dr. Paul Okano

Objectives:
1. To define specific stages in the evolution of neoplasia in respiratory epithelium.

2. To develop and utilize in vivo-in vitro and in vitro model systems to define and quantitate cellular changes that identify particularly the preneoplastic stages of carcinogenesis.

3. To determine the effects of retinoids on the differentiation and progression of neoplasia in these systems and thereby contribute to the understanding of the mechanism of action of vitamin A.

Major Findings:
1. Using the tracheal implant-organ culture-cell culture model, the following studies were carried out:

a. A study was completed to show the utility and accuracy of the organ culture exfoliation technique for identifying lesions on tracheal implants exposed from 4 to 12 months to 200 µg dimethylbenz(a)anthracene (DMBA)-beeswax pellets. This was done by comparing the cytopathology of cells which exfoliated into the medium during 24 hours of organ culture of 60 explants cut from the implants, to the histopathology of sections of the explants fixed immediately after organ culture. Lesions which ranged from regular metaplasia through mild, moderate, marked atypia, carcinoma in situ, and invasive carcinoma were detected with 92% accuracy.

b. A study was carried out to determine the relationship between the number of lesions as determined morphologically, to the number of carcinogen-altered cell populations. These were identified as primary cell cultures with altered growth control in vitro, in tracheal implants exposed for 2 weeks to 200 µg DMBA (~150 µg DMBA released; a subcarcinogenic dose), as compared to tracheal implants exposed continuously to the 200 µg DMBA pellets (all DMBA released by 2 months; highly tumorigenic dose). This was done by cutting the tracheal implants into 10-12 explants/trachea and identifying the lesions on the explants from the cytopathology of cells which exfoliated from them during organ culture as described above. The explants were then placed on the bottom of tissue culture dishes to establish primary cultures from the outgrowths of epithelial cells. Carcinogen-altered cell populations were detected as the primary cell cultures which survived a selection medium in which non-carcinogen exposed tracheal epithelial cells do not survive. At 2 and 6 months after the initiation of carcinogen exposure, tracheal implants exposed for 2 weeks to the DMBA had an average of 1 carcinogen-altered cell population/trachea, and no lesion greater than a mild atypia. In contrast, the continuously exposed tracheas had an average of 8 carcinogen-altered cell populations/trachea at 2 and 6 months, and lesions ranging from mild atypia to carcinoma were found on the explants. Other markers for the stage of progression of neoplasia, such as anchorage independent growth and tumorigenicity upon inoculation of cells into nude mice of the carcinogen-altered cell populations, are being tested and correlated with the severity of lesions as determined from the exfoliated cell cytopathology.

2. A tracheal organ culture-cell culture model was used to study the early events in the in vitro transformation of benzo(a)pyrene (B(a)P) exposed epithelium. In this model, tracheal explants are exposed to carcinogen, then expanding primary cell cultures are generated from the explants. Carcinogen-altered cells are identified by the appearance of rapidly proliferating foci of morphologically transformed cells in the epithelial cell sheets. The aim of the present experiment was to look for carcinogen-induced changes which precede the appearance of the morphologically altered foci in primary cultures established from tracheal explants exposed to 2 µg B(a)P/ml from day 3 to 6 of organ culture. Therefore, a selection step which would isolate cell populations with the capacity to survive in a restricted culture medium was introduced. In addition, a non-destructive quantitative method for monitoring the growth of the primary cultures was developed in order to detect differences in the growth rate of the individual cultures before and after the selection step. With these techniques, it was possible to identify "transformed" cell populations which survived the selection medium before the appearance of the morphologically altered foci. Most of the B(a)P-cultures which survived the selection medium were already distinguished by their rapid growth rate before the selection step. However, rapid growth rate was not essential for survival in the selection medium, or for the later appearance of morphologically altered foci in the cell cultures.

3. The properties of non-tumorigenic and tumorigenic tracheal epithelial cell lines are being studied as part of a program to develop a rapid, highly quantitative system for studying differentiation and neoplastic transformation in tracheal epithelial cells. Recently, it has been determined that a good marker for transformation may be a change in response to cytocalasin B. The tumorigenic cell lines exhibit a high level of multinucleated cells. Cells of non-tumorigenic cell line, C-18, exposed to N-methyl-N'-nitro-N-nitrosoguanidine showed a marked decrease in toxicity to cytochalasin B and an increase in multinucleation during subculture. Some of the carcinogen-exposed cell cultures also showed a shift to a lower or higher DNA content compared to the parent line as determined from flow cytometry. The data from many tests (changes in growth rate and calcium dependence; growth in soft agar; tumor formation in nude mice; changes in differentiation and neoplasia in repopulated tracheal implants, etc.) are being analyzed for the carcinogen-exposed cell cultures to determine successful markers for transformation in these cells.

4. By using a serum-free, defined medium developed by the contractor's group for maintaining the growth, differentiation, and continuous culture of tracheal epithelial cell lines, they have demonstrated a marked growth stimulatory effect of retinoic acid on non-tumorigenic and adenocarcinoma cell lines, but a growth inhibiting effect on a squamous carcinoma cell line.

Significance to Biomedical Research and the Program of the Institute:
Lung cancer is one of the common diseases in man. Current data, mostly from skin and liver carcinogenesis studies, suggest that the development of cancer is a multistage event. These studies should:

1. Define cellular and biochemical changes in epithelial cells which will mark stages in the progression of neoplasia in respiratory tissue.

2. Identify ways retinoids may alter these cellular processes and thereby alter the progression of neoplasia.

Proposed Course: This contract ended February 28, 1982.

Date Contract Initiated: September 1, 1980

Current Annual Level: 0

DEPARTMENT OF ENERGY/OAK RIDGE NATIONAL LABORATORY (YO1-CP-90208)

Title: Genetic Analysis of DNA Repair in Man with Cell Hybrids

Contractor's Project Director: Dr. Peter A. Lalley

Project Officer (NCI): Dr. David G. Longfellow

Objectives: The long-range goal of this project is to investigate the expression and individual genetic components involved in DNA repair in man. Such an elucidation of the genetic basis of DNA repair is fundamental if we are to understand its pivotal role in carcinogenesis. Therefore, the primary objectives of this project will be:

1. To genetically dissect the DNA repair systems in man.

2. To identify the number and kinds of genes required for DNA repair.

3. To assign these genes to specific human chromosomes.

4. To identify and determine the chromosomal assignment(s) of the genetic defect in XP.

Major Findings: Man x mouse somatic cell hybrids have been employed as the experimental system and bromodeoxyuridine photolysis, radiochromatography, and molecular weight analysis as the assays for DNA repair to pursue this project. The use of somatic cell hybrids allows for the mapping of genes to specific human chromosomes and the genetic dissection of this polygenic system due to the isolation of its component parts as a result of the preferential segregation of human chromosomes in proliferating human x mouse somatic cell hybrids.

Sixty-five human x mouse primary hybrid clones were isolated from five separate fusion experiments employing human cells from five unrelated individuals. Three of these human cell lines were fibroblasts, one was a lymphoblastoid cell, and the other human cells were unstimulated lymphocytes. Human and mouse cell lines' ability to repair UV-induced DNA damage was differentiated quantitatively with the mouse cell repairing DNA damage at 5-10% of the magnitude of human cells, and individual hybrid clones having one of three categories of repair:

1. Those having human-like repair capacity.

2. Those having mouse-like repair.

3. Hybrids intermediate between the two.

Segregation of the ability to repair UV-induced DNA damage was compared to the segregation of human chromosomes using 35 enzyme markers in these hybrids. When the data were analyzed from the three sets of hybrids made between human fibroblasts and mouse cells, there was a strong correlation between the presence of human

chromosome 3 and the ability to repair UV-induced DNA damage, and the loss of chromosome 3 and the loss of repair capacity. These data suggest that a gene or a series of genes required for DNA repair are located on human chromosome 3. In these same clones, there was a correlation between chromosome 14 and excision repair capacity. However, there was one clone which was positive for chromosome 14 but negative for repair. This clone is being checked to be sure it does not contain a fragment of chromosome 14.

When the data were analyzed from two sets of hybrids made with human lymphocytes and lymphoblastoid cells, the correlation was not so strong. There were two clones which were intermediate in their repair capacity but were negative for chromosome 3 markers. This may be due to chromosome breakage or to differences in the relative sensitivities of the enzyme assay for ACY, a gene located on chromosome 3 and the DNA repair assay. There was only one exception to a correlation between chromosome 14 and repair capacity in these hybrids. This hybrid was negative for chromosome 14 but positive for the ability to repair DNA damage. A possible explanation for the discrepancy of results in hybrids derived from human fibroblasts versus hybrids derived from lymphocytes or lymphoblastoid cells, may be that there is a tissue specificity in the expression of the repair system as is seen for several other enzyme systems. This possibility is being actively investigated.

In the course of these studies, the contractor addressed the problem of whether or not the different levels of repair ability that is seen in the hybrids is due to different amounts of DNA in each hybrid clone. This could be possible since the number of chromosomes (human) present in a hybrid cell varies considerably from clone to clone. However, this does not appear to be the case based on two sets of data. The repair capacity for each hybrid clone with the total number of chromosomes present was correlated. The levels of excision repair achieved is independent of the number of chromosomes (and therefore the amount of DNA) present in the cell. Secondly, repair capacity versus dose of UV was measured, and it was found that as the dose is increased, the amount of repair increases for the range of doses employed in this study. This indicates that for the dose employed (20 J/m^2) we have not saturated the enzyme system.

Studies into the complementation of the defective repair of UV-induced DNA damage in xeroderma pigmentosum (XP) by mouse cells were continued. It was found that mouse cells will complement the defective repair in 4 XP complementation groups; namely, Groups A, B, C, and D. However, mouse cells will not complement XP Group E cells indicating that the defect in Group E is qualitatively different from the defect in Groups A-D. In these experiments, hybrids between XP cells and mouse cells were formed under conditions which allow for the segregation of human chromosomes and retention of mouse chromosomes. When XP x mouse hybrid clones lost certain human chromosomes, they also lost the ability to be complemented by the mouse genome. The data suggest that the human chromosomes lost carried gene(s) coding for enzyme(s) in the repair pathway.

Hybrids have also been generated between XP cells and mouse spleen cells under conditions which allow for the segregation of the mouse chromosomes and retention of the human chromosomes. These experiments were carried out to determine which mouse chromosomes carry genes involved in the complementation of defective repair in XP. Data derived from XP-A x mouse spleen cell hybrids is consistent with published data from Ruddle's lab which suggest that mouse chromosome 4 carried a repair gene(s). However, the contractor's data cannot rule out other mouse chromosomes as possible sites for repair genes.

Preliminary experiments were carried out to determine whether this complementation of defective repair in XP cells by mouse cells is biologically significant. This was done by testing hybrid clones for resistance to UV irradiation. XP cells are highly sensitive to UV irradiation. Using this approach, viable XP x mouse hybrid clones were isolated following UV irradiation. The defective DNA repair capacity of the XP cells had been complemented by the mouse cells both enzymatically and biologically.

Significance to Biomedical Research and the Program of the Institute:
The importance of these studies lies in:

1. The fact that most organisms, including man, possess several complex DNA repair systems.

2. The demonstrated association between defective DNA repair, cancer proneness, and increased sensitivity to physical and chemical environmental mutagens and carcinogens.

3. The need to elucidate the genetic basis of this polygenic system in order to understand the interactions of the numerous repair enzymes.

4. The fact that a knowledge of the chromosomal assignment of the genes required for DNA repair and the gene or genes defective in XP will be extremely useful in prenatal diagnosis and genetic counseling.

Thus, the potential importance of this project is that it will yield information on the genetic structure of the DNA repair mechanisms in man. This information is essential if we are to fully understand the functional relationships between DNA repair, mutagenesis, and carcinogenesis.

Proposed Course: To continue these experiments utilizing human x mouse somatic cell hybrids to determine the number and kinds of genes required for the repair of UV-induced damage in man as well as the chromosomal assignment of these genes. This system will serve as a model for the genetic analysis of human repair mechanisms involved in repairing DNA damage induced by other agents.

During the past year, the contractor has identified a specific human chromosome which carries gene(s) involved in DNA repair of UV-induced DNA damage. In the coming year, he intends to make use of these inhibitory techniques that were developed which inhibit specific steps of the DNA repair process. These are Ara-C arrest, aphidicolin arrest, and novobiocin arrest. It is hoped that by assaying hybrids which are positive for repair, it will be possible to assign genes coding for specific steps in DNA repair to specific chromosomes. This will serve to give a clearer picture of the individual repair contribution of the gene(s) on each chromosome. This Interagency Agreement is scheduled to terminate on September 16, 1982.

Date Contract Initiated: September 17, 1979

Current Annual Level: 0

DEPARTMENT OF ENERGY/OAK RIDGE NATIONAL LABORATORY (YO1-CP-90211) (Formerly YO1-CP-50200)

Title: Respiratory Carcinogenesis - Dynamics of Neoplastic Development in the Respiratory System

Contractor's Project Director: Dr. Margaret Terzaghi

Project Officer (NCI): Dr. Paul Okano

Objectives: To develop in vivo and in vitro techniques for studying the development of respiratory tract cancers in vivo. To identify cellular changes occurring early during neoplastic development in carcinogen-exposed tracheal epithelium. To develop models for studying the effects of x-radiation and promoters on the dynamics of neoplastic development in tracheal mucosa.

Major Findings:

1. Initiation-promotion studies, exposure of intact tracheal grafts.

   a. No agarose-positive EF were isolated from vehicle-exposed control tracheas.

   b. The principal effect of TPA on the dynamics of neoplastic development as measured by the EF-assay in vitro was to increase the rate of progression to a potentially neoplastic state and/or inhibit reversal of the potentially neoplastic state in the intact trachea. This effect was most marked 12-18 months after DMBA exposure. Exposure to TPA following exposure to DMBA was associated with a 10-fold (4% to 40%) increase in tumor incidence within 20 months of exposure. TPA did not appear to affect the tumor latency period. This latter observation is consistent with the "late" effect of TPA on the development of potentially neoplastic cells detected in culture.

2. Initiation-promotion studies, exposure in vivo of EF-cell populations isolated in culture following initation. TPA did not appear to affect the tumor incidence or latency period of EF "cultured" up to 6 months in denuded tracheal grafts.

3. Effect of radiation on neoplastic development in cell populations with altered in vitro growth potential. A consistent decrease (> 50%) in latency period was observed in initiated cell populations when exposed to 400 rads X-ray prior to inoculation into denuded tracheal grafts.

Significance to Biomedical Research and the Program of the Institute:
Bronchogenic carcinoma, the prevalent neoplasm of the respiratory tract in man, most likely develops as a result of chronic exposure to various carcinogens and other agents present in the environment which act as co-factors in the carcinogenic process. The increased susceptibility to carcinogens or co-carcinogens of individuals already exposed to subcarcinogenic doses of one or more agents is clearly of practical importance.

The EF assay in vitro, developed in this laboratory, allows the quantitating of the emergence of carcinogen altered and neoplastic cell populations in tracheal epithelium following exposure in vivo to varied doses of one or more carcinogens and co-factors, delivered at controlled dose rates. Using this in vivo-in vitro model, the effect of dose rate and co-factors on the dynamics of neoplastic development in vivo in respiratory tract tissues can be studied effectively. It is expected that

these studies will help define those environmental factors which are of primary importance in the pathogenesis of respiratory neoplasms.

Proposed Course: All work proposed in this contract has been completed, and the contract terminated on December 31, 1981.

Date Contract Initiated: September 30, 1979

Current Annual Level: 0

JOHNS HOPKINS UNIVERSITY (NO1-CP-55713)

Title: Studies on Significance of Mutation in Carcinogenesis

Contractor's Project Director: Dr. Paul O. P. Ts'o

Project Officer (NCI): Dr. David G. Longfellow

Objectives:
1. To quantitatively characterize the entire process of neoplastic transformation of normal diploid Syrian hamster cells in culture by:

a. The statistical evaluation of the correlation between neoplasia-associated growth properties and tumorigenicity of chemically, physically, and spontaneously neoplastically transformed hamster cells.

b. The characterization of the process of neoplastic transformation in culture to identify markers of various stages of neoplastic progression and to determine the mechanism of progression from one stage to the next.

2. To use these studies on Syrian hamster cells as a paradigm for future investigations on the neoplastic transformation of human cells in culture.

Major Findings:
1. Correlation between Tumorigenicity and in vitro Growth Characteristics of Hamster and Human Cells--As part of a comprehensive study of neoplastic transformation, the contractor has quantitated the tumorigenicity and in vitro growth properties of more than 30 clonal Syrian hamster tumor cell lines. Statistical evaluation of the data thus far collected and tabulated shows:

a. At least two parameters appear to consistently correlate well with tumorigenicity (anchorage-independence and enhanced fibrinolysis with Kendall coefficients of rank correlation in the range of 0.75 to 0.98).

b. Some parameters clearly do not correlate with tumorigenicity (generation time in 10% serum).

c. A third group of parameters consists of those which correlate well within a group of cells transformed by the same perturbation but do not appear yet to correlate in all cases. These studies are currently being extended to evaluate statistically the correlation(s), if any, between the in vitro growth properties and the xenotumorigenicity in nude mice of three normal diploid human fibroblast cell strains and several clonal cell lines of various types of human sarcomas.

2. Loss of Post-confluence Inhibition of Cell Division Marks an Early Stage in Neoplastic Progression in vitro--The contractor has developed a qualitative and quantitative assay system for detecting cells lacking post-confluence inhibition of cell division (contact-insensitivity, CS^-) in Syrian hamster embryo (SHE) cells in culture by measuring the number of cells able to form colonies on a lethally irradiated, confluent monolayer of a contact-sensitive (CS^+) established cell line. A subpopulation in normal low passage cultures of SHE cells temporarily exhibits this CS^- phenotype at very low frequency (4×10^{-3}) but quickly loses the property within a few passages in vitro. This phenotype is invariably exhibited by various tumorigenic cell lines at very high frequency ($7\text{-}50 \times 10^{-2}$), and appears to correlate with the anchorage independent growth phenotype (Aga^+). The temporal acquisition of the CS^- phenotype by tertiary passage SHE cells following exposure to N-methyl-N'-nitro-N-nitroso-guanidine (MNNG) has been examined. Cells with a stably heritable CS^- phenotype are detected after approximately 20 post-treatment population doublings (PTPD). In contrast, AGA^+ cells are not detected until 35-95 PTPD. These CS^- cells appear to be preneoplastic cells, since clonally isolated CS^- cells do not exhibit anchorage-independent growth until after further passaging in vitro. The results suggest that acquisition of the CS^- phenotype represents an early stage in neoplastic progression. Similar CS^- cells are now being studied in human embryonic skin and lung fibroblast cell strains and in human sarcoma cell lines.

Other altered growth characteristics whose expression times after carcinogen treatment are currently under investigation include reduced requirements for Ca^{+2} and reduced requirement for serum. Both of these altered phenotypes appear also to be acquired within 20 population doublings following exposure to MNNG or benzo(a)pyrene.

3. Ploidy Dependence of in vitro Neoplastic Transformation--In vitro senescence and neoplastic transformation of Syrian hamster embryo (SHE) cells have been studied with respect to their dependence on ploidy. In vitro senescence is independent of ploidy, i.e., near-tetraploid clones of SHE cells senescence in culture after as many population doublings as do near-diploid clones. On the other hand, neoplastic transformation induced by chemical carcinogens (MNNG or ethyl methane sulfonate) measured by cloning efficiency in reduced serum or soft agar, or by tumorigenicity in newborn hamsters, is ploidy dependent; near-diploid clones become transformed upon treatment with carcinogens while near-tetraploid clones do not.

4. Somatic Genetic Analysis of Neoplastic Transformation--Gene mutation has been hypothesized as the basis for the hereditable cellular alterations characteristic of cancer. However, unlike single-gene mutation (which occurs in a single step, wild-type → mutant), neoplastic transformtion is a multistep, progressive process, characterized by the temporally distinct emergence of cellular subpopultions with altered phenotypic characteristics frequently associated with neoplastic cells. This progressive process, analogous to the in vivo progression of cancer, cannot be explained by single-gene mutations controlling either X-linked recessive or autosomally-linked codominant phenotypes. The acquisition of the capacity for anchorage independent growth occurs late after exposure of cells to carcinogens, and is well-correlated with the neoplastic conversion of preneoplastic cells. By applying Luria-Delbruck fluctuation analysis to the problem of in vitro carcinogenesis, studies with a preneoplastic, subtetraploid, anchorage dependent cell line demonstrate that Aga^+ cells arise spontaneously, and at a rate (5×10^{-7} Aga^+ variants/cell/generation) similar to single locus (Na^+/K^+ ATPase) codominant mutation to ouabain resistance in the same cells. Point (MNNG), frameshift (ICR-170, B(a)P diol-epoxide) and deletion (^{137}CS γ-irradiation) mutagens were

unsuccessful, however, in inducing the Aga^+ trait in these cells. A similar inability to induce $HPRT^-$ (recessive) mutants of these subtetraploid cells suggests a ploidy dependent, recessive nature of the anchorage-independent phenotype. By this hypothesis, the spontaneous rate of emergence of Aga^+ variants of these aneuploid cells can be explained by the measured frequency of chromosomal nondisjunction, which can permit the conversion of putative heterozygous preneoplastic cells into anchorage independent homozygotes at the rate observed. These studies thus implicate chromosome variability and random allelic assortment as providing a potential pathway for neoplastic progression. As an extension of these observations, the relationship between induced aneuploidy and susceptibility to MNNG-induced neoplastic transformation in normal human cells is under active investigation.

Significance to Biomedical Research and the Program of the Institute: Quantitative measurement of and an understanding of the basic mechanisms of neoplastic transformation are of primary importance both for basic research purposes and for the practical aspects of cancer cause and prevention. For the first time, the complete process of the transformation of a normal diploid cell to a tumorigenic cell is being described quantitatively, and the relationship of neoplastic transformation to somatic mutation is gradually being understood. The importance of the epigenetic process and chromosomal events is clearly indicated in these studies. Further, the research described provides a direct test of the importance of chromosomal variability and induced karyotypic abnormality as a factor in the development of neoplasia.

Proposed Course: The contract terminated on December 31, 1981.

Date Contract Initiated: June 27, 1975

Current Annual Level: 0

JOHNS HOPKINS UNIVERSITY (NO1-CP-85670)

Title: Identification of Heterozygous Carriers of DNA Repair Defects

Contractor's Project Director: Dr. Lawrence Grossman

Project Officer (NCI): Dr. David G. Longfellow

Objectives: To immunologically assess the level, distribution, and nature of DNA repair enzymes in cell lines derived from patients with DNA repair deficiencies.

Major Findings: During the tenure of this contract, it has been the contractor's purpose to prepare antibodies to a series of enzymes from human placenta, which could repair and restore the integrity of damaged DNA. To meet these objectives a great deal of effort was expended in purifying:

1. An AP endonuclease - an enzyme that recognizes either apurinic or apyrimidinic sites.

2. An enzyme that could sequentially remove damaged nucleotides in a 3' direction.

3. An enzyme that could sequentially remove damage in a 5' direction.

4. Two DNA polymerases that participate in replication and repair - DNA polymerases α, β.

5. The enzymes that are responsible for restoring the integrity of the DNA strands - polynucleotide ligases I, II.

The AP endonuclease that has been isolated represents the only such enzyme in human placenta. Unlike prokaryotes which have a number of different such enzymes, this AP endonuclease has been purified to homogeneity, and its role is to recognize apurinic or apyrimidinic sites arising either as a consequence of spontaneous depurination, depyrimidination, or enzymatic removal of modified purines or pyrimidines. The enzyme recognizes these sites and unlike all recorded such enzymes, hydrolyzes the phosphodiester bond 5' and 3' to these AP sites generating termini containing nucleotides eithout a nitrogenous base. Unlike enzymes reported in the literature for mammalian and bacterial enzymes, this one will not remove a terminal AP site. The contractor has reason to believe that the findings of others regarding the removal of terminal AP sites may be nonenzymatic and stimulated by presence of thiols or primary or secondary amines in reaction mixtures.

The exonuclease that hydrolyzes damaged DNA from the 3' direction (DNase VII) has also been purified to homogeneity, and this enzyme seems to assume a role in editing incorrect nucleotides that are misincorporated at the 3' growing end of the chain. This enzyme, although it acts in a distributive fashion, will not act on RNA nor will it act on RNA: DNA hybrids. However, the enzyme will remove a misincorporated ribonucleotide at the 3' end of a chain as well as other modified nucleotides.

The enzyme which acts in a 5' to 3' direction (DNase VIII) is able to remove approximately 10 nucleotides for each pyrimidine dimer which is introduced into DNA by ultraviolet irradiation. The enzyme removes short oligonucleotides and seems to be able to show preference for certain sequences.

The 3' to 5' exonuclease (DNase VII) is able to hydrolyze DNA in an almost unabated manner whereas the 5' to 3' exonuclease (DNase VIII) only removes approximately 10 nucleotides. The limitation of the 3' directed exonuclease seems to be determined by the presence of DNA polymerase β. This purified enzyme will limit the course of excision in 3' direction DNase VII.

The integrity of the DNA strands is restored by one of two enzymes isolated from human placenta referred to as polynucleotide ligase I and II. These enzymes can be distinguished according to their molecular weights, their position on chromatograms, and in terms of their sensitivity to the presence of dATP in reaction mixtures in which rATP is the required cofactor for ligation. In the presence of high concentrations of AMP the enzyme can act reversibly. Using this reaction, it has been possible for the contractor to demonstrate the reaction intermediate as adenylated DNA. The role of poly-ADP-ribosylation in stimulating ligase activity is currently under investigation. The site on the enzyme or in the DNA intermediates in which APP ribosylation occurs is currently under investigation.

A great deal of effort has been expended in defecting very low levels of antibody against these enzymes for the purposes of characterizing their presence, distribution, and nature in cells derived from patients with repair deficiencies. Although antibodies have been obtained against polynucleotide ligase, it has been extremely difficult to obtain antibodies against the other enzymes. For this reason, it appears not to be a viable approach to characterizing the level of these

enzymes in this repair deficient state. However, the contractor's knowledge of the properties of these as enzymes and as proteins allows him to examine the level of these enzymes in normal and repair deficient cell lines.

Significance to Biomedical Research and the Program of the Institute: The purification and characterization of seven enzymes from human placenta which repair and restore the integrity of damaged DNA should now permit an examination of the level of these enzymes in normal and repair deficient cell lines.

Proposed Course: Contract terminated March 31, 1982.

Date Contract Initiated: September 30, 1978

Current Annual Level: 0

SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH (NO1-CP-85665)

Title: Identification of Heterozygous Carriers of DNA Repair Defects

Contractor's Project Director: Dr. Raju S. K. Chaganti

Project Officer (NCI): Dr. David G. Longfellow

Objectives: The overall objective of this contract is to develop, using chromosome instability (chromosome breakage and sister chromatid exchange) as the endpoint, methods for the detection of heterozygous genotypes for the genes causing the autosomal recessive disorders: Fanconi anemia (FA), ataxia telangiectasia (AT), Bloom syndrome (BS), and xeroderma pigmentosum (XP). FA, AT, BS, and XP homozygotes have an increased predisposition to cancer, while cancer incidence in AT heterozygotes is also reported to be increased.

Major Findings: Cumulative total of cell lines established during the contract period:

FA homozygous	29
FA heterozygous	25
AT homozygous	4
AT heterozygous	2
BS homozygous	4
XP homozygous	4
XP heterozygous	1
Diskeratosis congenita	12
Other	5
TOTAL	86

Breakdown of cell lines established by tissue:

Fibroblast	78
Lymphoblastoid	8
TOTAL	86

During the past three years, the contractor's major effort went into the study of Fanconi anemia and has resulted in development of a rapid method for the detection of affected individuals, pre- and post-natally, and identification of the carrier state in affected families by the diepoxybutane (DEB)-stress method. Phenotypic and cellular heterogeneity, presumably based on genetic heterogeneity, has become apparent from our studies; and it underscores the difficulties implicit in attempts to develop tests for heterozygote detection applicable to the general population in disorders which manifest heterogeneity. AT and XP, as is well known, also exhibit considerable genetic, cellular, and phenotypic heterogeneity. While earlier, the contractor planned to tackle XP after FA, the priority was changed, and last year work was begun with AT instead. There were two reasons for this. The FA studies convinced the contractor of the value of the blood lymphocytes as the target cells for the development of these tests, and XP patients were just unavailable. The second reason was the fact that AT heterozygotes are now considered cancer-prone, whereas the cancer-proneness of XP heterozygotes is unknown. The contractor was able to establish a working relationship with 10 AT families in which the obligate heterozygotes were willing to participate in these studies. It is pleasing to report that the initial results of these studies of AT heterozygous lymphocytes stressed with the radiomimetic drug bleomycin were most promising; the trend appears to parallel the results with FA.

A bank of fibroblast and lymphoblastoid cell lines has been developed which includes affected and carrier genotypes of the four mutations; FA is best represented.

In summary, the contractor's achievements so far are the following:

1. Development of a method to recognize heterozygotes in families with the FA mutation that causes cellular hypersensitivity to DEB.

2. Recognition of genetic heterogeneity and its actual and potential import on attempts to develop tests for heterozygote detection.

3. Recognition that AT and most probably XP will behave in a manner similar to FA in this regard.

Significance to Biomedical Research and the Program of the Institute:
Chromosome instability possibly is a reflection of the abnormal DNA metabolism documented or postulated in these disorders. By studying the cytogenetic responses of cells carrying these genes to mutagens that will elicit the specific chromosomal responses, it was expected to gain insights into the nature of the defects themselves. In terms of the cancer program, methods for detection of carrier individuals will enable recognition of individuals at risk.

Proposed Course: The contract officially terminated on December 29, 1981.

Date Contract Initiated: September 30, 1978

Current Annual Level: 0

TEXAS, UNIVERSITY OF (M.D. ANDERSON HOSPITAL AND TUMOR INSTITUTE) (NO1-CP-85671)

Title: Isolation and Purification of Human Polycyclic Hydrocarbon Metabolizing Enzymes and the Production of Antisera to the Pure Enzymes

Contractor's Project Director: Dr. Marilyn S. Arnott

Project Officer (NCI): Dr. Paul Okano

Objectives:
1. Isolation and purification of three carcinogen metabolizing enzymes: glutathione-S-transferase (GST), phenolsulfotransferase (PST), and UDP-glucuronyl-transferase (UDPGT) - from human autopsy liver.

2. Preparation of specific antisera to each purified enzyme.

Major Findings: Three enzymes which facilitate conjugation of a wide variety of xenobiotics, as well as endogenous substrates, have been isolated from normal adult human livers. UDP-glucuronyltransferase has been purified from detergent solubilized microsomes by ion exchange and affinity chromatography. Gel electrophoresis under denaturing conditions indicates a subunit molecular weight of approximately 58,000 daltons. Conjugating activity with p-nitrophenol, methylumbelliferone, 3-hydroxybenzo(a)pyrene, morphine, and estrone copurify throughout this procedure. This material elicits a modest immunological response when injected into rabbits.

Six isozymes of glutathione-S-transferase have been purified by chromatographic techniques. Five of these have alkaline isoelectric points, while one is acidic. All are active with 1-chloro-2,4-dinitrobenzene as substrate, but only two of the isozymes catalyze glutathione conjugation to styrene oxide. These two forms are immunologically distinct from the others, and in some systems, can be distinguished from each other. One of these two isozymes appears to be a heterodimer consisting of subunits of 23,000 daltons and 26,000 daltons. Subunits of the other isozymes all exhibit molecular weights of approximately 26,000 daltons.

A phenolsulfotransferase (PST) has been purified to apparent homogeneity. It has a molecular weight of 58,000 daltons, consisting of two 31,000 dalton subunits. A similar preparation of rat PST revealed a slightly higher molecular weight and a more alkaline isoelectric point. Kinetic studies using two acceptors, phenol and 3-hydroxybenzo(a)pyrene, and the sulfate donor, adenosine-3'-phospho-5'-phosphosulfate (PAPS), showed that in spite of the observed physical and chemical differences between the human and rat enzymes, functionally the PSTs from the two species are similar. Additional substrate specificity studies with the human enzyme revealed activity with dopamine as acceptor, and a low level of sulfation of N-hydroxy-2-acetylaminofluorine was detected. Antisera to the human enzyme, prepared in rabbits, exhibit a titer too low for detailed immunological studies.

Significance to Biomedical Research and the Program of the Institute:
The availability of human carcinogen metabolizing enzymes in purified form:

1. Provides a means for examining in detail the metabolic activation and detoxication of polycyclic aromatic hydrocarbon carcinogens in humans.

2. Facilitates comparison of carcinogen metabolism between human and experimental animal systems, thus, aiding in animal-to-human extrapolation of carcinogenicity data.

3. Allows for the preparation of monospecific antibodies, opening the way for development of radioimmunoassays for key enzymes in human tissues.

These achievements will contribute to the overall goals of the National Cancer Institute, as they should lead to reliable methods for assessing individual cancer risk in humans, based on their ability to metabolize carcinogens.

Proposed Course: Contract terminated March 31, 1982.

Date Contract Initiated: September 30, 1978

Current Annual Level: 0

SUMMARY REPORT
RESEARCH RESOURCES

The Chemical Research Resources program provides chemical standards of research interest to the carcinogenesis research community at large. Through a number of resource contracts the program has chemical carcinogen reference standards prepared, analyzed and distributed to cancer researchers around the world. Labeled forms of retinoids which have shown promise in studies conducted for the Chemoprevention Program are made available for pharmacologic and metabolic investigations. The program also manages instrument loan arrangements which provide 11 NCI-owned thermal energy analyzers to research laboratories around the world for studies on the environmental occurrence and relevance of nitrosamines.

Chemical syntheses for the program are provided by seven contractors, five of whom provide chemical carcinogens and their derivatives for the Chemical Carcinogen Reference Standard Repository at IITRI (NO1-CP-05612). The other two contractors provide retinoids for the Chemoprevention Program.

A new resource effort, which began this year at the American Health Foundation (NO1-CP-15747), will prepare key derivatives of the benzofluoranthenes for distribution by the Repository. The benzofluoranthenes are among the most widely occurring of the polynuclear aromatic hydrocarbon carcinogens, however relatively little is known about their mechanisms of metabolic activation. The availability of these compounds will enable basic research on this important group.

At the Midwest Research Institute (NO1-CP-05613) a wide spectrum of derivatives of polynuclear aromatic hydrocarbons (PAH) are synthesized and purified. These derivatives, both nonlabeled and labeled (^{3}H, ^{14}C), are prepared by unequivocal methods to produce adequate quantities of well-characterized compounds of high purity ($\geq$ 98%) for distribution as metabolite standards through the NCI Chemical Carcinogen Reference Standard Repository. During the last year 34 new PAH derivatives were added to the inventory. This contractor also serves as the Radiochemical Repository for the program. From an inventory of 40 compounds more than 200 samples have been sent to 115 authorized investigators in the United States, Japan, England, France, Canada, Sweden, Finland, Switzerland and Germany during the last year.

Companion contract efforts at MRI (NO1-CP-05719) and at SRI International (NO1-CP-05614) provide for the resynthesis of PAH derivatives in order to maintain the inventory at the Repository. Once an unequivocal route has been developed and tested several times by the previously mentioned contractors, then contractors at MRI and SRI International provide a continuing supply. Each contractor has specific parent PAH compounds for which he is responsible for providing derivatives. A second objective for these contractors is the syntheses of compounds from other classes that are needed in the Repository. Nitrosamines, aromatic amines, additional parent polynuclear aromatic hydrocarbons, aflatoxins, steroid derivatives, and physiologically active natural products are among the chemical classes made.

One contract effort at SRI International (NO1-CP-85612) has synthesized and purified heterocyclic analogs of polycyclic aromatic hydrocarbons. A series of nitrogen, sulfur, and oxygen heteroanalogs have been prepared that are either known to be constituents of environmental pollution or are analogs of known PAHs. The availability of these compounds should be an aid in assessing the potential harm to

man, animals, and the environment resulting from airborne emissions from power plants that will be burning increasing amounts of coal. Work on this contract was completed this year.

Compounds provided by the above five contractors are distributed as reference standards by the Chemical Carcinogen Reference Standard Repository operated by ITT Research Institute (NO1-CP-05612). About 1800 samples were distributed to researchers who requested them during 1981. The inventory includes over 585 compounds derived from the synthesis contractors and from surplus stocks of the National Toxicology Program. The Repository has coordinated the chemical coding and shipping logistics for several blind-coded chemical evaluation studies each year. This has enabled investigators to provide an unbiased evaluation of new screening methods and/or chemicals being considered for long-term bioassay. This contract enables the NCI to provide compounds for pertinent experiments in Chemical Carcinogenesis which could not be carried out otherwise. Carcinogenesis research has been greatly stimulated by the availability of authentic reference standards and/or substrates. This can be attested to by the volume of published accounts of research citing the NCI Chemical Carcinogen and Radiochemical Repositories (IITRI and MRI) as the source of standards.

Retinoids for testing by Chemoprevention Program contractors are synthesized in kilogram quantities by the Southern Research Institute (NO1-CP-85616). Radiolabeled retinoids are prepared for selected compounds and those compounds which show chemopreventive efficacy in tests such as the tracheal organ culture system performed at IITRI (NO1-CP-05610). SRI International (NO1-CP-05601) prepares radiolabeled retinoids which are subsequently made available to the research community for use in basic metabolic and pharmacokinetic studies.

Contractors at the Southwest Research Institute (NO1-CP-85601) have been preparing retinoids in encapsulated form for stabilization of the molecules for delivery into biological systems.

Ten Thermal Energy Analyzers (TEA) have been placed under loan agreements in laboratories around the world. An eleventh instrument currently awaits assignment. The instruments, which are very sensitive, selective analyzers for N-nitrosamines, were developed in part under contract to NCI by the Thermo-Electron Corporation (Waltham, Mass.). The instruments were purchased in 1975 and a loan program initiated to stimulate research and collaboration on the environmental and occupational occurrence of N-nitroso compounds. The major emphasis has been on determining the incidence of these compounds in products for human consumption. This has involved inter-laboratory comparisons of analysis of the various samples. The recipients involved have used these instruments with gas chromatographic and with high pressure liquid chromatographic equipment in making a variety of important discoveries concerning environmental distribution of N-nitroso compounds. These discoveries have included the detection of nitrosamines as normal constituents of human blood, as contaminants in beer, foods, cosmetics, in flame retardants, hydraulic fluids, machine cutting and grinding fluids, in deionized water, and in the occupational environments of leather and rubber workers. The current location of the ten instruments is as follows: Oregon State University; U.S. Department of Agriculture, Agricultural Research Service; Laboratory of the Government Chemist, London, England; U.S. Food and Drug Administration; Deutsches Krebsforschungszentrum Institute fur Toxikologie und Chemotherapie, Heidelberg, West Germany; Tallinn Polytechnical Institute, Estonia, USSR; Massachusetts Institute of Technology; Eppley Institute; American Health Foundation; and the International Agency for Research on Cancer, Lyon France.

CONTRACT INDEX

AMERICAN HEALTH FOUNDATION (NO1-CP-15747)

Title: Synthesis of Derivatives of Polynuclear Aromatic Hydrocarbons

Contractor's Project Director: Dr. Stephen S. Hecht

Project Officer (NCI): Dr. David G. Longfellow

Objectives: To synthesize one-gram quantities of metabolites of benzo(b)fluoranthene, benzo(j)fluoranthene, and benzo(k)fluoranthene for the NCI Carcinogen Repository.

Major Findings: Since the beginning of the contract in October 1981, progress has been made on the synthesis of gram quantities of 8,9-dihydro-8,9-dihydroxybenzo(k)fluoranthene, 9,10-dihydro-9,10-dihydroxybenzo(b)fluoranthene, and 1,2-dihydro-1,2-dihydroxybenzo(b)fluoranthene. The ketone precursor to the latter, 1-oxo-1,2,3,3a-tetrahydrobenzo(b)fluoranthene was obtained by a new high yield synthesis from fluorene, an inexpensive starting material. One gram quantities of these dihydrodiols will be provided to the NCI Carcinogen Repository as they become available.

Significance to Biomedical Research and the Program of the Institute: The benzofluoranthenes are among the most widely occurring of the polynuclear aromatic hydrocarbon carcinogens, but little is known about their mechanisms of metabolic activation. The limited data which are available indicate that they are activated by pathways which may be quite different from those operative for a number of other polynuclear aromatic hydrocarbons such as benzo(a)pyrene. The present program will provide key benzofluoranthene metabolites to the research community. This will stimulate basic research on cancer causation by this important group of carcinogens.

Proposed Course: During the next year, the synthesis of specific dihydrodiol, dihydrodiol epoxide, or epoxide metabolites of benzo(b)fluoranthene and benzo(k)fluoranthene will be completed.

Date Contract Initiated: June 19, 1981

Current Annual Level: $142,781

IIT RESEARCH INSTITUTE (NO1-CP-05610)

Title: Bioassay of Retinoid Activity by Tracheal Organ Culture System

Contractor's Project Director: Dr. Leonard J. Schiff

Project Officers (NCI): Dr. Carl E. Smith

Objectives: The objective of this project is the bioassay of new retinoid compounds by the tracheal organ culture assay. Hamster tracheal organ cultures provide an experimental assay system for determining whether new retinoids can alter epithelial cell differentiation. Under conditions of vitamin A deficiency, the tracheobronchial epithelium forms keratinized squamous metaplastic lesions but in the presence of active retinoids, the process of keratinization is reversed toward columnar ciliated and mucus secreting cells similar to those observed in vitamin A normal animals.

Major Findings: The chemical structure-biological activity relationship of new retinoid compounds was evaluated using the hamster tracheal organ culture assay. Retinoids were received from the following sources: Marcia I. Dawson, SRI International, Menlo Park, CA; William H. Okamura, University of California, Riverside; John McMurry, Cornell University, Ithaca, NY; BASF Aktiengesellschaft, Ludwigshafen am Rhein, Germany; and Thomas J. Curphey, Dartmouth Medical School, Hanover, NH,. A total of 137 assays (one retinoid dose response per assay) was performed using approximately 5,000 hamster tracheas, and their ability to reverse keratinization was compared to all-trans-retinoic acid, the reference substance. Dose-response curves were made for the retinoids and the 50% effective dose (dose effective in suppressing keratinization in one-half of the cultures) determined.

A new class of retinoids synthesized by BASF which may be considered as benzoic acid derivatives (RBAD's) were the most active of the retinoids tested. When compared to the reference substance all trans retinoic acid, many of these compounds were equal to or more potent in reversing keratinization. A number of the analogs showed activity detectable in the 10^{-12} M range.

Significance to Biomedical Research and the Program of the Institute: Studies performed using the tracheal organ culture assay to measure the intrinsic ability of retinoids to control epithelial cell differentiation, provides significant predictive value for the potential use of a new retinoid for prevention of epithelial cancer. Results from these bioassays can provide information for animal studies to determine whether biologically active retinoids have prophylactic and therapeutic properties against a number of epithelial tumors.

Proposed Course: As they become available, newly synthesized retinoids will be evaluated for biological activity in tracheal organ culture system.

Date Contract Initiated: August 30, 1980

Current Annual Level: $153,559

IIT RESEARCH INSTITUTE (NO1-CP-05612)

Title: Chemical Carcinogen Standard Reference Repository

Contractor's Project Director: Dr. James N. Keith

Project Officer (NCI): Dr. David G. Longfellow

Objectives:
1. To provide reference samples of carcinogens and related chemicals to research laboratories, for use in identificaton of such compounds in metabolic products, environmental samples, consumer products, etc.

2. To provide for the safe handling, storage, and shipment of these samples with appropriate safety information and analytical data.

Major Findings: Over 300 requests were received, and 1800 samples were provided during calender year 1981. At the end of the year, 585 compounds were in stock, consisting of 35 nitrosamines, 27 polycyclic aromatic hydrocarbons (PAH), 176 PAH (including methyl and nitro derivatives and a variety of oxidized metabolites and analogues), and a variety of pesticides, drugs, and industrial chemicals. Of these, the most frequently requested compounds are the PAH derivatives, most of which are not commercially available, but are provided to the repository by four synthesis contracts.

During the past year, the Repository began participation in the new program In Vitro Evaluation of Chemical Candidates for In Vivo Screening. Candidates selected by a working group are purchased or otherwise acquired by the Repository and shipped as coded samples to the two participating contractors at the rate of about 6-8 samples per month. Specially prepared safety data packages, including emergency envelopes, are provided.

An ongoing task initiated under the previous contract, is the preparation and distribution of analytical standards for the USDA-FSQS Recognized Laboratory Program for Nitrosamine Analysis. New standards are prepared and shipped every six months, with supplemental samples at more frequent intervals to more active laboratories.

Significance to Biomedical Research and the Program of the Institute: Together with the cooperating synthesis contractors, the repository represents a valuable resource providing reference samples of many important research chemicals which would otherwise be unavailable to investigators. In many cases, positive identification of these metabolites, etc. would be very difficult or impossible without the use of the reference samples the program supplies. By distribution through the repository, a single, small-scale synthesis can be used to the advantage of scores of research programs.

Proposed Course: Continue the program, with emphasis on research chemicals which are vital for current research programs but commercially unavailable. Analyze the frequency and type of requestor, and investigate methods of making the program more cost-effective. Investigate possible payback systems.

Date Contract Initiated: September 30, 1980

Current Annual Level: $274,739

MIDWEST RESEARCH INSTITUTE (NO1-CP-05613)

Title: Synthesis of Derivatives of Polynuclear Aromatic Hydrocarbons

Contractor's Project Director: Mr. James C. Wiley, Jr.

Project Officer (NCI): Dr. David G. Longfellow

Objectives: The major objective of this program is the synthesis and purification of NCI-selected nonlabeled and labeled (^{3}H, ^{14}C) polynuclear aromatic hydrocarbon (PAH) derivatives of the following types: phenols; quinones; epoxides;

dihydrodiols; diolepoxides; alkyl and hydroxyalkyl substituted parent hydrocarbons; nitro-PAH derivatives; PAH-DNA adducts; and sulfate, glucuronide, and glutathione conjugates. These derivatives are prepared by unequivocal methods to produce adequate quantities of well-characterized compounds of high purity ($\geq$ 98%) for distribution as metabolite standards through the NCI Chemical Carcinogen Standard Reference Repository. Activities in support of the NCI Repository include the initial synthesis, maintenance of inventory through resynthesis, and shipments of compounds to authorized recipients of isotopically labeled PAH metabolites from a Radiochemical Repository at Midwest Research Institute (MRI). In addition, selected polycyclic hydrocarbon derivatives are synthesized for the National Institute of Environmental Health Sciences via an interagency agreement with the NCI.

Major Findings: During the last year 34 polynuclear aromatic hydrocarbon derivatives have been synthesized, purified, characterized, and either shipped to the NCI Chemical Carcinogen Standard Reference Repository or, in the case of the isotopically labeled derivatives, placed in the Radiochemical Repository at MRI. These derivatives have included ^{14}C- and ^{3}H-labeled racemic anti- and syn-dihydrodiol epoxides of benzo(a)pyrene (BP); ^{3}H-labeled and unlabeled enantiomers of anti-dihydrodiol epoxide of BP; major and minor tetrols and triols of BP; glucuronide and glutathione conjugates of BP; glucuronide conjugates of benz(a)-anthracene and 7,12-dimethylbenz(a)anthracene (DMBA), K-region epoxides of indeno(1,2,3-c,d)pyrene; resynthesis of labeled and unlabeled BP phenols, dihydrodiols and epoxides; and non-K-region A-ring phenols and epoxides of DMBA. The Radiochemical Repository, maintained and operated by MRI, has from its inventory of 40 compounds shipped 205 ^{3}H- and ^{14}C-labeled PAH metabolite samples to 115 authorized investigators in the United States, Japan, England, France, Canada, Sweden, Finland, Switzerland, and Germany during the last year.

Significance to Biomedical Research and the Program of the Institute: The cause, and ultimately the prevention, of cancer require a detailed understanding of the chemical and biological events at the molecular level. Polycyclic aromatic hydrocarbons and their metabolites, many of which have been shown to be potent carcinogens, provide ideal systems for a study of molecular carcinogenesis. It is virtually impossible for a laboratory lacking specific synthetic experience to synthesize any one of these compounds for a particular metabolic study. This contract allows the NCI to provide compounds for pertinent experiments in chemical carcinogenesis which could not be carried out otherwise. Carcinogenesis research has been greatly stimulated by the availability of authentic metabolite derivatives (labeled and unlabeled) for use as reference standards and/or substrates.

Proposed Course: Novel compounds will continue to be synthesized upon the request of the NCI. A major effort will be devoted to the synthesis, purification, and characterization of the following types of PAH derivatives:

1. Metabolites of parent PAH currently in the Repository (e.g., phenols, epoxides, quinones, dihydrodiols, triols, tetrols, dihydrodiolepoxides, derivatized methyl and nitro analogs and conjugates).

2. Potential metabolites of other classes of parent PAH not currently in the Repository with emphasis on classes of wide environmental distribution (e.g., indeno(1,2,3-c,d)pyrene).

3. Sulfate, glucuronide, and glutathione conjugates of selected PAH phenols, alcohols, dihydrodiols, quinones, and epoxides.

4. Resynthesis of expended PAH Repository derivatives, up to two to three times to provide for modifications and improvements of reaction procedures and optimization of yields of the original synthesis.

5. Radiolabeled (^{3}H, ^{14}C) and mass-labeled (^{2}H, ^{13}C) parent PAH, their metabolites, and derivatives for distribution through the Radiorepository located at MRI.

6. Tetrols and triols of selected PAH (e.g., benzo(a)pyrene and 7,12-dimethylbenz(a)anthracene) for use as standard markers of diolepoxide formation in biological systems.

7. Mono-, di-, and trimethyl derivatives and metabolites of a selected group of parent PAH (e.g., chrysene, phenanthrene, fluoranthrene, pyrene, dibenz(a,h)anthracene, benzo(a)pyrene, benzo(e)pyrene, and benz(a)anthracene).

8. PAH-DNA adducts of selected PAH derivatives (e.g., BP-7,8-diol-9,10-epoxide-$2NH_2$ guanine, BP-7,8-diol-9,10-epoxide-$2NH_2$ guanosine, and BP-7,8-diol-9,10-epoxide-phosphoesters) for use as standards in DNA-carcinogen binding studies.

9. Nitro PAH derivatives of selected PAH of wide environmental distribution with special emphasis on PAH that appear on the EPA priority pollutant list.

Most of these PAH derivatives will be prepared by the same or extensions of methods which MRI has used in preparation of over 180 unlabeled and labeled PAH metabolites and derivatives on the present and a previous NCI contract. In some cases, new techniques and procedures will be employed.

Date Contract Initiated: September 30, 1980

Current Annual Level: $499,780

MIDWEST RESEARCH INSTITUTE (NO1-CP-05719)

Title: Synthesis of Selected Chemical Carcinogen Standards

Contractor's Project Director: Dr. Richard S. Bodine

Project Officer (NCI): Dr. David G. Longfellow

Objectives: The major objective of this program is the resynthesis of NCI-selected derivatives of benzo(a)pyrene, 7,12-dimethylbenz(a)anthracene, and cyclopenta-(c,d)pyrene for inventory maintenance of the NCI Chemical Carcinogen Standard Reference Repository. The syntheses of the selected derivatives use previously established, unambiguous methods to provide well-characterized compounds of high purity and include unlabeled and ^{3}H- and ^{14}C-labeled compounds. A second objective of this program is the syntheses of compounds from other chemical classes including aromatic amines, steroid derivatives, additional polynuclear aromatic hydrocarbons, and physiologically active natural products.

Major Findings: In the past 12 months, 12 derivatives of benzo(a)pyrene have been prepared, characterized, and sent to the NCI Chemical Repository. These derivatives have included the 12-phenol, the 7-glucuronide, the 1,6-, 3,6-, and 7,8-quinones, the 4,5- and 7,8-epoxides, the syn- and anti-7,8-dihydrodiol-9,10-epoxides, the

trans-7,8-dihydrodiol, and the (+)- and (-)-enantiomers of the trans-7,8-dihydrodiol.

Significance to Biomedical Research and the Program of the Institute: This contract provides the National Cancer Institute with pertinent compounds for the Chemical Carcinogen Standard Reference Repository. In response to the goals of the program, the preparation of these compounds allows researchers lacking synthesis capabilities to have available authentic samples of substrates and probable metabolites for their investigations of chemical carcinogenesis.

Proposed Course: The synthesis of unlabeled and ^{3}H and ^{14}C-labeled metabolites and conjugates of benzo(a)pyrene, 7,12-dimethylbenz(a)anthracene, and cyclopenta-(c,d)pyrene will continue to supply the NCI Chemical Carcinogen Standard Reference Repository. The labeled physiologically active natural product capsaicin-1'-^{14}C will be prepared, and as determined by NCI, additional labeled and unlabeled syntheses may include aromatic amines, steroid derivatives, and other polynuclear aromatic hydrocarbons and physiologically active natural products.

Date Contract Initiated: September 30, 1980

Current Annual Level: $192,030

SOUTHERN RESEARCH INSTITUTE (NO1-CP-85616)

Title: Synthesis of Kilogram Amounts of Retinoids for Long-Term Animal Studies

Contractor's Project Director: Dr. Y. Fulmer Shealy

Project Officer (NCI): Dr. Carl E. Smith

Objectives: The objectives of this contract are the synthesis and complete characterization of certain retinoids selected by the National Cancer Institute. These retinoids are synthesized in large quantities for long-term evaluation in animals.

Major Findings: Several retinamides were synthesized for long-term studies of chemoprevention of cancer in animals and for toxicological evaluation in animals. Large amounts of all-trans-N-butylretinamide (> 1 kg.), all-trans-N-(4-(pivaloyl-oxy)phenyl)retinamide (> 0.8 kg.), and all-trans-N-(4-hydroxyphenyl)retinamide (> 2.8 kg.) were synthesized. The following all-trans retinoids were synthesized in quantities ranging from 270-370 grams. (±)-N-(2,3-dihydroxypropyl)retinamide, N-(4-hydroxybutyl)retinamide, N-(2-hydroxyethyl)retinamide, N-(2-hydroxypropyl)-retinamide, N-(3-hydroxypropyl)retinamide, N-propylretinamide. All of these retinoids were fully characterized, and various amounts were supplied to ten different investigators for the biological studies mentioned above. Observations on the sensitivity of five all-trans retinamides to atmospheric oxygen showed that the 2,3-dihydroxypropyl amide absorbed oxygen much more rapidly than the other four compounds. The 2-hydroxyethyl and 4-hydroxybutyl amides also absorbed oxygen readily. The N-butyl and N-(4-hydroxyphenyl) amides were more stable.

Developmental studies on a synthesis of 13-cis-retinoic acid on a large scale culminated in reproducible procedures and the synthesis of about 850 grams of this compound.

Significance to Biomedical Research and the Program of the Institute: The retinamides comprise a group of active chemopreventive agents, some of which have very low toxicity. 13-Cis-retinoic acid is also active, less toxic than all-trans-retinoic acid, and a precursor of other potential chemopreventive agents. Agents that prevent carcinogenesis, or the progression of the carcinogenic process to full malignancy in epithelia, have enormous potential for the control of human cancer. Studies in animals and in organ cultures show that retinoids have this potential; but new retinoids with improved chemopreventive action, decreased toxicity, and more favorable pharmacokinetic properties are needed. To achieve these goals, long-term experiments in animals must be performed. This contract provides large amounts of retinoids selected by the National Cancer Institute for these long-term studies of the chemoprevention of cancer.

Proposed Course: Retinoids requested by the NCI for its program on the chemoprevention of cancer will be synthesized, fully characterized, and sent to investigators designated by the NCI.

Date Contract Initiated: September 30, 1978

Current Annual Level: 0

SOUTHWEST RESEARCH INSTITUTE (NO1-CP-85601)

Title: Encapsulation of Retinoids for Administration in Laboratory Diets

Contractor's Project Director: Dr. Donald J. Mangold

Project Officer (NCI): Dr. Carl E. Smith

Objectives: The objective of this program is the stabilization of certain synthetic retinoids by microencapsulation in food or pharmaceutical grade materials and a demonstration of bioavailability of the encapsulated retinoids using the rat as the animal model. The stability of the encapsulated methods is being determined at 25° and 55°C with and without light.

Major Findings: Based upon previously reported results of the determination of the optimum microsphere formulations for the protection of trans-retinyl acetate and trans-retinoic acid, samples of microspheres of two experimental retinoids, all-trans-N((4-hydroxyphenyl))-retinamide (4HPR) and all-trans-(N-4-(Pivaloyloxy)-phenyl))-retinamide (4-PPR) have been prepared using the most promising formulations. A 210g sample of 4-HPR and a 220g sample of 4-PPR in a gelatin matrix, with Tenox 20 antioxidant and sodium benzoate preservative, was forwarded to an NCI contractor for further evaluation in animal studies. A sample of the mirospheres admixed with rat feed, followed by aging of the mixture at room temperature in light, gave a significantly lower decomposition of retinoid as compared to a standard stabilized retinoid formulation containing trioctanoin, Tenox 20, and tocopherol deposited from solvent on rat feed.

Significance to Biomedical Research and the Program of the Institute: Retinoids, vitamin A analogs, have been shown to have some ability to prevent chemical carcinogenesis in certain epithelial tissues of animals; however, the natural retinoids cannot be readily used because of toxicity and limited tissue distribution at the high dietary amounts required. Recently, it has been shown that

several synthetic retinoids have high activity and less toxicity for the prevention of cancer in animals in the results of work conducted by the National Cancer Institute. In order to extensively study the effect of these synthetic retinoids by feeding in animal diets, the materials must be stabilized, since most retinoids must be protected from oxidation, light, heat, moisture, and bacterial decomposition.

With the availability of relatively stabilized 4-HPR and 4-PPR in the form of microspheres, larger scale and simpler animal feeding studies can be undertaken by other investigators. The formulations found promising for stabilizing the retinoids investigated to date should be useful in the preparation of stabilized formulations of many other experimental retinoids as microspheres.

Proposed Course: No future work is planned as the program is to be completed on March 31, 1982.

Date Contract Initiated: September 30, 1978

Current Annual Level: 0

SRI INTERNATIONAL (NO1-CP-05601)

Title: Synthesis of Radiolabeled Retinoids for Metabolic and Pharmacologic Studies

Contractor's Project Director: Dr. Hans H. Kaegi

Project Officer (NCI): Dr. Carl E. Smith

Objectives: The objective of the research is to synthesize adequate quantities of radioisotopically labeled retinoid compounds. These are to be used as tracers in the investigation of the metabolic and pharmacologic action of retinoid compounds as anticancer agents.

Major Findings: A fresh supply of all-trans-retinoic acid labeled with tritium in position 11 has been prepared according to our standard method (Kaegi, H. H., and DeGraw, J. I., J. Labelled Compds. Radiopharm. 18, 1099 (1981)). Parts of this compound were converted into tritiated all-trans-N-(4-hydroxyphenyl)-retinamide. All-trans-retinol-11-^{3}H was prepared by saponification of all-trans-retinyl acetate with sodium methoxide in methanol, followed by purification on HPLC. Cold work leading to a preparation of all-trans-retinoic acid labeled with carbon-14 in positions 10 and 11 has been completed. Work is progressing on a second approach for the preparation of tritiated all-trans-retinoic acid of high specific activity (30-50 Ci/mmole), labeling positions 7 and 8. A method for labeling all-trans-retinoic acid in position 10 with tritium has been worked out and found to be suitable for lower level (~5 Ci/mmole) preparations. High specific activity preparations do not seem to be feasible using this methodology. A sample of tritiated retinoic acid was prepared in very low yield having a specific activity of 15.5 Ci/mmole but unfortunately was found to be very unstable. The stock of labeled retinoids was stored and maintained and distributed to users as requested by the Project Officer. The compounds were periodically analyzed and when needed repurified by micropreparative HPLC.

Significance to Biomedical Research and the Program of the Institute: Retinoid deficiency enhances the susceptibility of experimental animals to chemical

carcinogenesis. The application of retinoids can reverse carcinogen-induced hyperplasia or lesions, but the mechanism of action of the retinoids is largely unknown. Mechanistic studies with radiolabeled retinoids may enable investigators to design more therapeutically useful retinoids suitable for clinical application in cancer prevention.

Proposed Course: The synthesis of all-trans-retinoic acid labeled with ^{14}C and tritiated at a high specific activity (30-50 Ci/mmole) in positions 7 and 8 will be carried out. Additional compounds will be prepared as requested by the Project Officer and the stock of already prepared retinoids maintained.

Date Contract Initiated: September 30, 1980

Current Annual Level: $189,645

SRI INTERNATIONAL (NO1-CP-05614)

Title: Synthesis of Selected Chemical Carcinogens

Contractor's Project Director: Dr. Elmer J. Reist

Project Officer (NCI): Dr. David G. Longfellow

Objectives: To synthesize quantities of known and suspected carcinogenic materials and their metabolites.

Major Findings: During the past year, the following compounds have been prepared: syn 1,2-epoxy-trans-3,4-dihydroxy-1,2,3,4-tetrahydrobenz(a)anthracene; aflatoxin B_1 2,3-diol, aflatoxin Q_1, trans-1,2-dihydroxy-1,2-dihydrobenz(a)anthracene (precurser to the 3,4-epoxy diols, syn and anti) and N-butyl-3-hydroxybutyrolactam (precurser to butyl 3 carboxy-3-hydroxypropyl nitrosamine).

Significance to Biomedical Research and the Program of the Institute:
It is believed that the majority of cancers that occur in man are caused by substances in the environment. By studying these substances and their mechanisms of action, information may be derived that will lead to means of prevention of cancer in man.

Proposed Course: Additional carcinogen suspects will be prepared.

Date Contract Initiated: September 30, 1980

Current Annual Level: $171,286

SRI INTERNATIONAL (NO1-CP-85612)

Title: Syntheses of Hetero-Substituted Polyaromatic Hydrocarbons

Contractor's Project Director: Dr. Elmer J. Reist

Project Officer (NCI): Dr. David G. Longfellow

Objectives: The synthesis of heteroaromatic compounds that are analogs of carcinogenic aromatic hydrocarbons and as a result are reasonable candidates to possess carcinogenic activity. They are also potential environmental pollutants.

Major Findings: During the past year, the following compounds have been prepared: phenanthro(4,5-bcd)thiophene, 2-azabenz(a)anthracene, N-tosyl-2-aza-1,2,3,4-tetrahydrobenz(a)anthracene-7,12-dione (precurser for 2-aza-7-methylbenz(a)-anthracene and 2-aza-7,12-dimethylbenz(a)anthracene) and 3-aza-3,4-dihydro-phenanthrene (precurser for 1-azapyrene).

Significance to Biomedical Research and the Program of the Institute: A number of aza-aromatic hydrocarbons have been implicated as carcinogenic agents. Others have been identified as environmental pollutants, forming during combustion of organic material, e.g. in tobacco smoke. The carcinogenic potential of many of these compounds has not yet been demonstrated. The availability of these compounds as standard references will assist in identifying them in the atmosphere and also permit evaluation of their carcinogenic potential.

Proposed Course: Additional heteroaromatic compounds that are analogs of carcinogenic hydrocarbons will be prepared, characterized, and sent to NCI.

Date Contract Initiated: September 30, 1980

Current Annual Level: 0

SUMMARY REPORT
SPECIAL PROJECTS

Contracts included in the Special Projects Program are those which emphasize multidisciplinary research, together with those in the Chemical and Physical Carcinogenesis Branch (CPCB) which are not assigned to other CPCB Programs. A principal thrust of this Program is to search the broad expanse of carcinogenesis research, with the object of identifying areas of emergent high promise relative to human cancer causation.

The Program includes 5 contracts which are all scheduled to be completed during FY 1982. Due to incremental funding in the previous fiscal year, no funds were expended during FY 1982 on this Program.

The several contracts assigned to the Special Projects encompass a diversity of subject matter. One contract concerning the development and characterization of human cell and organ culture systems relevant to carcinogenesis was completed early this year at the University of North Carolina (NO1-CP-75956). The contractor identified three cell types in cultured human primary endometrial tissue and achieved partial transformation of one cell type after repetitive exposure to MNNG. In experimental studies with human endometrial tissue it was demonstrated that the metabolism profile for benzo(a)pyrene varied with the stage of the menstrual cycle from which the tissue was derived.

Three contactors have been studying the heritable factors in mice which influence susceptibility to carcinogens. For work in the area of photobiology and photocarcinogenesis, ten representative stocks and strains of non-haired mice have been characterized at Temple University (NO1-CP-85603) for their comparative UV photocarcinogenic sensitivity. In addition, several immunologic traits and the comparative breeding efficiency of these recessive mutant lines was recorded. The inbred strain designated HRA/skh has emerged as a particularly attractive animal model.

At the Texas A&M Research Foundation (NO1-HD-92839) work has been underway which is attempting to identify the chromosomal location of the Ah locus in mice by establishing linkage relationships to previously mapped biochemical loci. The Ah locus is one of very few genetic entities known to play a significant role in mammalian carcinogenesis. Preliminary data are suggestive of a loose linkage of Ah with Glo-1 on chromosome 17.

The third contractor, Microbiological Associates (NO1-HD-92840), has been testing various recombinant inbred and congenic mice to determine the role of certain known genes in controlling or influencing susceptibility for both 3-methylcholanthrene (MCA) induced subcutaneous tumors and MCA-induced pulmonary carcinomas. Various other enzymes and antigen marker levels have been followed after induction with 5,6 benzoflavone. In these tests the Ah locus appears to be the primary determinant of susceptibility to carcinogenesis, however there are apparently other background genes which play a significant role.

At the University of Nebraska, Eppley Institute (NO1-CP-05628), a two generation study on the dietary modulation of spontaneous cancer incidence in Syrian Golden hamsters is near completion. In these studies, three levels of protein (lactalbumin) have been followed. While histopathological information is not yet available, it is apparent that protein level has a dramatic impact on longevity in these animals. The 20% and 40% lactalbumin diets significantly increase survival compared to the 10% protein or commercial chow diets.

SPECIAL PROJECTS

CONTRACT INDEX

MICROBIOLOGICAL ASSOCIATES (NO1-HD-92840)

Title: The Genetics of Chemical Carcinogenesis

Contractor's Project Directors: Dr. Richard E. Kouri
Dr. C. J. Henry
Dr. R. A. Lubet

Project Officers (NCI): Dr. David G. Longfellow
Dr. Daniel Nebert

Objectives: The objective of this project is to determine the role of certain known genes in controlling or influencing susceptibility to chemical carcinogenesis.

Major Findings: The initial study employed the various recombinant inbred (RI) and congenic (CI) animals in studying susceptibility to 3-methylcholanthrene (MCA)-induced subcutaneous tumors. When studying RI lines derived from crossing C57B1/6N and AKN animals, two clearcut groups were established. Three of the individual lines were highly susceptible to MCA-induced tumors (incidence > 60%, average latency < 150 days), while 8 of the lines were minimally susceptible to MCA-induced tumors (incidence < 20%). When the genetic makeup of these lines were investigated, the three susceptible lines were aryl hydrocarbon hydroxylase (AHH)-inducible while all eight of the nonsusceptible lines were AHH noninducible. In contrast, the effects of retrovirus expression (i.e. p30 expression in the spleen) of H-2 haplotype, appeared to play a limited function in determining susceptibility. When similar studies were performed using the C57B1/6 x C3H RI lines, we found once again that the Ah locus seemed to be directly linked with tumor susceptibility. However, when comparing tumor susceptibility between AHH inducible strains of the (B6 x AKN) RI lines and (B6 x C3H) RI lines the AHH inducible B6 x C3H RI lines yielded a higher tumor incidence and shorter latency than their (B6 x AKN) counterparts. These results imply that there are background genes which play a significant role in determining tumor susceptibility, although the Ah locus itself appears to be the primary determinant of susceptibility. The congenic animals have been employed in subcutaneous MCA studies and have shown that merely changing the Ah locus in otherwise genetically identical animals increases the susceptibility to MCA-induced tumors by greater than four fold. The B6 x AKN RI lines are presently being studied for their susceptibility to MCA induced carcinomas in the lung.

In addition to studying MCA-induced carcinogenesis, other compounds for possible linkage between their biological effects and the Ah locus were investigated. These investigations have included a variety of azo dyes (discussed below) as well as the two isomeric polycyclic hydrocarbons, dibenz(a,h)anthracene and dibenz(a,c)-anthracene. Dibenz(a,h)anthracene causes 50% tumorigenesis in AHH-inducible animals while causing very few tumors (< 2% incidence) in non-inducible animals. In contrast, dibenz(a,c)anthracene induced tumors in only 2 of 60 inducible animals while giving no tumors in the non-inducible animals. Most recently the contractor has begun to investigate the possible role of the Ah locus in the biological effects of certain sudan (azo) dyes. These compounds were found to induce AHH activity and are activated by the liver microsomes to mutagenic forms in vitro.

These compounds therefore become primary candidates to display biological effects which are closely associated with the Ah phenotype of the animals. The role of these (or similar chemical) in causing toxicity as measured by histopathologic, cytogenetic or immunotoxicologic endpoints are presently being evaluated.

Significance to Biomedical Research and the Program of the Institute:

1. Linkage of Ah locus to hydrocarbon mediated carcinogenicity and toxicity
The initial objective of this contract has been to establish the significant involvement of the Ah locus in polycyclic hydrocarbon mediated effects in a variety of biological models including:

a. Induction of subcutaneous tumors

b. Induction of lung carcinomas

c. Induction of toxicity (acute)

2. Linkage of Ah locus to biological effects of various classes of compounds
Although the polycyclic hydrocarbons are significnt environmental pollutants the question of general applicability of the model still arises. Preliminary evidence shows that the biological effects (carcinogenicity, toxicity) or certain azodyes as well as polyhalogenated biphenyls are likely to be associated with the Ah locus.

3. Possible linkage of Ah locus to biologic effects in humans
Previous studies have indicated a significant "genetically" determined polymorphism for AHH inducibility in the human population. These studies have been hindered by the requirement of using mitogen activated peripheral blood lymphocytes, although more recent improvements in the assay may allow for substantial improvement. Nonetheless taking together the finding that the Ah locus is associated with the biological effects of polycyclic hydrocarbons and polychlorinated biphenyls, and that the human population shows a polymorphic response for this locus, we might expect:

a. Persons with highly inducible levels might be hypersusceptible to tumors induced by Ah-determined chemicals (polycyclic hydrocarbons, smoke, etc.), i.e. lung tumors.

b. Persons who are highly inducible might be hypersusceptible to the toxic effects of polyhalogenated biphenyls.

c. Persons of low inducibility may be at greater risk to chemically-induced tumors of the lymphoreticular system.

Proposed Course: Two primary groups of experiments will continue to be investigated in the third year of this contract. First the contractor will continue to induce lung carcinomas in the RI lines. This should be able to confirm the previous finding that the Ah locus is a primary determinant in MCA induction of tumors intratracheally. Secondly a more thorough study of one of the Sudan dyes to study the effects of the Ah locus and its biological effect will be done. Ah inducible and Ah non-inducible congenic strains will be employed to study acute toxicity and varied toxic effects (i.e., histopathology, immunotoxicity, and clinical chemistry) following i.p. administration of the dye. Future areas of study might include the role of the Ah locus following systemic administration of hydrocarbon, and the role

of the Ah locus in the carcinogenic or cocarcinogenic effects of azo dyes and polychlorinated biphenyls.

Date Contract Initiated: September 30, 1979

Current Annual Level: 0

NEBRASKA, UNIVERSITY OF (EPPLEY INSTITUTE FOR RESEARCH IN CANCER AND ALLIED DISEASES) (NO1-CP-05628)

Title: The Possible Influence of Diet in Carcinogenesis

Contractor's Project Director: Dr. Diane D. Birt

Project Officer (NCI): Dr. Carl E. Smith

Objectives:
1. Establish three levels of a well-utilized protein for the Syrian golden hamster. These levels will be close to the requirement, slightly below the requirement, and in excess of the requirement. Once established in preliminary studies, these levels will be fed in chronic studies.

2. Study the influence of the three levels of dietary protein established above (lactalbumin at 10, 20 and 40% of the diet was studied) on:

a. Spontaneous diseases in two generations of hamsters, thereby following animals exposed to the three diets during fetal growth, lactation and through post weaning life, in addition to following animals exposed throughout post weaning life.

b. Protein utilization and nutritional status in two generations of hamsters. Protein utilization will be evaluated by following carcass nitrogen, serum proteins, and various liver microsome enzymes.

Major Findings: Nutritional data on the chronic studies was evaluated, and the remaining survivors on the spontaneous disease investigation were allowed to die. Growth of female hamsters generally increased with each increment in lactalbumin, with only small differences between the generations. Male growth was greatest in hamsters that consumed the 20% lactalbumin diet and higher in the F_1 than in the F_0 generation. Reproduction was superior in hamsters fed commercial diet and differed by season in those fed purified diets, with the best performance following the fall matings. Litter size and body weights of offspring at weaning increased from hamsters fed 10% to those fed 20% or 40% lactalbumin diets. Longevity differed between the two generations of females. In the F_0 generation the longest lifespans were observed in the 20% and 40% lactalbumin groups, with shorter survivals in the 10% and commercial groups, respectively. The F_1 generation females experienced increasing survival with each increase in lactalbumin. In contrast with females, male survival increased from the 40% to the 10% lactalbumin diet and was greastest in males fed the 20% level. Male longevity did not differ between the two generations and was shortest with the commercial diet. Data on hepatic microsomal systems indicated increases in cytochrome P_{450} content between 4 and 64 weeks of age and decreases in aryl hydrocarbon hydroxylase and aniline hydroxylase activity between maturity and senescence. Dietary lactalbumin level influenced both the

magnitude and timing of these age related changes. The activities of the hydroxylases were modified by diet primarily at 6 and 18 weeks, but effects on cytochrome P_{450} increased as the hamsters aged. Cytochrome P_{450} values increased with increases in protein, and hydroxylase activities were highest in hamsters consuming the medium and/or high protein diets.

Significance to Biomedical Research and the Program of the Institute: The role of diet in cancer is becoming more apparent as research efforts in this area are increased. This project evaluates the role of dietary protein on spontaneous tumor yields in a species not previously studied for this purpose, the Syrian hamster. In addition, at several phases of the study we are evaluating the influence of dietary protein on related systems of the hamster, the microsomal drug metabolizing systems.

Several lines of investigation have suggested that transplacental and translactal effects may be of importance in carcinogenesis. The involvement of these phases of life will be evaluated in this project by studying two generations of hamsters. The parent animals began on the studies at weaning and their offspring. The detailed evaluation of the protein needs of the Syrian hamster will improve this species as a model for biological research.

Proposed Course: The evaluation of the influence of dietary lactalbumin level on spontaneous diseases will be completed.

Date Contract Initiated: March 19, 1968

Current Annual Level: 0

NORTH CAROLINA, UNIVERSITY OF (NO1-CP-75956)

Title: Studies of Carcinogenesis in Human Endometrial Tissue

Contractor's Project Directors: Dr. David G. Kaufman
Dr. Leslie A. Walton

Project Officer (NCI): Dr. Lea I. Sekely

Objectives: The overall objective of this project is to determine whether specific insights into the causation of human endometrial carcinoma can be derived by direct study of the effects of chemical carcinogens on human endometrial tissue. Specific objectives for the current year of this project were to further characterize the biologic properties of this tissue in vitro and to evaluate the effects of chemical carcinogens on human endometrial tissue in culture.

Major Findings: The focus of this project for the past year has been on the monolayer cell cultures derived from endometrium. In previous studies we have identified three cell types which routinely arise from primary specimens: a differentiated mature epithelial cell, a fibroblast, and a polygonal undifferentiated cell. The contractor has partial transformation of one cell type, the polygonal undifferentiated cell, after repetitive exposure to MNNG. The contractor's primary concern was to characterize this cell type in vitro and, if possible, to identify its in vivo origin. The contractor's previous experiments had indicated that at early passage, these polygonal cells responded to cycling of

estrogen and progesterone by forming aggregates and, in some cases, producing glycogen. These are features of endometrial stromal cells in vivo, and since numerically the stromal cells are the dominant endometrial cell type, this was the most likely origin of the polygonal cell. Electron microscopy studies of these cells after hormonal cycling showed the presence of distinctive markers for stromal cells in vivo during late secretory phase; long nexi or gap junctions, and solitary cilia were detected. This finding, together with a lack of desmosomes and extensive microvilli, point to a stromal cell origin for these polygonal cells. Indirect evidence to support this conclusion is that the polygonal cell is capable of changing into spindle cell shape in different medium. This is also indicative of a stromal cell origin because during the menstrual cycle the stromal cells do undergo such changes: they are spindle shaped during the proliferative phase but they become rounded under the influence of progesterone. In the predicidual reaction, the stromal cells are crowded and plump. The contractor believes that different media may mimic different endometrial phases. The morphology of these cells reverses from spindle to polygonal and back again if placed in DMEM then CMRL and back to DMEM. The contractor is currently screening histochemically for various enzymes which are known to fluctuate in the stroma during the menstrual cycle. The results indicate a predecidual stage for the cells in CMRL medium; high acid phosphatase activity, no alkaline phosphatase activity, presence of 5' nucleotidase and succinic dehydrogenase, and lack of peroxidase activity all correlate with in vivo findings for endometrial stroma.

In order to quantitate the effects of carcinogens on the stromal cells, the contractor has developed a clonal assay. The contractor has determined that the stromal cells will form colonies from single cells in DMEM media supplemented with 20% fetal calf serum. CMRL will not support this growth. The colony forming efficiency is about 10-20%. The contractor is screening various media and growth supplements to optimize the conditions for clonal growth. To date, every media screened (except CMRL) supports clonal growth. Several common differences exist between CMRL and other media, and the contractor is in the process of screening these factors to determine if any are the determinant to preventing clonal growth. Since carcinogen-treated cells do form colonies in CMRL, it may be extremely important to identify what factors prevent clonal growth in normal cells. These observations raise the possibility that the predecidual-like change of endometrial stroma cells in vitro may represent terminal differentiation of these cells and therefore is incompatible with clonal growth.

Recent studies have also focused on the effects of the tumor promoter TPA on stromal cells. The contractor has found that stromal cells previously exposed to carcinogens are more sensitive to morphology changes induced by TPA than control cells and do not become refractory to the effects of TPA, as control cells do. The contractor has also found that prolonged exposure to TPA causes almost complete inhibition of colony-forming ability in acetone-treated cultures, while cells exposed to carcinogen either experience no change in colony forming ability or become better able to form colonies after prolonged TPA exposure. These findings indicate that selective effects may occur to promote the growth of carcinogen-treated cells during long-term TPA treatment.

Significance to Biomedical Research and the Program of the Institute:
The overall objective to this program is to malignantly transform human endometrial tissue by treatment with chemical carcinogens in vitro. These studies may contribute to our understanding of the causation of human endometrial cancer and help explain interindividual variation in susceptibility to this disease. The

accomplishments to date in the performance of this contract represent tangible steps in efforts to achieve these objectives.

Proposed Course: This contract has terminated.

Date Contract Initiated: September 30, 1977

Current Annual Level: 0

TEMPLE UNIVERSITY (NO1-CP-85603)

Title: Hairless Mice for Carcinogenesis Studies

Contractor's Project Director: Dr. Paul Donald Forbes

Project Officer (NCI): Dr. David G. Longfellow

Objectives: The overall objectives of this project include:
1. Deriving the comparative UV photocarcinogenic sensitivity of selected stocks and strains of non-haired mice.

2. Characterizing several immunologic traits of the selected animals.

3. Evaluating breeding efficiency of stocks or strains which might be designated as preferred test organisms for photocarcinogenesis studies.

4. Completing special studies on four of the strains.

Major Findings: The primary purpose of this contract was to examine several strains of hairless mice in order to select and develop appropriate animals for work in the area of photobiology and photocarcinogenesis. By the time the project was well under way the inbred strain designated HRA/Skh had emerged as a particularly attractive animal model. These mice are robust and prolific, overcoming many of the economic difficulties and handling disadvantages of hairless mice. This strain of animals is currently being used in the National Toxicology Program's bioassay of 8-methoxypsoralen and related psoralen derivatives. Although the HRA/Skh strain is no more acutely sensitive to ultraviolet radiation than a number of other stocks and strains of hairless mice, this strain is particularly prone to early skin tumor formation following repeated exposure to ultraviolet radiation.

Each of ten stocks and strains of non-haired mice is being subjected to a dose-response study, involving five different daily doses of simulated sunlight. The ten representative stocks and strains each appear to have a characteristic sensitivity, and the sensitivities form a continuum from the HRA/Skh at one extreme to the HRS/J at the other extreme. For example, HRS/J animals require about twice the dose that is needed to produce a given tumor response in HRA/Skh mice. The contractor has determined that F_1 hybrid mice (HRA/Skh x HRS/J) are intermediate to the parental strains in UV sensitivity. A number of immunologic responses have been evaluated in each of these stocks and strains, and in both the irradiated and the unirradiated mice, the derived values are within normal limits. The tests include the ability to respond to various mitogens as well as to T-cell dependent and T-cell independent antigens. The number of T lymphocytes and B lymphocytes in the spleen, thymus and lymph nodes were found to be comparable among the strains. The antibody forming

ability as measured by plaque-forming cells in the spleen was demonstrable in all strains. The animals are able to mount a delayed contact-sensitivity response.

Significance to Biomedical Research and the Program of the Institute: Heritable factors influence susceptibility to carcinogens, as clearly evidenced in animal studies. This study identified several sources of material for isolating and evaluating such components as immunologic and cellular repair.

Date Contract Initiated: September 1, 1978

Current Annual Level: 0

TEXAS A&M RESEARCH FOUNDATION (NO1-HD-92839)

Title: Mapping of the Ah Locus in the Mouse

Contractor's Project Director: Dr. James E. Womack

Project Officer (NCI): Dr. David G. Longfellow

Objectives: The primary objective of this research is to find the chromosomal location of the Ah locus in mice by establishing linkage relationships to previously mapped biochemical loci.

Major Findings: Laboratory strains of Mus musculus molossinus and Mus musculus castaneus are valuable linkage testing strains because they carry a number of unique alleles at biochemical genetic loci. The contractor has used M. m. molossinus and M. m. castaneus to construct backcrosses with strain DBA/2J to test the possible linkage of the Ah locus with biochemical markers segregating in these strains. The contractor has found Ah to segregate independently of Idh-1 and Pep-3 on chromosome 1, Amy-1 on chromosome 3, Gpd-1 on chromosome 4, Pgm-1 on chromosome 5, Es-8, Mod-2, and Hbb on chromosome 7, Es-1 and Es-2 on chromosome 8, Mpi-1 on chromosome 9, Apk on chromosome 10, Es-3 on chromosome 11, Np-1 and Es-10 on chromosome 14, Gpt-1 and Gdc-1 on chromosome 15, and Got-1 on chromosome 19. These markers are distributed over eleven of the mouse chromosomes and their testing swept approximately 30% of the mouse gene map. Although these data are negative with respect to Ah linkage, they are extremely important in that they demonstrate where Ah is not located. Thus, further attempts to map Ah should be concentrated on the chromosomal regions not tested in these experiments. Unfortunately, these regions are essentially void with respect to biochemical markers although many are marked with mapped morphological or coat color mutants.

Another important aspect of this study has been the determination of the strain distribution patterns (SDP) for Ah and seven biochemical loci in a set of N x 8 recombinant inbred (RI) strains. Since mapping data in RI strains is cumulative, the SDP for Ah may be matched in the future with the SDP of some yet to be discovered gene and thus accomplish the task of locating Ah on the mouse gene map.

Significance to Biomedical Research and the Program of the Institute: The Ah locus is one of the very few genetic entities known to play a significant role in mammalian carcinogenesis. The location of its position in the mouse genome will aid in the understanding of its regulation and its comparative relationship to its homologue in the human genome.

Proposed Course: The contractor will continue to concentrate mapping efforts to regions that have not been eliminated by previous testing. Of special interest is chromosome 17, where preliminary data are suggestive of loose linkage of Ah with Glo-1.

Date Contract Initiated: September 30, 1979

Current Annual Level: 0

CHEMICAL AND PHYSICAL CARCINOGENESIS BRANCH

GRANTS ACTIVE DURING FY 82

BIOLOGICAL AND CHEMICAL PREVENTION

Investigator/Institution/Grant Number	Title
AUERBACH, Arleen D. Sloan-Kettering Institute for Cancer Research 1 RO1 CA 32503-01	Effects of Anticarcinogens on Fanconi Anemia Chromosomes
AWASTHI, Yogesh C. University of Texas Med Br Galveston 5 RO1 CA 27967-03	Mechanism of Anti-Carcinogenic Effect of Antioxidants
BENEDICT, William F. Children's Hospital of Los Angeles 1 RO1 CA 31574-01	Ascorbic Acid Transformation and Oncogenic Progression
BERTRAM, John S. Roswell Park Memorial Institute 5 RO1 CA 25484-03	Inhibition of In Vitro Transformation by Retinoids
BLACK, Homer S. Baylor College of Medicine 5 RO1 CA 13464-09	Photochemical Reactions Related to Skin Cancer
BRINCKERHOFF, Constance E. Dartmouth College 1 RO1 CA 32476-01	Action of Retinoids on Synovial Cells
BUEDING, Ernest Johns Hopkins University 5 RO1 CA 18251-06	Protection Against Mutagenic Effects of Drugs
CHOPRA, Dharam P. Southern Research Institute 5 RO1 CA 26696-02	Biology of Airway Epithelial Lesions
CHUNG, Fung-Lung American Health Foundation 1 R23 CA 32272-01	Screening for Inhibitors of N-Nitrosamine Carcinogenesis
CROCE, Carlo M. Wistar Institute 1 RO1 CA 32495-01	Retinoic Acid Induced Differentiation
CURPHEY, Thomas J. Dartmouth College 1 RO1 CA 32478-01	Pancreatic Cancer and Retinoids: Model and Mechanism

DAWSON, Marcia I. SRI International 1 R01 CA 30512-01	Novel Retinoids for Chemoprevention of Epithelial Cancer
GIFFORD, George E. University of Florida 5 R01 CA 22183-03	Nutritional Aspects of Vitamin A and Cancer
HILL, Donald L. Southern Research Institute 1 R01 CA 30604-01	Prevention of ENU-Induced Brain Cancer By Retinoids
HILL, Donald L. Southern Research Institute 5 R01 CA 26815-02	Biotransformation of Retinoids In Vitro
HILL, Donald L. Southern Research Institute 5 R01 CA 26389-03	Disposition and Chemopreventive Activity of Retinoids
HORNSBY, Peter J. University of California La Jolla 1 R01 CA 32468-01	Antioxidant Action In a Model Cell Culture System
LUDLUM, David B. Albany Medical College 1 R01 CA 32446-01	Repair of Carcinogenic Lesions in DNA
MATHEWS-ROTH, Micheline M. Peter Bent Brigham Hospital 5 R01 CA 23053-05	Carotenoids as Antitumor Agents for Skin Tumors
MAYS, Charles W. University of Utah 5 R01 CA 28314-02	Reducing Cancer Risk By Radionuclide Chelation
McCORMICK, Anna M. University of Texas Health Science Center at Dallas 1 R01 CA 31676-01	Metabolism of Chemopreventive Retinoids
McCORMICK, David L. IIT Research Institute 1 R01 CA 30646-01A1	Interactions Among Modifiers of Mammary Carcinogenesis
McCORMICK, J. Justin Michigan State University 1 R01 CA 32490-01	Inhibition of Carcinogen - Transformation of Human Cells
MEDINA, Daniel Baylor College of Medicine 5 R01 CA 11944-11	Biology of Mammary Preneoplasias

MEDINA, Daniel Baylor College of Medicine 1 RO1 CA 32473-01	Selenium Inhibition of Mouse Mammary Tumorigenesis
MEHTA, Rajendra G. IIT Research Institute 5 RO1 CA 26030-03	Retinoids and Mammary Carcinogenesis
ONG, David E. Vanderbilt University 5 RO1 CA 20850	Cancer and Vitamin A
PETERSON, Per A. University of Uppsala 9 RO1 CA 32583-04	Retinol Metabolism With Special Regard to the Eye
PROUGH, Russell A. University of Texas 1 RO1 CA 32511-01	Inhibitor Effects on Monooxygenase Function
REDDY, Janardan K. Northwestern University 1 RO1 CA 32504-01	Antioxidants and Paroxisomas Proliferator Carcinogenesis
ROGERS, Adrianne E. Massachusetts Institute of Technology 1 RO1 CA 32498-01	Azaserine Carcinogenesis: Effects of Methionine, Cholin
RUDDLE, Nancy H. Yale University 1 RO1 CA 32447-01	Lymphotoxin and Interferon Inhibition of Carcinogenesis
SHKLAR, Gerald Harvard University 5 RO1 CA 23524-03	Oral Carcinogenesis, Vitamin A and Retinoids
STRAUSS, Bernard S. University of Chicago 1 RO1 CA 32436-01	Plasminogen Activator and Error-Prone DNA Synthesis
SULLIVAN, Paul D. Ohio University Athens 5 RO1 CA 22209-03	Antioxidants Interaction With Benzopyrene & Derivatives
THOMPSON, Henry J. University of New Hampshire 1 RO1 CA 32465-01	Breast Cancer Chemoprevention and Polyamine Biosynthesis
WATTENBERG, Lee W. University of Minnesota of Minneapolis-St. Paul 5 RO1 CA 09599-23	Microsomal Induction and Response to Carcinogens

WATTENBERG, Lee W.
of Minnesota of
Minneapolis-St. Paul
5 RO1 CA 14146-09

Inhibition of Chemical University
Carcinogenesis

WIEBEL, Friedrich J.
GSF-Research Center
1 RO1 CA 32541-01

Carcinogen Inactivation By
Conjugation With Glutathione

WOLF, George D.
Massachusetts Institute of Technology
5 RO1 CA 13792-05

Vitamin A and Glycoproteins
of Skin Tumors

YANG, Chung S.
University of Medicine & Dentistry
of New Jersey
1 RO1 CA 28298-01A1

Effects of BHA on Carcinogen
Metabolism

CHEMICAL AND PHYSICAL CARCINOGENESIS BRANCH

GRANTS ACTIVE DURING FY 82

CARCINOGENESIS MECHANISMS

Investigator/Institution/Grant Number	Title
ALWORTH, William L. Tulane University of Louisiana 5 RO1 CA 23014-03	Modification of Mammalian Epoxide Hydrase Activity
ARCHER, Michael C. Ontario Cancer Institute 5 RO1 CA 26651-03	Mechanism of Nitrosamine Alkylation of DNA and RNA
ASTLE, Lynn University of Utah 7 RO1 CA 32628-01	Gastrointestinal Carcinogenicity of Malondialdehyde
BAIRD, William M. Purdue University West Lafayette 2 RO1 CA 28825-02	Modifiers of Chemical Carcinogenesis in Cell Culture
BAKER, Donald G. University of Virginia Charlottesville 1 RO1 CA 25890-01A1	Influence of Hyperthermia on X-Ray Carcinogenesis
BRAND, K. Gerhard University of Minnesota of Minneapolis-St. Paul 2 RO1 CA 10712-14	Initiation & Promotion in Foreign Body Tumorigenesis
BRESNICK, Edward University of Vermont & St. Agric College 5 RO1 CA 20711-06	Polycyclic Hydrocarbon Metabolism and Carcinogenesis
BRYAN, George T. University of Wisconsin Madison 5 RO1 CA 11946-11	Carcinogenicity and Metabolism of 5-Nitrofurans
BUHLER, Donald R. Oregon State University 5 RO1 CA 22524-04	Metabolism and Toxicity of Pyrrolizidine Alkaloids
COATES, Robert M. University of Illinois Urbana-Champaign 5 RO1 CA 20436-06	Hydroxylamine Rearrangements and Carcinogenesis
COHEN, Arthur M. University of Southern California 5 RO1 CA 24518-03	Inhibition of Carcinogenesis by Selenium

COHEN, Samuel M. University of Nebraska Lincoln 1 RO1 CA 32313-01	Non-Mutational Multistage Urinary Bladder Carcinogenesis
CORBETT, Michael D. University of Florida 7 RO1 CA 32395-01	Hydroxamic Acid Production by Marine Organisms
COSTA, Max University of Texas Health Science Center Houston 1 RO1 CA 29581-01A1	Surface Charge & Phagocytosis of Toxic Metal Particulates
ERNSTER, Lars University of Stockholm 5 RO1 CA 26261-03	Metabolism of Polycyclic Hydrocarbons and Cancer
FIALA, Emerich S. American Health Foundation 5 RO1 CA 26395-03	Single Ring Arylamine Carcinogens: Mechanism of Action
FIELD, Lamar Vanderbilt University 1 RO1 CA 30321-01	Thiono-Type Compounds and Their Relation to Cancer
FLOYD, Robert A. Oklahoma Medical Research Foundation 5 RO1 CA 18591-07	Carcinogen Free Radicals In Arylamine Metabolism
FORD, George P. Pacific Northwest Research Foundation 1 RO1 CA 30475-01	The Prediction of Nucleoside- Carcinogen Reactivity
FRANK, Arthur L. Mount Sinai School of Medicine 5 R23 CA 24271-03	Mineral Fiber Size and Carcinogenicity in Vitro
GIBSON, David T. University of Texas Austin 2 RO1 CA 19078-07	Microbial Degradation of Carcinogenic Hydrocarbons
GLUSKER, Jenny P. Institute for Cancer Research 5 RO1 CA 10925-33	Application of Crystallographic Techniques
GOLD, Avram University of North Carolina Chapel Hill 5 RO1 CA 28622-02	Activation of Polycyclic Environmental Mutagens
GOLD, Barry I. University of Nebraska Medical Center 5 RO1 CA 24554-03	Epoxidation in Chloro-Olefin Carcinogenesis

GOLDMAN, Peter
Beth Israel Hospital
5 RO1 CA 15260-09

Carcinogen Metabolism By Host Intestinal Bacteria

GOULD, Michael N.
University of Wisconsin Madison
5 RO1 CA 28954-02

Carcinogen Activation By Cultured Mammary Cells

GRIBBLE, Gordon W.
Dartmouth College
5 RO1 CA 24422-03

Fluorinated Benzanthracene Synthesis and Screening

GURTOO, Hira L.
Roswell Park Memorial Institute
5 RO1 CA 25362-03

Genetics of Aflatoxin Metabolism --Role in Carcinogenesis

GUTTENPLAN, Joseph B.
New York University
5 RO1 CA 19023-06

Mechanism of Mutagenesis and Carcinogenesis

HANNA, Patrick E.
University of Minnesota of Minneapolis-St. Paul
5 RO1 CA 21659-03

Carcinogen Activation Via Acyl Transfer

HANRATTY, William P.
University of California Irvine
5 RO1 CA 24488-02

The Controlled Initiation of Neoplasms in Drosophila

HARVEY, Ronald G.
University of Chicago
5 RO1 CA 11968-09

Chemistry of Carcinogenic Hydrocarbons

HECHT, Stephen S.
American Health Foundation
1 RO1 CA 32242-01

Carcinogenic Methylated PAH: Structural Requirements

IRVING, Charles C.
University of Tennessee Center for Health Sciences
5 RO1 CA 26165-03

Conjugation Reactions in Arylamine Carcinogenesis

KAUFFMAN, Frederick C.
University of Maryland Baltimore
5 RO1 CA 20807-05

Pharmacology of Carcinogen Activation in Intact Cells

KOREEDA, Masato
University of Michigan Ann Arbor
2 RO1 CA 25185-04

The Bioorganic Chemistry of Arene Oxides

LANNACCONE, Philip M.
Nortwestern University
1 RO1 CA 29675-01

Effects of Exposure to Carcinogens on Blastocysts

LEHR, Roland E. University of Oklahoma Norman 5 RO1 CA 22985-06	Diol Epoxide/Other Derivatives of PAH & AZA-PAH: SAR'S
LEVINE, Walter G. Yeshiva University 5 RO1 CA 14231-09	Role of Metabolism in the Biliary Excretion of Drugs
LILL, Patsy H. University of South Carolina Columbia 5 RO1 CA 25361-03	Carcinogen Induced Facilitation of Tumor Growth
LIPKIN, Martin Sloan Kettering Institute for Cancer Research 1 RO1 CA 28805-01	Nitrate Metabolism in Gastrointestinal Cancer
LOEPPKY, Richard N. University of Missouri Columbia 5 RO1 CA 22289-05	Nitrosamine Fragmentation and Nitrosamine Carcinogenesis
LOEPPKY, Richard N. University of Missouri Columbia 5 RO1 CA 26914-03	Carcinogenesis: Nitrosamine Formation and Inhibition
LOTLIKAR, Prabhakar D. Temple University 5 RO1 CA 10604-14	Mechanism of Chemical Carcinogenesis
MAGEE, Peter N. Temple University 5 RO1 CA 23451-04	Formation and Metabolism of N-Nitroso Compounds
MALEJKA-GIGANTI, Danuta University of Minnesota of Minneapolis-St. Paul 5 RO1 CA 28000-02	Mammary Carcinogenesis By Arylhydroxamic Acids
MANDEL, Richard Boston University 5 RO1 CA 27324-03	Additive and Synergistic Effects of Mutagens
MARNETT, Lawrence J. Wayne State University 2 RO1 CA 22206-04A1	Studies on Malondialdehyde
MC MURTREY, Kenneth D. University of Southern Mississippi 5 RO1 CA 29903-02	Toxicology of Polynuclear Heterocyclic Carcinogens
MILLER, Richard K. University of Rochester 2 RO1 CA 22335-05	Transplacental Carcinogenesis

MIRVISH, Sidney S.
University of Nebraska Medical Center
5 RO1 CA 24776-03

Mechanism of Liver Carcinogenesis By Two Nitrosoureas

MOOLTEN, Frederick L.
Boston University
2 RO1 CA 23534-04

Protective Immunity To Chemical Carcinogens

MORRISON, Harry A.
Purdue University West Lafayette
2 RO1 CA 18267-04

Cutaneous Photobiology and Drug Phototoxicity

NAGEL, Donald L.
University of Nebraska Medical Center
1 RO1 CA 31016-01

An In Vitro Model For Alkylation By Pancreas Carcinogens

NEWMAN, Melvin S.
Ohio State University
5 RO1 CA 07394-17

Synthesis of Substituted Polycyclic Hydrocarbons

O'FLAHERTY, Ellen J.
University of Cincinnati
5 RO1 CA 29917-02

Quantitative Considerations In Urethan Carcinogenesis

PAQUETTE, Leo A.
Ohio State University
2 RO1 CA 12115-11

Unsaturated Polyolefins and Hydrocarbon Carcinogenesis

PARTHASARATHY, Rengachary
Roswell Park Memorial Institute
2 RO1 CA 23704-04A1

Stereochemistry of Thiol-Disulfide Interchanges

REICH, Edward
Rockefeller University
2 RO1 CA 08290-17

Chemotherapeutic Deoxynucleosides And Other Agents

REINKE, Lester A.
University of Oklahoma Health Sciences Center
5 RO1 CA 30137-02

Influence of Ethanol on Carcinogen Activation

RICHARDSON, Arlan G.
Illinois State University
5 RO1 CA 24856-03

Age-Related Changes In Chemical Carcinogenesis

RILEY, Edgar F.
University of Iowa
5 RO1 CA 26511-02

Assay of Tumor Induction By X-Ray and Drug Modalities

ROMAN-FRANCO, Angel A.
University of Puerto Rico Med Sciences
5 RO1 CA 28894-02

Mechanism of Action of Carcinogenic Fibers

SARDELLA, Dennis J. Boston College 5 R01 CA 23454-03	Probing Carcinogens' Active Sites By F-Substitution
SCHAAP, A. Paul Wayne State University 5 R01 CA 15874-08	Enzymatically Generated Singlet Oxygen In Carcinogenesis
SCHWARTZ, Arthur G. Temple University 5 R01 CA 14661-09	Actions of Chemical Carcinogens On Cultured Cells
SCRIBNER, John D. Pacific Northwest Research Foundation 5 R01 CA 23712-04	Early and Critical Events in Chemical Carcinogenesis
SIMENHOFF, Michael L. Thomas Jefferson University 5 R01 CA 26571-03	In Vivo Nitrosamines and Cancer in Renal Failure
SIMS, Peter University of London 5 R01 CA 21959-05	Mechanisms of Activation of Polycyclic Hydrocarbons
SINSHEIMER, Joseph E. University of Michigan Ann Arbor 5 R01 CA 25770-03	Epoxide Toxicity in Alkene Metabolism
SLAGA, Thomas J. University of Tennessee Knoxville 5 R01 CA 20076-06	Polycyclic Hydrocarbon Metabolism and Binding in Skin
SPECK, William T. Case Western Reserve University 5 R01 CA 23692-05	Potential Hazards of Phototherapy
STERNSON, Larry A. University of Kansas Lawrence 5 R01 CA 28782-02	Chemical Characterization of Arylhydroxylamines
STOMING, Terrance A. Medical College of Georgia 7 R01 CA 33586-01	Metabolism of 3-Methylcholanthrene in Liver and Lung
SUN, Albert Y. University of Missouri Columbia 5 R01 CA 26586-03	Chlorinated Water and Membrane Functions Neoplasia
SUZUKI, Yasunosuke Mount Sinai School of Medicine 5 R01 CA 24311-01	Pathogenesis of Experimental Malignant Mesothelioma

SUZUKI, Yasunosuke Mount Sinai School of Medicine 5 RO1 CA 29432-02	Carcinogenic and Fibrogenic Effects of Zeolites
TANNENBAUM, Steven R. Massachusetts Institute of Technology 5 RO1 CA 26156-03	Carcinogenic Nitrosamines From Primary Amines
TAYLOR, K. Grant University of Louisville 5 RO1 CA 22365-03	Radical Reactions of Mutagens and Carcinogens
THURMAN, Ronald G. University of North Carolina at Chapel Hill 5 RO1 CA 23080-05	Pharmacology of Carcinogen Activation in Intact Cells
UNDERWOOD, Graham R. New York University 5 RO1 CA 25073-03	Study of Ultimate Carcinogen From Aromatic Amines
VAN DUUREN, Benjamin L. New York University 5 RO1 CA 24124-03	Carcinogenic Acylating Agents and Mode of Action
VOLLHARDT, K. Peter University of California Berkeley 5 RO1 CA 20713-05	Activated Mutagenic and Aromatic Hydrocarbons
WALSH, Christopher T. Massachusetts Institute of Technology 5 RO1 CA 20574-06	Reactive Heterocycles--Cancer and Biomechanism
WEINKAM, Robert J. Purdue University West Lafayette 5 RO1 CA 28631-02	Chemotherapeutic and Carcinogenic Methlating Agents
WHALEN, Dale L. University of Maryland Baltimore 5 RO1 CA 17278-06	Kinetic Studies of Aryl Epoxide Reactions
WHEELER, Larry A. University of California Los Angeles 5 RO1 CA 22249-03	Oral Flora and Carcinogenesis of Dental Therapeutics
WONG, Lan K. University of Pittsburgh 5 R23 CA 27928-02	A Comparative Metabolism Study of PAH Analogues
YANG, NIEN-CHU C. University of Chicago 5 RO1 CA 10220-12	Molecular Mechanisms of Mutagenesis and Carcinogenesis

YANG, Shen K.
U.S. Uniformed Services University
of the Health Sciences
1 RO1 CA 29133-01A1

Metabolic Activations of
Monomethylbenz(A) Anthracenes

CHEMICAL AND PHYSICAL CARCINOGENESIS BRANCH

GRANTS ACTIVE DURING FY 82

MOLECULAR CARCINOGENESIS

Investigator/Institution/Grant Number	Title
ACS, George Mount Sinai School of Medicine 2 RO1 CA 16890-07A2	Studies on Chemotherapeutic Deoxyribonucleosides
ADAIR, Gerald M. University of Texas System Cancer Center 1 RO1 CA 28711-01A1	Expression of Genetic Variation in Cultured Cells
ANDREWS, Alan D. Columbia University 5 R23 CA 26894-03	DNA Repair Processes In Xeroderma Pigmentosum
AVADHANI, Narayan G. University of Pennsylvania 5 RO1 CA 22762-05	Cellular & Molecular Targets of Chemical Carcinogens
BANERJEE, Mihir R. University of Nebraska Lincoln 5 RO1 CA 25304-03	Chemical Carcinogenesis Mammary Gland Organ Culture
BECKER, Frederick F. University of Texas System Cancer Center 5 RO1 CA 20659-06	Analysis of Cellular Events In Chemical Carcinogenesis
BECKER, Frederick F. University of Texas System Cancer Center 5 RO1 CA 28263-02	Chromosomal Proteins in Chemical Carcinogenesis
BECKER, Frederick F. University of Texas System Cancer Center 5 RO1 CA 20657-06	Phenotypic Analysis of Chemical Carcinogenesis
BERNHARD, William A. University of Rochester 9 RO1 CA/GM 32546-07	Effects of Ionizing Radiation on Nucleic Acids
BERTRAM, John S. Roswell Park Memorial Institute 5 RO1 CA 18197-06	Mechanisms of Carcinogenesis in Cell Culture

BHANOT, Opinder S.
New York University
5 RO1 CA 16319-07

Mutagenesis and Carcinogenesis

BHARGAVA, Madhu M.
Yeshiva University
5 RO1 CA 24842-03

Ligandin: Structure, Regulation, Role in Hepatocarcinoma

BLOOM, Stephen E.
Cornell University Ithaca
5 RO1 CA 28953-02

Chick Embryos For Detecting Environmental Mutagens

BLUMER, Jeffrey L.
Case Western Reserve University
1 R23 CA 30067-01

Lymphocyte Carcinogen Metabolism In Acute Leukemia

BOUCK, Noel P.
Northwestern University
5 RO1 CA 27306-03

Genetic Analysis of Malignant Transformation

BOWDEN, George T.
University of Arizona
5 RO1 CA 26972-03

Postreplication Repair in Cultured Mammalian Cells

BOX, Harold C.
Roswell Park Memorial Institute
5 RO1 CA 29425-02

Molecular Studies of Carcinogenesis and Mutagenesis

BOYNTON, Alton L.
National Research Council of Canada
5 RO1 CA 28340-02

Assays For, And Actions Of, Carcinogens and Promoters

BRANSCOMB, Elbert W.
University of California Livermore
1 RO1 CA 31714-01

Monitoring In Vivo Somatic Mutations in Animals and Man

BRESNICK, Edward
University of Vermont &
St. Agric College
5 RO1 CA 23514-05

DNA Repair After Polycyclic Hydrocarbon Administration

BROOKES, Peter
University of London
5 RO1 CA 25807-03

Biological Result of Carcinogen Induced Damage to DNA

BROYDE, Suse B.
New York University
5 RO1 CA 28038-02

Carcinogen-DNA Adducts: Linkage Site and Conformation

CHEN, Fu-Ming
Tennessee State University
1 RO1 CA 29817-01

Binding of Benzo(a)Pyrene Metabolites to DNA

CHEN, Lan B. Sidney Farber Cancer Institute 1 RO1 CA 29793-01	Chemical Carcinogenesis Of Epithelial Cells
CLARKE, Richard H. Boston University 5 RO1 CA 17922-05	Carcinogen-DNA Complexes: Structure and Interactions
CLARKSON, Judith M. University of Texas System Cancer Center 5 RO1 CA 19281-06	Cell Cycle Related DNA Repair Mechanisms
CORBETT, Michael D. University of Florida 7 RO1 CA 32385-01	Hydroxamic Acid Production By Marine Organisms
COVEY, Douglas F. Washington University 5 RO1 CA 23582-04	Suicide Substrates: Cancer and Endocrine Applications
COX, Ray University of Tennessee Center for Health Sciences 5 RO1 CA 15189-09	DNA Repair and Chemical Carcinogenesis
DAVIDSON, Richard L. University of Illinois Medical Center 5 RO1 CA 31781-02	Chemical Mutagenesis Mechanisms In Mammalian Cells
DI MAYORCA, Giampiero University of Medicine & Dentistry of NJ 5 RO1 CA 25013-04	Molecular Mechanism of Chemical Carcinogenesis
DIEBOLD, Gerald J. Brown University 1 RO1 CA 29912-01	Optoacoustic Detection of Carcinogens
DIXON, Kathleen University of California Los Angeles 5 RO1 CA 28449-02	Probing DNA Repair With SV40 Virus and Mutant Cells
DUKER, Nahum J. Temple University 5 RO1 CA 24103-03	Pathology of Repair of Carcinogenic DNA Damage
EISENSTADT, Eric Harvard University 5 RO1 CA 26135-03	Mammary and Liver Prolactin Receptors

EVANS, Helen H. Case Western Reserve University 5 RO1 CA 23427-03	Radiation Induced Mutagenesis and Carcinogenesis
FAHL, William E. Northwestern University 5 RO1 CA 25189-03	Hydrocarbon Carcinogenesis In Mouse and Human Cells
FARBER, Emmanuel University of Toronto 5 RO1 CA 25094-03	A Short Term In Vivo Assay For Carcinogens
FARBER, Emmanuel University of Toronto 5 RO1 CA 21157-06	Pathogenesis of Liver Cancer Induced By Chemicals
FARBER, John L. Hahnemann Medical College & Hospital of Philadelphia 7 RO1 CA 32610-01	Hepatocarcinogenesis: A Role For Liver Necrosis
FELDBERG, Ross S. Tufts University 5 RO1 CA 19419-06	Nature and Repair Of A New Form of DNA Damage
FIALA, Silvio E. Shepherd College 5 RO1 CA 14084-09	The Role of Carcinogen In Nucleic Acid Metabolism
FINK, Gerald R. Cornell University Ithaca 5 RO1 CA 23441-05	Chemical Carcinogens and Frameshift Mutation in Yeast
FRAENKEL-CONRAT, Bea University of California Berkeley 2 RO1 CA 12316-12	Alkylation of Polynucleotides In Vitro and In Vivo
FRANKLIN, Michael R. University of Utah 5 RO1 CA 15760-08	Modification of Procarcinogen Enzymatic Activation
FREEDMAN, Herbert A. Downstate Medical Center 5 RO1 CA 29052-02	H-2 Locus and Local Tumorigenesis By Methylcholanthrene
FRIEDBERG, Errol C. Stanford University 5 RO1 CA 12428-11	DNA Repair and Its Relationship To Carcinogenesis
GEACINTOV, Nicholas E. New York University 5 RO1 CA 20851-05	Characterization of Carcinogen-Nucleic Acid Complexes

GESSNER, Teresa Roswell Park Memorial Institute 5 RO1 CA 24127-04	Conjugations and Carcinogen Metabolism
GOLD, Barry I. University of Nebraska Medical Center 1 RO1 CA 29088-01A1	Activation and Transportation of Nitrosamines
GOLDFARB, Stanley University of Wisconsin Madison 2 RO1 CA 15664-07A1	Cholesterol Metabolism Of Hepatic Neoplasms
GOLDTHWAIT, David A. Case Western Reserve University 5 RO1 CA 18747-05	Repair of X-Irradiated DNA In Normal and Cancer Cells
GOLDTHWAIT, David A. Case Western Reserve University 5 RO1 CA 27528-02	Chemical Carcinogenesis and DNA Repair
GOODMAN, Jay I. Michigan State University 1 RO1 CA 30635-01	Genetic Toxicology--The Role of Non-Random Gene Damage
GREENBERGER, Joel S. Sidney Farber Cancer Institute 2 RO1 CA 25412-04	Stem Cell Age and X-Ray/ Chemotherapy Leukemogenesis
GRIFFIN, Martin J. Oklahoma Medical Research Foundation 5 RO1 CA 24459-03	Role of Epoxide Hydrase In Chemical Carcinogenesis
GRISHAM, Joe W. University of North Carolina Chapel Hill 1 RO1 CA 32036-01	DNA Methyl Adducts: Toxicity, Mutation, & Transformation
GRISHAM, Joe W. University of North Carolina Chapel Hill 5 RO1 CA 29323-02	Analysis of Tumor Progression In Liver Cells In Vitro
GUDAS, Lorraine J. Sidney Farber Cancer Institute 5 RO1 CA 27953-02	Genetics/DNA Precursor Metabolism, Mutagenesis, Repair
GUPTA, Ramesh C. Baylor College of Medicine 1 RO1 CA 30606-01	Reaction of Carcinogenic Aromatic Amines With DNA
HANKINSON, Oliver University of California Los Angeles 2 RO1 CA 28868-03	Carcinogen Activation And Screening in Variant Cells

HANNA, Patrick E. University of Minnesota of Minneapolis-St. Paul 5 RO1 CA 24427-03	Bioactivation of Arylhydroxamic Acids: SAR Studies
HARD, Gordon C. Temple University 5 RO1 CA 24216-03	Experimental Pathology of Renal Carcinogenesis
HARRINGTON, George W. Temple University 5 RO1 CA 18618-07	Electroanalytical Studies of N-Nitrosamines
HARTMAN, Philip E. Johns Hopkins University 5 RO1 CA 26328-03	Detection Systems for Mutagens & Carcinogens
HASELTINE, William A. Sidney Farber Cancer Institute 5 RO1 CA 29240-02	Complementation Group A Locus of Xeroderma Pigmentosum
HASELTINE, William A. Sidney Farber Cancer Institute 5 RO1 CA 26716-03	DNA Damage/Repair By Environmental Carcinogens/Mutagens
HECHT, Stephen S. American Health Foundation 2 RO1 CA 23901-04	Environmental Nitrosamines--Metabolism & Carcinogenesis
HENDERSON, Earl E. Temple University 5 RO1 CA 23999-03	Characterization of Unique Lymphoblastoid Cell Lines
HERRIOTT, Roger M. Johns Hopkins University 5 RO1 CA 25167-03	Pyrolytic Products of Proteinous Foods as Mutagens
HITTELMAN, Walter N. University of Texas System Cancer Center 5 RO1 CA 27931-02	Molecular Basis of Chromosome Aberrations
HNILICA, Lubomir S. Vanderbilt University 5 RO1 CA 26412-04	Experimental Hepatocarcinogenesis
HOLLENBERG, Paul F. Northwestern University 2 RO1 CA 16954-06	Hemoprotein-Catalyzed Oxygenations of Carcinogens
HOLOUBEK, Viktor University of Texas Medical Branch Galveston 5 RO1 CA 22559-03	Interference of Azocarcinogens With RNA Processing

HOWARD FLANDERS, Paul Yale University 5 RO1 CA 26763-03	Excision Enzymes and The Repair of Damaged DNA
HUMAYUN, M. Zafri University of Medicine & Dentistry of New Jersey 5 RO1 CA 27735-03	Mutagenesis By Carcinogens: A Molecular Approach
HURWITZ, Jerard Yeshiva University 5 RO1 CA 21622-05	Carcinogens on Pro- And Eucaryotic DNA Replication
HYLEMON, Phillip B. Virginia Commonwealth University 2 RO1 CA 17747-07	Bile Acids and Large Bowel Carcinogenesis
JACOBS, Lois J. University of Wisconsin Madison 5 RO1 CA 30450-02	Quantitative Mutagenesis Studies In Human Fibroblasts
JACOBSON, Myron K. North Texas State University 2 RO1 CA 23994-05	Alteration of NAD Metabolism By Chemical Carcinogens
JACOBSON, Myron K. North Texas State University 5 RO1 CA 29357-02	Poly(ADP-Ribose) Metabolism In Xeroderma Pigmentosum
JEFCOATE, Colin R. University of Wisconsin Madison 5 RO1 CA 16265-08	DNA Modification By Polycyclic Hydrocarbons
JENSEN, David E. Temple University 1 RO1 CA 31503-01	Nitrosocimetidine--DNA Methylation & Cellular Response
JENSEN, Ronald H. University of California Livermore 1 RO1 CA 31549-01	Detection of Somatic Cell Mutations in Humans
JIRTLE, Randy L. Duke University 5 RO1 CA 25951-03	Survival and Carcinogenesis In Transplanted Hepatocytes
KALLENBACH, Neville R. University of Pennsylvania 2 RO1 CA 24101-04	Specificity in Frameshift Mutagenesis
KALLENBACH, Neville R. University of Pennsylvania 5 RO1 CA 31027-02	Frameshift Mutagenesis By Covalently Reacting Mutagens

KAN, Lou-Sing Johns Hopkins University 5 RO1 CA 27111-03	Model Alkylated Decanucleotide DNA Helices
KAUFFMAN, Shirley L. Downstate Medical Center 2 RO1 CA 17569-07	Lung Preneoplastic Hyperplasia and Chemical Carcinogens
KAUFMAN, David G. University of North Carolina Chapel Hill 5 RO1 CA 20658-06	Chemical Carcinogenesis And Cell Proliferation
KAUFMAN, David G. University of North Carolina Chapel Hill 1 RO1 CA 32238-01	Factors Influencing Initiation of Hepatocarcinogenesis
KENNEDY, Ann R. Harvard University 5 RO1 CA 22704-04	Radiation and Chemical In Vitro Malignant Transformation
KIM, Sung-Hou University of California Berkeley 5 RO1 CA 27454-03	Crystalline Complexes of RNA With Small Molecules
KIMBALL, Paul C. Ohio State University 7 RO1 CA 33554-01	Chemical Cocarcinogenesis In The Rat: Gene Activation
KING, Charles M. Michigan Cancer Foundation 5 RO1 CA 23386-05	Mechanistic Approaches To Carcinogenesis
KOESTNER, Adalbert Michigan State University 7 RO1 CA 32594-01	Neurooncogenesis By Resorptive Carcinogens
KOHEN, Elli Papanicolaou Cancer Research Institute 2 RO1 CA 21153-04	Intracellular Enzyme Kinetics and Carcinogens
KULESZ-MARTIN, Molly Roswell Park Memorial Institute 1 RO1 CA 31101-01	Quantitative Carcinogenesis In Cultured Epithelial Cells
LAISHES, Brian A. University of Wisconsin Madison 2 RO1 CA 24818-04	Proliferation Control During Hepatocarcinogenesis
LAPEYRE, Jean-Numa University of Texas System Cancer Center 1 RO1 CA 31487-01	Regulation and Enzymology of DNA Methylase in Cancer

LARCOM, Lyndon L. Clemson University 5 RO1 CA 21479-05	Biological Effects of DNA-Protein Crosslinks
LIEBERMAN, Michael W. Washington University 1 RO1 CA 31734-01	Methylation of DNA During Repair of Carcinogen Damage
LIEBERMAN, Michael W. Washington University 5 RO1 CA 20513-06	Chemical Carcinogen-Induced DNA Repair In Human Cells
LIEHR, Joachim G. University of Texas Health Science Center Houston 5 RO1 CA 27539-03	Mechanism of Estrogen-Induced Renal Carcinogenesis
LIPSKY, Michael M. University of Maryland at Baltimore 5 RO1 CA 28951-02	Multi-Stage Renal Carcinogenesis In Rats
LITMAN, Gary W. Sloan Kettering Institute for Cancer Research 5 RO1 CA 24861-03	Interaction of Benzoapyrene With Nuclear Proteins
LOMBARDI, Benito University of Pittsburgh 5 RO1 CA 23449-05	Choline Deficiency, Oval Cells and Hepatocarcinogenesis
LONGNECKER, Daniel S. Dartmouth College 5 RO1 CA 17843-06	Studies of DNA Change Induced By Pancreatic Carcinogens
LOTLIKAR, Prabhakar D. Temple University 1 RO1 CA 31641-01	Modulation of Mycotoxin Carcinogenesis By Glutathione
LOWE, Nicholas J. University of California Los Angeles 5 RO1 CA 25970-03	UV Light & Epidermal Polyamine & DNA Synthesis
MACMANUS, John P. National Research Council of Canada 1 RO1 CA 31898-01	Incidence and Quantitation of a Tumour Protein
MAHER, Veronica M. Michigan State University 5 RO1 CA 21247-06	Role of Mutagenesis In Chemical Carcinogenesis
MAHER, Veronica M. Michigan State University 5 RO1 CA 21253-05	Interaction of Carcinogens With DNA--Repair of Lesions

MANGEL, Walter F. University of Illinois Urbana-Champaign 5 RO1 CA 25633-03	A New Assay For Transformed Cells
MARCHOK, Ann C. Oak Ridge National Laboratory 1 RO1 CA 30529-01	Preneoplastic Markers In Specific Lesion Cells
MCCORMICK, J. Justin Michigan State University 2 RO1 CA 21289-04A1	In Vitro Transformation of Human Cells By Carcinogens
MEEHAN, Thomas D. Michigan Molecular Institute 5 RO1 CA 25106-03	Specificity In BAP Diol Epoxide Covalent Binding To DNA
MILO, George E. Ohio State University 5 RO1 CA 25907-03	Chemical Carcinogen Induced Neoplastic Transformation
MULLINS, Dail W. University of Alabama in Birmingham 1 RO1 CA 30547-01	The Role of Poly (ADP-Ribose) Polymerase in DNA Repair
MURRAY, Michael L. Louisiana State University Medical Center New Orleans 5 RO1 CA 26355-02	Mediation of Nitrous Acid Mutagenesis By Polyamines
NAKANISHI, Koji Columbia University 5 RO1 CA 11572-13	Structural and Bioorganic Studies of Bioactive Compounds
OHLSSON-WILHELM, Betsy M. University of Rochester 5 RO1 CA 25731-03	Radiation Sensitive Haploid Frog Cells
OLIVE, Peggy L. Johns Hopkins University 5 RO1 CA 28793-02	Mutagenicity and DNA Damage Using Spheroids
OLSON, Jack W. University of Kentucky 1 RO1 CA 31099-01	Hepatocarcinogenesis And Ornithine Decarboxylase
OLSON, Wilma K. Rutgers The State University New Brunswick 5 RO1 CA 25981-03	Carcinogenesis By Hydrocarbons: A Molecular Approach

PEGG, Anthony E. Pennsylvania State University Hershey Medical Center 5 RO1 CA 18137-07	Persistence of Alklylated DNA in Carcinogenesis
PIETTE, Lawrence H. University of Hawaii at Manoa 5 RO1 CA 10977-17	ESR Studies of Biological Free Radical Mechanisms
PLANCK, Stephen R. University of Arizona 1 R23 CA 30466-01	Enzymology of Mammalian DNA Replication and Repair
PRAKASH, Satya University of Rochester 1 RO1 CA 32514-01	Repair of DNA Damaged By Psoralen +360 NM Irradiation
RANDERATH, Kurt Baylor College of Medicine 5 RO1 CA 25590-03	Effects of Carcinogens On Nucleolar DNA
RANDERATH, Kurt Baylor College of Medicine 1 RO1 CA 32157-01	32P-Labeling Test For Nucleic Acid Damage By Carcinogens
RICH, Alexander Massachusetts Institute of Technology 1 RO1 CA 29753-01	Chemical Carcinogenesis And DNA Structure
ROGAN, Eleanor G. University of Nebraska Medical Center 5 RO1 CA 25176-03	Binding of Aromatic Hydrocarbons to Nucleic Acids
ROSSMAN, Toby G. New York University 5 RO1 CA 29258-02	Mutagenesis By Metals Of Environmental Significance
SARMA, D. S. University of Toronto 2 RO1 CA 23958-04	DNA Repair/Replication In Chemical Carcinogenesis
SCHENDEL, Paul F. University of Connecticut Storrs 1 RO1 CA 32182-01	Mismatch Repair In Mutagenesis By Alkylating Carcinogens
SCHROEDER, Alice L. Washington State University 5 RO1 CA 26314-03	Post-Replication-Repair of DNA In Neurospora
SEDWICK, W. David Duke University 1 RO1 CA 31110-01	Antifolate-Induced Misincorporation Of UDR In Human Cell

SELL, Stewart
University of California San Diego
5 R01 CA 29368-02

Radioimmunoassay of
Alphafetoprotein

SHIM, Sang C.
Korea Advanced Institute of Science
2 R01 CA 21729-04A1

Photochemistry of
5,M-Dimethoxycoumarin

SICILIANO, Michael J.
University of Texas System
Cancer Center
5 R01 CA 28909-02

Genetics of Chemical
Carcinogenesis In Fish

SINCLAIR, Peter R.
Dartmouth College
5 R01 CA 25012-04

Liver Cell Culture For
Study of Carcinogen Activation

SIRICA, Alphonse E.
University of Wisconsin Madison
1 R01 CA 30102-01

Hepatic Oval Cells In Culture
And In Vivo

SIRICA, Alphonse E.
University of Wisconsin Madison
5 R23 CA 29401-02

Isolation Of "Preneoplastic"
Cell Populations

SIROVER, Michael A.
Temple University
5 R01 29414-02

Regulation of DNA Repair In
Chemical Carcinogenesis

SMUCKLER, Edward A.
University of California San Francisco
5 R01 CA 21141-06

Pathology of Chemical
Carcinogenesis

SMULSON, Mark E.
Georgetown University
5 R01 CA 25344-03

Carcinogens and Chromatin
Structure and Function

SOLT, Dennis B.
Harvard University
5 R01 CA 28620-02

Sequential Analysis Of
Oral Carcinogenesis

SONG, Pill-Soon
Texas Tech University
5 R01 CA 13598-09

Skin-Sensitizing & Carcinogenic
Furocoumarins

SOROF, Sam
Institute for Cancer Research
5 R01 CA 05945-19

Macromolecules In Chemical
Carcinogenesis

STOHRER, Gerhard
Sloan Kettering Institute for
Cancer Research
5 R01 CA 22458-03

Metabolic and Chemical Studies
of Carcinogenesis

TEEBOR, George W. New York University 2 RO1 CA 16669-07A1	Repair Of Radiation-Induced Carcinogenic Damage To DNA
TESSMAN, Irwin Purdue University West Lafayette 5 RO1 CA 22239-05	Effect of Ultraviolet Light on Cellular Processes
TOPAL, Michael D. University of North Carolina Chapel Hill 5 RO1 CA 28632-02	Effects of Carcinogen Modification of DNA Precursors
WALBORG, Earl F., Jr. University of Texas System Cancer Center 5 RO1 CA 27377-02	Membrane Glycoproteins During Hepatocarcinogenesis
WALKER, Graham C. Massachusetts Institute of Technology 5 RO1 CA 21615-06	Mutagenesis and Repair of DNA
WALLACE, Susan S. New York Medical College 5 RO1 CA 24953-03	Radiation Repair in Drosophila Melanogaster
WEBBER, Mukta M. University of Colorado Health Sciences Center 5 RO1 CA 28279-02	Human Prostatic Growth Regulation and Carcinogenesis
WHALEN, Dale L. University of California Los Angeles 5 RO1 CA 26086-03	Oral Flora and Carcinogenesis of Dental Therapeutics
WHITLOCK, James P., Jr. Stanford University 5 RO1 CA 24580-03	Chemical Carcinogens and Their Cellular Receptors
YAGER, James D., Jr. New York University 7 RO1 CA 32175-01	Error-Prone DNA Repair In Hepatocarcinogenesis
YANG, Chung S. University of Medicine & Dentistry of New Jersey 5 RO1 CA 16788-08	Monoxygenase: Properties and Carcinogen Activation
YU, Fu-Li Rockford School of Medicine 5 RO1 CA 30093-02	Aflatoxin B1 and Nucleolar RNA Synthesis

CHEMICAL AND PHYSICAL CARCINOGENESIS BRANCH

GRANTS ACTIVE DURING FY 82

SPECIAL PROJECTS

Investigator/Institution/Grant Number	Title
ALBERT, Roy E. New York University 5 PO1 CA 26724-03	Inhalation Carcinogenesis of Environmental Agents
ALBERTINI, Richard J. University of Vermont & St. Agric College 1 RO1 CA 30688-01	Direct Mutagenicity Testing In Man
BAIRD, William M. Purdue University West Lafayette 1 PO1 CA 30234-01	Molecular Mechanisms of Carcinogen-DNA Interactions
BANERJEE, Mihir R. University of Nebraska Lincoln 5 RO1 CA 11058-12	Chemical Carcinogenesis Mammary Gland Organ Culture
BARKA, Tibor D. Mount Sinai School of Medicine 5 RO1 CA 24023-03	Tumor Promoters, Growth and Differentiation
BAXTER, C. Stuart University of Cincinnati 5 RO1 CA 24022-03	Cell Culture Studies of Environmental Promoters
BELMAN, Sidney New York University 5 RO1 CA 18536-06	Role of Cyclic Nucleotides in Tumor Promotion
BENFIELD, John R. City of Hope National Medical Center 5 RO1 CA 28045-02	Esophageal and Pancreatic Carcinogenesis
BENFIELD, John R. City of Hope National Medical Center 5 RO1 CA 29373-02	Model of Bronchogenic Lung Cancer
BERENBLUM, Isaac Weizmann Institute of Science 5 RO1 CA 21088-05	New Approaches to Systemic Two-Stage Carcinogenesis
BLOCK, Ronald E. Papanicolaou Cancer Research Institute 5 RO1 CA 13056-07	Characterization of Biological Tissues By NMR

BLUMBERG, Peter M.
Harvard University
2 RO1 CA 22895-04

Mechanism of Action Of Phorbol Diesters In Vitro

BOKKENHEUSER, Victor D.
St. Luke's-Roosevelt Institute for Health Science
2 RO1 CA 25763-07

Bacteria and Steroid Metabolism

BRANSCOMB, Elbert W.
University of California Berkeley
1 RO1 CA 30613-01

Somatic Point Mutation Monitoring

BRASITUS, Thomas A.
Columbia University
5 RO1 CA 28040-02

Colonic Epithelial Cell Plasma Membranes

BRESNICK, Edward
University of Vermont & St. Agric College
5 RO1 CA 20711-06

Polycyclic Hydrocarbon Metabolism and Carcinogenesis

BROWN, Clark E.
New England Deaconess Hospital
5 RO1 CA 24893-03

Cancer of Rat Prostate: Its Induction and Prevention

BROWN, Nigel A.
George Washington University
1 RO1 CA 32306-01

Effects of Tumor Promoters on Mammalian Embryogenesis

BRUEGGEMEIER, Robert W.
Ohio State University
5 RO1 CA 28578-02

Biotransformation of Estrogens and Cancer

COSTLOW, Mark E.
St. Jude Children's Research Hospital
5 RO1 CA 23956-03

Hormone Receptor Regulation In Mammary Tissue

COSTLOW, Mark E.
St. Jude Children's Research Hospital
5 RO1 CA 25170-03

Prolactin Receptor Regulation In Cultured Mammary Cells

CURPHEY, Thomas J.
Dartmouth College
1 RO1 CA 30650-01

Pancreas & Liver Carcinogen Metabolism In Three Species

DIAMOND, Leila
Wistar Institute of Anatomy and Biology
1 RO1 CA 30446-01

Hydrocarbon Activation By Cells

DIAMOND, Leila
Gordon Research Conferences
1 R13 CA 31175-01

Gordon Research Conference on Cancer, 1981

DIAMOND, Leila Wistar Institute of Anatomy and Biology 2 RO1 CA 23413-04A1	Tumor Promoter and Cell Differentiation
DIAMOND, Leila Wistar Institute of Anatomy and Biology 5 PO1 CA 21778-03	Environmental Factors In The Induction of Cancer
EBNER, Kurt E. University of Kansas College of Health Science and Hospital 5 RO1 CA 17478-06	Mammary and Liver Prolactin Receptors
EPSTEIN, Samuel S. University of Illinois Medical Center 5 RO1 CA 24959-03	Quantitative Carcinogenesis Projection Between Species
ESTENSEN, Richard D. University of Minnesota of Minneapolis-St. Paul 5 RO1 CA 22195-05	PMA--A Cocarcinogen As A Lymphocyte Mitogen
FIALA, Emerich S. American Health Foundation 1 RO1 CA 31012-01	Disposition of Hydrazines: Species and Strain Effects
FISHMAN, Jack Rockefeller University 5 PO1 CA 22795-05	Specialized Center For Cancer Endocrinology
FRANKEL, Fred R. University of Pennsylvania 5 RO1 CA 17301-07	Mammary Cancer And The Nuclear Estradiol Receptor
FRANTZ, Andrew G. Columbia University 5 RO1 CA 11704-11	Prolactin and Other Peptides
FREEMAN, Aaron E. Center for Neurologic Study 5 RO1 CA 30220-02	Organoid In Vitro Model Of Liver Carcinogenesis
GARTE, Seymour J. New York University 5 R23 CA 23806-03	Biochemical Mechanisms of Tumor Promoters
GORSKI, Jack University of Wisconsin Madison 5 RO1 CA 18110-07	Prolactin in Normal and Neoplastic Pituitary Tissue
GOULD, Michael N. University of Wisconsin Madison 1 RO1 CA 30295-01	Human Vs. Rodent Mammary Mediated Mutagenesis Assay

GRANDJEAN, Carter J. Midwest Research Institute 5 RO1 CA 27934-03	Diallylnitrosamine Carcinogenesis: Species Differences
GRIFFITH, O Hayes University of Oregon 2 RO1 CA 11695-13	Photoelectron Microscopy of Cell Membranes
GUENGERICH, F. Peter Vanderbilt University 5 RO1 CA 30907-02	Purified Human Enzymes And Carcinogen Metabolism
GURPIDE, Erlio Mount Sinai School of Medicine 5 RO1 CA 15648-09	Steroid Dynamics In Human Endometrial Cancer
HAM, Richard G. University of Colorado at Boulder 5 RO1 CA 30028-02	Defined Medium For Human Mammary Epithelial Cells
HICKS, Ruth M. University of London 1 RO1 CA 31082-01	Carcinogenesis in Human and Rat Bladder Tissues
HILF, Russell University of Rochester Medical Center 5 RO1 CA 16660-06	Role of Insulin In Hormonal Control of Breast Cancer
HILL, Donald L. Southern Research Institute 1 RO1 CA 30296-01	Carcinogen Metabolism In Sensitive and Resistant Species
HOLLANDER, Vincent P. Hospital for Joint Diseases Ortho Inst. 7 RO1 CA 32581-01	Hormonally Sensitive Tumors
HOLLANDER, Vincent P. Hospital for Joint Diseases Ortho Inst. 7 RO1 CA 32631-01	Endocrine Factors In The Development of Plasmacytoma
HOMBURGER, Freddy Bio-Research Institute 5 RO1 CA 24696-03	Syrian Hamster Model of Pancreatic Carcinogenesis
HUGGINS, Charles B. University of Chicago 5 RO1 CA 11603-12	Endocrinology of Experimental Leukemia
JENSEN, Elwood V. University of Chicago 5 RO1 CA 02897-26	Steroids and Growth

KAIGHN, M. Edward
Pasadena Foundation for Medical Research
5 RO1 CA 25089-03

Culture and Carcinogenesis of Human Bladder Urothelium

KAUFMAN, David G.
University of North Carolina
Chapel Hill
1 RO1 CA 31733-01

Promotion of Chemical Carcinogenesis in Uterine Tissue

KAUFMAN, David G.
University of North Carolina
Chapel Hill
1 RO1 CA 32239-01

Factors Influencing Initiation of Hepatocarcinogenesis

KERR, Sylvia J.
University of Colorado Health
Sciences Center
5 RO1 CA 12742-10

Study of Methylations In Neoplasia

KLEIN-SZANTO, Andres J.
Oak Ridge National Laboratory
1 RO1 CA 29556-01

Importance of Dark Cells In Skin Carcinogenesis

LEHRER, Robert I.
University of California Los Angeles
1 RO1 CA 30526-01

Blood Cell Receptors For Tumor-Promoting Phorbol Esters

LEUNG, Benjamin S.
University of Minnesota of
Minneapolis-St. Paul
5 RO1 CA 25998-03

Hormonal Interaction In Mammary Carcinoma

LI, Jonathan J.
University of Minnesota of
Minneapolis-St. Paul
5 RO1 CA 22008-05

Estrogen Carcinogenicity And Hormone Dependent Tumors

LILLY, Frank
Albert Einstein College of Medicine
1 PO1 CA 31855-01

Mechanisms of Chemical Lymphomagenesis

LINDAHL, Ronald G.
University of Alabama
In University
5 RO1 CA 21103-03

Gene-Enzyme Relationship Of Liver Aldehyde Dehydrogenase

LING, Gilbert N.
Pennsylvania Hospital
5 RO1 CA 16301-07

Water In Cancer And In Normal Tissues

MAGUN, Bruce E.
University of Arizona
5 RO1 CA 29290-02

Mechanisms of Tumor Promotion In Vivo And In Vitro

MARKLAND, Francis S., Jr.
University of Southern California
5 RO1 CA 22910-03

Characterization of Mammary Glucocorticoid Receptor

MC ELHENY, Victor K.
Cold Spring Harbor Laboratory
1 R13 CA 31384-01

The Possible Role of Nitrosamines In Human Cancer

MC GRATH, Charles M.
Michigan Cancer Foundation
5 RO1 CA 25482-03

Hormonal Control of Metastasis

MC GUIRE, William L.
University of Texas Health Science Center San Antonio
5 RO1 CA 11378-13

Mechanism of Hormonal Control Of Mammary Carcinoma

MEITES, Joseph
Michigan State University
5 RO1 CA 10771-15

Neuroendocrine Control of Mammary And Pituitary Tumors

MENDELSOHN, Naomi
Mount Sinai School of Medicine
5 R23 CA 27154-03

Maturation-Dependent Responses Of Myeloid Cells

MICHALOPOULOS, George K.
Duke University
1 RO1 CA 30241-01

Cell Culture And Transplantation Of Human Hepatocytes

MILLER, James A.
University of Wisconsin Madison
5 PO1 CA 22484-04

Biochemical Studies In Chemical Carcinogenesis

MILLER, Jon P.
SRI International
5 RO1 CA 24588-02

Effects of Tumor Promoters On Protein Kinases

MIRVISH, Sidney S.
University of Nebraska Medical Center
5 PO1 CA 25100-03

N-Nitroso Compounds

OFNER, Peter
Tufts University
5 RO1 CA 15776-06

Prostatic Differentiation And Sex Hormone Metabolism

OFNER, Peter
Tufts University
5 RO1 CA 29513-02

Androgens In Prostatic And Epididymal Culture

PARDEE, Arthur B.
Sidney Farber Cancer Institute
5 PO1 CA 22427-04

Molecular Analysis of Malignant Transformation

PARSA, Ismail Downstate Medical Center 1 RO1 CA 30354-01	Interspecies Comparisons Of Pancreas Carcinogenesis
PARSONS, Donald F. New York State Department of Health 5 RO1 CA 29255-02	Squamous Cell Carcinoma-- Invasion Mechanisms
PURDY, Robert H. Southwest Foundation For Research and Education 2 RO1 CA 24629-04	Mutagenic and Carcinogenic Potential Of Estrogens
RIVERA, Evelyn M. Michigan State University 5 RO1 CA 17862-06	The Biology Of Rat Mammary Hyperplasias
ROSEN, Jeffrey M. Baylor College of Medicine 5 RO1 CA 16303-07	Hormonal Regulation of Breast Cancer
SAXENA, Brij B. Cornell University Medical Center 5 RO1 CA 13908-06	Gonadotropin Receptors
SCHECHTER, Joel E. University of Southern California 5 RO1 CA 21426-05	Rathke's Pouch-Derived Tumors: Effects of Hormones
SCHUT, Herman A. Medical College of Ohio at Toledo 1 RO1 CA 30514-01	In Vitro Carcinogenesis Studies In Colon and Esophagus
SCOTT, Robert E. Mayo Foundation 5 RO1 CA 21722-05	Membrane Pathology In Carcinogenesis
SELKIRK, James K. Oak Ridge National Laboratory 1 RO1 CA 30355-01	Comparative Dynamics Of Benzo(a)pyrene Metabolism
SINGER, Bea A. Gordon Research Conference 1 R13 CA 32017-01	Gordon Research Conference On Chemical and Biochemical Mechanisms On Mutagenesis
SIPERSTEIN, Marvin D. University of California San Francisco 5 RO1 CA 15979-08	Cholesterol Metabolism In Normal And Malignant Liver
STONER, Gary D. Medical College of Ohio at Toledo 1 RO1 CA 30133-01	Carcinogenesis Studies In The Human Bronchus

STONER, Gary D. Medical College Of Ohio At Toledo 5 R01 CA 28950-02	Carcinogenesis Studies In Cultured Rat Esophagus
STUART, Robert K. Johns Hopkins University 1 R01 CA 30491-01	Tumor Promoters And Regulation Of Hematopoiesis
TANG, Frank Y. University of Rochester 5 R01 CA 25455-03	Regulation Of Mammary Gland Growth And Regression
TANNENBAUM, Steven R. Massachusetts Institute of Technology 5 P01 CA 26731-03	Endogenous Nitrite Carcinogenesis In Man
TROSKO, James E. Michigan State University 5 R01 CA 21104-05	Mutation And Derepression Of Genes In Carcinogenesis
TS'O, Paul O. Johns Hopkins University 5 P01 CA 16043-06	Biomedical Risks Caused By Nucleic Acid Perturbation
VESSELINOVITCH, Stan D. University of Chicago 2 R01 CA 25522-04	Role of Sex Hormones In Hepatocarcinogenesis
VESSELINOVITCH, Stan D. University of Chicago 5 R01 CA 25549-03	Synthetic Steroids And Hepatocarcinogenesis
VILLEE, Claude A. Harvard University 5 R01 CA 24615-03	Hormone Induced And Dependent Tumors
WALKER, Bruce E. Michigan State University 5 R01 CA 27535-03	Tertogenicity of Transplacental DES In Mice
WEBER, George Indiana Univ-Purdue Univ At Indianapolis 5 P01 CA 13526-10	Correlated Study of Metabolic Regulation In Neoplasia
WEINSTEIN, I. Bernard Columbia University 2 P01 CA 21111-05	Molecular Events In Chemical Carcinogenesis
WEINSTEIN, I. Bernard Columbia University 5 R01 CA 26056-03	Cellular And Biochemical Effects Of Tumor Promoters

WEISBURGER, John H. American Health Foundation 1 RO1 CA 30658-01	Strain Differences In Carcinogenesis
WENDER, Paul A. Stanford University 7 RO1 CA 31841-01	Synthetic Studies On Tumor Promoters And Inhibitors
WENNER, Charles E. Roswell Park Memorial Institute 5 RO1 CA 13784-09	The Effect Of Cocarcinogens On Cellular Membranes
WILLIAMS, Jerry R. George Washington University 1 RO1 CA 31015-01	Mechanisms Of Procarcinogenic Metabolism In Rat And Man
WOLF, George D. Federation of American Societies For Experimental Biology 1 R13 CA 31915-01	Conference On Micronutrients: Vitamin A And Retinoids
WOTIZ, Herbert H. Boston University 1 PO1 CA 28856-01	The Role Of Hormones And Binding Proteins In Cancer
WYNDER, Ernst L. American Health Foundation 5 PO1 CA 12376-09	Environmental Carcinogenesis

SUMMARY REPORT

SPECIAL PROGRAMS BRANCH

The Special Programs Branch (1) plans, develops, directs and manages a national extramural program of basic and applied research in the special emphasis areas of biometry, diet/nutrition, epidemiology, and preventive oncology as well as smoking and health; (2) establishes program priorities and evaluates program effectiveness, (3) provides a broad spectrum of information, advice and consultation to individual scientists and institutional science management officials relative to NIH and NCI funding as well as scientific review policies and procedures, preparation of grant applications, and choice of funding instruments; (4) provides NCI management with recommendations regarding funding needs, priorities and strategies for the support of relevant research areas consistent with the current state of development of individual research activities and the promise of new initiatives; (5) plans, develops and manages research resources necessary for the conduct of the coordinated research program; and (6) plans, organizes and conducts meetings and workshops to further program objectives, and maintains contact with the relevant scientific community to identify and evaluate new research trends relating to its program responsibilities.

The Special Programs Branch (SPB) was established February 11, 1979 by relocation of extramural efforts in diet/nutrition, epidemiology, and smoking and health from other areas within the Division of Cancer Cause and Prevention (DCCP), National Cancer Institute (NCI). Biometry was subsequently identified as a disciplinary area distinct from epidemiology. The programs were brought together in one branch to facilitate multidisciplinary approaches to research in these special areas of carcinogenesis. While the development of biometry has relevance to research in all of biology, it also contributes specifically to each of the SPB programs. Both Diet/Nutrition and Smoking and Health programs benefit directly from an interface with the Epidemiology Program which is, in turn, strengthened through efforts to better characterize studied populations by increasing use of laboratory determinations. This symbiotic potential extends beyond the internal activities of SPB to the extramural community where interest in multidisciplinary efforts is increasingly evident. The Preventive Oncology Academic Award (POAA) Program, addresses this same need by providing an outstanding faculty member the opportunity to develop, coordinate, and expand research and educational programs related to cancer prevention at the sponsoring institution.

No rigid boundaries exist between the individual programs comprising the SPB and indeed, as is evident from the program descriptions to follow, the activities of the branch involve a high degree of integration and cooperative interaction between the respective program directors.

During this fiscal year two workshops have been held. The first on the "Role of Natural Inhibitors in Carcinogenesis", was held on December 18, 1981 to assess the state-of-the-art in this area and identify aspects of the research area which might benefit from specific stimulation by NCI. The other workshop, the "Annual Meeting of Preventive Oncology Awardees", was held June 1-2, 1981, for the purpose of reviewing progress of investigators holding these awards and to exchange information on the specific activities ongoing at their respective institutions. Areas of high priority for research stimulation have been identified by the branch, in consultation with members of the extramural scientific

community. These new initiatives have now been approved by the Board of Scientific Counselors for the division. We anticipate the announcement of RFA's (research grant application solicitations) later in this fiscal year, with awards to be made as soon as possible in the next fiscal year. The initiatives include: 1) Studies on the Epidemiology of Rare Tumors, 2) Studies of Acquired Immunodeficiency Syndrome (Kaposi's Sarcoma and Opportunistic Infections); 3) Studies in Biochemical Epidemiology, and 4) Studies to determine the "Accuracy" of Questionnaire Derived Historic Dietary Information. An additional RFA in the area of nicotine toxicology is in the process of being prepared.

Biometry: This Program was established in late 1979 by transfer of approximately 25 percent of the research activity from the older, established Epidemiology Program. Although primarily comprised of research grant activities, contracts and/or interagency agreements are being utilized to determine the feasibility of linking existing federal data sources to provide resources to the scientific community. The Program content includes a variety of research activities including, but not limited to, mathematical models relevant to cancer biology, statistical techniques of use in evaluating the effects of potential carcinogens, determining the effects of patient characteristics on survival analysis or the analysis of competing risks, record linkage for investigations involving special population groups and cancer registries or death lists, cytogenetics and somatic cell genetics, techniques to evaluate cancer screening tests and procedures, and improved methodologies for evaluating estimates of cancer risk from low-dose exposure to carcinogens.

Diet/Nutrition: The Diet, Nutrition and Cancer Program (DNCP) was established in 1974 to "collect, analyze and disseminate information concerning the relationship between cancer and nutrition that would be beneficial in the prevention, diagnosis and treatment of cancer." This activity was initially supported entirely through use of the contract mechanism. In view of the increasing importance given to nutrition in all phases of the Cancer Program, the DNCP was reorganized in 1978 and specific elements were divided among three divisions and the Office of the Director (OD, NCI). At this time, all DCCP nutrition related research activities are concerned with etiology and/or prevention and supported under the research grant mechanism.

The range of activities supported under this program range from basic investigation of the carcinogenic and anticarcinogenic effects of diet and specific nutrients in animal systems to epidemiologic investigations focused on the effects of dietary factors on human carcinogenesis.

Epidemiology: Since 1967 the extramural Epidemiology Program of the NCI has consisted of five components: traditional epidemiology, human genetics, biomedical communications, behavioral research, and biometry. In 1973, the Division of Cancer Control and Rehabilitation was established (now the Division of Resources, Centers and Community Activities) with strong program interests in motivation, communication, and coping as behavioral problems. This led to a reduction in our Epidemiology Program activities in the area of behavioral research, with an increased level of effort in the area of etiology and prevention. During 1979, biometry (including human genetics) was identified as a component deserving special attention leading to its development as a new program within the Special Programs Branch. Areas of research of interest to the epidemiology program (currently supported entirely by the research grants mechanism) include investigations focusing on the natural history of neoplasia

in humans, the incidence and prevalence of various human cancers as a function of geographic location, etiologic factors related to human cancer (including intrinsic and extrinsic risk factors), opportunities for preventive intervention, and improved methodologies for the design and conduct of epidemiologic studies.

Preventive Oncology Academic Award (POAA): The initial review of proposals for this program was conducted by the Cancer Research Manpower Review Committee, Division of Extramural Activities, NCI on June 5-7, 1980. Eight of nineteen POAA proposals were funded in late FY 1980 following mail ballot review by the National Cancer Advisory Board.

From its inception, a major strength of the POAA has involved the open recognition that institutional programs, like people, may vary substantially in their level of development. Some, with a stronger tradition in preventive medicine, may be nearer the stage where formal implementation of courses in preventive oncology represents a primary thrust; most appear to require the opportunity provided by the POAA to allow the single most suitable candidate (determined by joint agreement among involved Deans, Dept. Chairmen, etc.) the opportunity to explore and facilitate development of in-depth ongoing research as well as potential new interdisciplinary research areas. The POAA has not been primarily concerned with formal course development, but rather sought to provide an appropriate institutional mileau among established scientists and educators in which such development could reasonably be expected. An equally important POAA goal is to provide outstanding students with an opportunity for a "hands-on" introduction to prevention related research disciplines during a time period before their career decisions are finalized. A revised Program Announcement has been prepared and was reissued during this fiscal year.

Smoking and Health: The NCI Smoking and Health Program (SHP) was begun in 1968 solely as a contract effort. Since its relocation from the OD, DCCP to the Chemical and Physical Carcinogenesis Branch in 1978 and subsequent transfer to SPB in 1979, the SHP has sought to identify groups of individuals at high risk to tobacco-related diseases, to develop supplementary aids for assisting in smoking cessation, and for means by which to reduce the hazards of smoking products for persons unable to give up their habit or addiction. In a manner similiar to that described above for Diet/Nutrition, the SHP activities have been divided between two NCI Divisions (DRCCA and DCCP) with coordination responsibilities transferred in 1981 from OD, NCI to DRCCA. The larger effort now termed the Smoking, Cancer and Health Program/ NCI (SCHP/NCI) includes those tobacco related research activities in epidemiology and toxicology of the Special Programs Branch (SHP-SPB). Response to the FY'81 SCHP/NCI Program Announcement for investigator-initiated research grant proposals was less than anticipated, although several meritorious proposals, including an epidemiologic study of smoking in relation to hepatocellular carcinoma and a multidisciplinary program project on tobacco-specific nitrosamines, have been funded. An RFP to investigate smoker compensation when changing smoking materials among cigarettes having various tar and nicotine delivery levels has been recently issued.

SPECIAL PROGRAMS BRANCH

FY 1982 Funds*

PROGRAM	GRANTS Number R01	R13	23	P01	K07	TOTAL	Dollars (in Millions)	CONTRACTS Number	Dollars (in Millions)	Total Dollars (in Millions)
Biometry	33	0	0	2	0	35	5.42	1	.15	5.57
Diet/Nutrition	42	0	0	4	0	46	6.11	--	--	6.11
Epidemiology	37	1	2	4	0	44	7.53	--	--	7.53
Smoking and Health	6	0	0	1	0	7	1.33	6	1.86	3.19
Preventive Oncology	0	0	0	0	9	9	.61	--	--	.61
TOTALS	118	1	2	11	9	141	$21.00	7	$ 2.01	$23.01

* Estimated Total Dollars as of June 1982.

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Description

The extramural Biometry Program currently supports 38 grants in biostatistics and genetically oriented carcinogenesis research. These activities are developmental (innovative) in nature and share a common denominator; their reliance on recent advances in computer technology (both hardware and software). They seek to encourage collaboration among biostatisticians, oncologists, geneticists, and members of related disciplines in their quest for insight into the unique problems of analysis of carcinognesis data and the carcinogenic process itself.

Fifty percent (19) of the activities stress theoretical biostatistical problems related to a variety of topics such as survival analysis, competing risks, and dose-response phenomena. Such basic topics are being studied in detail and with great sophistication. For example, survival analysis, which in the past was considered primarily as determining the probability of death given disease/event, now denotes a family of statistical methods appropriate for analysis of a very large number of oncology related problems. The data can include survival time per se, response to a given treatment, time to relapse or tumor development, length of remission, as well as sequential entries and progressive censoring. Biostatisticians are investigating these problems in the context of complex determinants, and developing methods appropriate for their analyses. Much of the work going on involves non-parametric theory, and how best it can be utilized to counter the fact that assumptions of normality (underlying parametric theory) may not be realistic in light of the vast array of variables. Once theoretical work in the area has been developed for specific cases, perhaps using computer simulated data, biostatisticians are being encouraged to test their methods against more conventional techniques using real-life data from oncology studies, tumor registries or other sources available to them. This work is most profitable when carried out in collaboration with oncologists whose task it is to lend credence to the physiological rationale for the ensuing results. In a sense, these investigations then become hypothesis generating exercises; clinicians gain insight into the coalescance of factors in given situations and begin to see avenues for future exploration.

The areas of competing risk and dose response are being investigated in much the same way. Problems are identified, conflicting or confounding factors determined, theoretical work initiated, tested and finally made available to applied biostatistians for use in handling analysis of laboratory/epidemiologic data.

As the search for competing risks and confounding factors in the natural history of cancer has been intensified there is a growing recognition of the need for familial (pedigree) information coupled with environmental and demographic information. Thus there has been a natural extension of the Biometry Program toward support of such projects. The genetic portion of the program now accounts for approximately 30 percent of the grants, including one large P01. Meanwhile, some of the theoretical statistical projects, noting the mathematics involved in cell marker analysis, have began to move in this direction. Hence, strict demarcations between the biostatistical/computing, and genetics portions of the program are disappearing.

Current genetics grants include two designed to identify lineages and subsequently examine them for kindreds which appear to be predisposed to cancer (in general or site specific). These efforts, undertaken in the Mormon population in Utah and a Mexican-American population in Laredo, Texas, have been built on information gleaned from years of church records. Both studies have been successful in establishing large data bases from which to work. Other grants are designed to attack the problem using the reverse strategy. Cases are identified and, subsequently, genealogies constructed. Here medical data is being collected in as much detail as possible and, in some instances, augmented with physiological specimens (urine, sera, tissue etc.). Most of these grants have reached the point where they are now seeking to develop risk factor profiles and/or biological markers for disease detection. Although the genetics grants within the Biometry Program are considered primarily pedigree studies, a few grantees are becoming involved in genetics at the cellular level. Findings from these studies will complement studies on the natural history of cancer (for particular sites), as cell biologists and biostatisticians attempt to formulate mathematical models of tumor development.

Among the remaining 20 percent of the grants are two large multidisciplinary projects, one large data base acquisition project and a P01. The multidisciplinary projects were originally Research Resources Grants for Biomedical Computing. While initially (prior to 1971) these two projects were designed to give mathematical support to research anywhere in their respective parent institutions, they are now totally devoted to problem solving in cancer research. When these particular grants come in for competitive renewal, attempts will be made to group the individual projects by subject matter for submission as separate R01 grants. The data base acquisition project is heavily involved in computer linkage of information from a wide variety of sources. Once the data is in place, the project proposes to address the effect of life style characteristics (including nutrition) on cancer incidence and mortality in Seventh Day Adventists. The P01 is unique to this program as it is directed toward a specific carcinogen, yet its component parts encompass the spectrum of the Biometry Program.

Research Accomplishments

Work dealing with long term survival estimation involving comparisons between "tail improved" curve estimation and the conventional lifetable is under investigation by Tartar. The significance of this stems from the need to compare new curve estimation procedures to lifetable methods in a context where the true survival curve is unknown. He has demonstrated that the new long term survival estimation procedures are from eight to ten times more efficient than the ideal lifetable. However, properties (other than tail estimation improvement) need to be further evaluated. This curve estimator allows a scientist to input his preference as to where he would like the estimation to be most accurate, e.g., right hand tail. This can be done by the investigator by selecting an appropriate weighting function as a component of the algorithm (Tartar).

As one segment of his project, Kozial has addressed the development of appropriate statistical methodology for the analysis of tumor growth curve data from experimental or clinical studies. The rate of growth of solid tumors, together with the time of metastatic spread, is an important factor in the diagnosis and prognosis of cancer. In addition, growth rate data from induced tumors

in laboratory animals subsequently subjected to various treatment regimens have been used to assess the efficacies of potential therapies for malignant disease since the kinetic characteristics of tumors in higher animals and humans are similar. Unfortunately, two inherent factors may render the analysis of tumor growth curve data difficult; the tumor size data may be incomplete, subjects may not survive the time course of the experiment and, therefore, may contribute only partial information to the tumor growth study; and within particular treatment groups, growth characteristics may not be homogeneous, tumor regressions being observed along with tumor growth. These factors obviate the utilization of parametric univariate and multivariate methods of analysis of growth curve data. Hence, Koziol has devised (and published the details of) an appropriate multivariate distribution-free method of analysis. It has been applied to data arising from a series of immunotherapy experiments conducted at the University of California San Diego Cancer Center (Fukushima).

Pierce and Tsutakawa are both working with researchers in Hiroshima on dose response in relation to incidence of cancer (delayed effects among high dose survivors of atomic bomb exposure being of particular interest). Human radiation exposure in contrast to laboratory studies where accurate measurements can be made, must be estimated. Hence, statistical models need to be developed for estimation using random effects models, incorporating unobservable random factors as well as known observable effects. The Hiroshima data is, of course, unique for evaluating such models. Neel is also in the process of establishing some collaborative work with the Hiroshima group. If Neel is successful it will allow him access to the Japanese data for making blind comparisons between mutation abnormalities detected using his high speed automated 2-D gel electrophoresis method (Skolnick) and those detected by the Japanese using a standard method of electrophoresis. Such collaboration should prove to be extremely valuable.

Moolgavkar continues to address theoretical statistical methods for cancer research in populations, be they people, animals or cells. His unique background in mathematics and medicine serves to make his team particularly productive. One example is the paper (Moolgavkar) recently published presenting a two-stage model for carcinogenesis which provides a framework for understanding the roles of spontaneous events, hereditary factors and environmental agents. The model can be fitted to age-specific incidence data on human cancers of both children and adults and may shed light on the relative importance of agents that effect transition rates, tissue growth and tissue differentiation. The model has been applied to lung cancer with emphasis on the rate and pattern of smoking and breast cancer with emphasis on the roles of hormones, radiation and heredity. The hypothesis of this two-stage model is that the first mutation occurs in an ancestral germ cell. This mutant gene then acts as a cancer gene in affected families. The second event always occurs in a somatic cell. Both events are rare at the cellular level in healthy persons, however, in individuals who carry the gene, there are numerous cells in which a single event (the second) can give rise to malignant transformation.

Elston has been working on computerization of a method for analyzing from pedigree data the etiology of any age dependent human disease suspected of having a genetic component or of any quantitative trait that may predispose to such disease. He is currently working with Dr. Henry Lynch on analysis of colorectal cancer pedigrees and Dr. Mary Claire King on breast cancer pedigrees. Results from such analysis could provide a sound basis for genetic counseling.

Tritiated thymidine (3HTdR) labeling reveals the locations within colonic crypts at which epithelial cells are undergoing proliferation. In disease-free individuals, such proliferation is found to be largely confined to the lower three-quarters of the crypts (away from the luminal surface), tailing off rapidly towards the luminal surface: in symptomatic individuals, the proliferation loci are generally found to be shifted slightly towards the luminal surface and to have a tail that tapers off less rapidly towards that surface. These differences in the distribution of labeled cells over the height of the crypt, have previously been shown to be statistically adequate for differentiating symptomatic from disease-free individuals within familial colon cancer kindreds. Lipkin, Lynch et al., have introduced an improved classification method for identifying disease prone cases on the basis of limited differences between epithelial cell labeling with 3HTdR. The new method has direct probabilistic theoretical basis, and simplifies and systemitizes the application of cell labeling statistics to decide the classification of individuals into alternative populations. The method has been tested and found successful when applied to Lynch's familial colon cancer kindreds (Lipkin).

Projections

Growth of the Biometry Program will continue at a rate acutely dependent upon the quality of the applications received. Theoretical statistics will need support to allow persons with appropriate expertise to study new and ongoing topics. There will be greater emphasis on application of new statistical methodologies to cancer data. There will be increasing emphasis on utilization of new computer hardware in graphics and image processing to recent advances in pattern recognition, statistical theory, and mathematical/genetic modeling. Genetic studies will continue to receive strong support (e.g., grants in the biostatistical/computer science area comprise half of the program but one-third of the budget). It is projected that this will even out however, as the biostatistical grants move into applications and genetic modeling, which by their very nature are more expensive. Attempts will be made utilizing the contract mechanism to continue to investigate the feasibility of linking existing data files to establish useful resources for occupational epidemiologic research.

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BIOMETRY PROGRAM

CONTRACT INDEX

INTERNAL REVENUE SERVICE (YO1-CP2-0510)

Title: Feasibility of Coding Occupation from the IRS Form 1040.

Contractor's Project Director: Dr. Frederick Scheuren

Project Officer (NCI): Dr. Marthana C. Hjortland

Objective: Continuation of efforts to study ways of using the tax return (Form 1040) to obtain occupational data on individuals.

Major Findings: Results to date indicate that it will be possible to produce a dictionary of occupational entries (from Form 1040) which may be used with the coded Continuous Work History Sample's (CWHS) information on industry to code occupation for a CWHS sample of about 19,000 individuals in the work force. Validation against 1980 census data will be required.

Significance to Biomedical Research and the Program of the Institute: There is a need to develop resources indicating carcinogenic exposures in the work place for conduct of occupational epidemiologic studies.

Proposed Course: Work will progress on several fronts affecting the development of a dictionary to be used to convert job title on Form 1040 to a numeric census-compatible standard occupation code. An agreement with SSA is in place to get Standard Industrial Classification codes and discussions will be held with the Census Bureau concerning validation of the IRS coding system. Due to delayed startup (January 1982 rather than October 1981) the one year project will be carried over to FY'83. DCCP funds have been set aside so that the project can be completed in twelve months as proposed.

Date Contract Initiated: January 11, 1982

Current Annual Level: $250,000

U.S. DEPARTMENT OF LABOR (YO1-CP1-0508)

Title: Feasibility of Using State Unemployment Insurance Records for Occupational Epidemiology.

Contractor's Project Director: Mr. Howard Vincent

Project Officer (NCI): Dr. Marthana C. Hjortland

Objectives: To determine the availability and usefulness of state unemployment insurance (UI) data for occupational epidemiologic studies.

Major Findings: It appears that it would be feasible to create a system of records from the state unemployment insurance records in certain states. This information is available for a greater or lesser period of time by state. Uniform information is not the rule. In order to access the UI records, it is necessary to work through the Department of Labor.

Significance to Biomedical Research and the Program of the Institute: There is a need to access the feasibility of developing resources for occupational epidemiology from as many existing sources as possible in an effort to find those most useful.

Proposed Course: The work on this contract has been completed. The final report will be submitted in the near future.

Date Contract Initiated: September 22, 1981

Current Annual Level: $31,170

Description

The Diet and Nutrition Program of the Special Programs Branch (DNP-SPB) is responsible for those aspects of diet and nutrition research related to cause and/or prevention of cancer in humans. Currently, there are 54 grants and two contracts in this program. It does not include those parts of the NCI-wide Diet/Nutrition and Cancer Program (DNCP) dealing with special nutritional needs of sick patients or tumor-bearing animals.

The DNP-SPB supports epidemiologic as well as laboratory investigations searching for etiologic factors related to diet and nutrition. These include mechanism studies of cancer induction by a variety of dietary constituents, (i.e., fats of varying sources and saturation levels, proteins of various types and levels, fiber, nitroso compounds, mycotoxins and other naturally occurring carcinogens, inhibitors of carcinogenesis, compounds associated with the gut including bile acids/fecal steroids and the influence of microflora). In addition, DNP promotes studies which focus on specific dietary factors, (i.e., nutrients, or micronutrients), biochemical epidemiology, host factors involved in pathogenesis and the development of methods or refinements of techniques for identifying putative carcinogens in foods, body fluids or feces as well as the influence of various methods of food processing and cooking.

Research Accomplishments

Epidemiological observations and studies in laboratory animals suggest that cancers of the stomach, colon, pancreas, prostate, breast, ovary and endometrium are associated with dietary components. Diet could influence cancer in several ways: through undernutrition, by affecting imunological systems; through carcinogens as contaminants of food, through the formation of carcinogens during the storage, processing or cooking of foods; through carcinogens produced in vivo from ingested food; and through the protective effect of certain dietary components by their influence on the carcinogen detoxification systems.

A case-control study in the multiethnic population of Hawaii was initiated in 1979 to test the hypothesis that a negative relationship exists between dietary intake of vitamin A and/or vitamin A precursors and risk of primary lung cancer in humans. The most intriguing findings to date, however, relate to dietary cholesterol rather than vitamin A. Preliminary analysis of the data on 203 cases and 342 controls indicate that the mean dietary cholesterol intake was substantially greater in cases than in controls in each ethnic group studied. There was a dose-response relationship for dietary cholesterol as well as a synergistic effect between cigarette smoking and dietary cholesterol intake. The predominant effect of cholesterol on lung cancer risk was among smokers.

An ongoing prospective epidemiologic study of nurses is evaluating the magnitude of the associations between dietary factors and site-specific cancer rates. The study focuses on the relationship of amount and type of dietary fat with breast cancer; vitamin A intake with cancer of all sites; consumption of fat, fiber,

and meat with colon cancer; and intake of cholesterol, vitamins C and E, coffee and artificial sweetners with a variety of cancers. A study of Mormons living in Utah is evaluating the influence of diet on colon cancer by correlating interview information with laboratory determinations of dietary lipids, proteins, fiber, and minerals; serum lipids, vitamins, and trace elements; and home-preserved and commercial food samples consumed by the study population. A second part of this study involves feeding of specific types of fiber in the diet to rats to determine their influence on the distribution and metabolism of selected toxicants.

A lower risk was found to be associated with increased frequency of vegetable consumption in a study involving 147 patients with cancer of the esophagus and 264 control subjects. The putative protective effect of vegetable intake was evident even after controlling for its possible association with smoking and drinking. These findings are consistent with evidence of lower risk associated with vegetable consumption in colon, lung, bladder, oral and laryngeal cancers, and with evidence of tumor inhibition by vegetable components in animals.

Several studies are underway to evaluate the influence of nutritional modulation on carcinogenesis. In one study in mice, dietary restriction started at or beyond midadulthood slowed immunologic aging, inhibited cancer and prolonged life. Previous studies had produced similar effects in mice restricted dietarily since weaning. Adult-onset underfeeding provides a model of cancer inhibition which can be studied to identify contributing immunologic, endocrinologic, etc., influences.

A choline-devoid (CD) diet given to rats was observed to promote liver carcinogenesis initiated by a carcinogen. Inclusion of phenobarbital, pentobarbital, amobarbital, but not barbituric acid, in a CD diet resulted in a synergistic effect. In rats not exposed to a carcinogen, feeding the CD diet enhanced liver DNA synthesis and cell proliferation. Inclusion of phenobarbital or pentobarbital in the CD diet inhibited these effects, while barbituric acid exerted no inhibition. The synergism in promotion observed when barbiturates were used in combination with the CD diet may be due to stimulation of initiated cells by the CD diet and selective suppression of non-initiated cells by barbiturates.

A number of investigators are currently studying the influence of the amount and type of dietary fat on tumor production by chemical carcinogens. In one study, ingestion of diets high in corn oil or lard increased dimethylbenz(a)anthracene (DMBA) mammary carcinogenesis in female rats, while beef tallow, but not rapeseed oil, produced a similar but much smaller effect. Spontaneous mammary tumors were not increased by the diets high in corn oil or lard. Corn oil was as effective in increasing mammary tumorigenesis if fed after DMBA treatment as it was if fed throughout the experiment. Lard was not effective unless it was fed throughout the experiment. Another investigator found that both the concentration and the type of dietary fat had a profound influence on mammary carcinogenesis. No correlation was observed between tissue fatty acid composition and mammary tumor incidence.

Beta-naphthoflavone (BNF), fed at 500 ppm to rainbow trout for 6 weeks caused the formation or elevation of a previously undetected hepatic microsomal cytochrome P-448 and an increase in the associated mixed function oxidase (MFO) enzyme activities. In contrast, 6 weeks of 450 ppm dietary cyclopropenoid fatty acids (CPFA) caused a reduction in cytochrome P-450 content and in MFO enzyme activities.

Feeding 500 ppm BNF for 6 weeks and CPFA concurrently the last 3 weeks resulted in the complete inhibition and/or reversal in the formation of cytochrome P-448 and a reduction in cytochrome P-450.

In preliminary experiments using isolated hepatocytes from mice on a diet containing butylated hydroxyanisole (BHA) profound differences in the benzo(a)pyrene (BP) metabolic pattern were observed. There was no increase in levels of either the glucuronide or sulfate conjungates. The amount of BP metabolites bound to intracellular DNA decreased dramatically. This effect appeared to be related to a change in BP metabolism rather than an increase in the conjugation of reactive metabolites.

Several studies are underway to determine the role of selenium in carcinogenesis. In one study it was found that the degree of inhibition of mammary tumorigenesis in rats induced by DMBA was proportional to the level of dietary selenium up to 5 ppm. The inhibition was manifested by a lower tumor incidence, longer latency period, and fewer number of tumors per animal. Selenium was unable to counteract completely the enhancing effect of a high fat diet on mammary carcinogenesis. It has been hypothesized that at physiologicals levels selenium protects against tumor growth by regulating peroxidation, especially in animals receiving a high fat diet. Vitamin E supplementation alone had no prophylactic effect against tumorigenesis, but it potentiated the ability of selenium to inhibit chemically induced mammary tumorigenesis. In another study, selenium inhibition of the initiation stage of mammary tumorigenesis appeared to be dose dependent whereas its effect on post initiative phenomena was observed only above a critical pharmacologic threshold of the element.

Analytical methods are being developed for: the identification and quantification of barley malt alkaloids which upon nitrosation form N-nitroso compounds; analysis of non-volatile N-nitroso compounds in foods, and the characterization of N-nitroso derivatives formed from peptides with N-terminal proline. The critical parameters which influence the formation of N-nitrosamines in direct-fire dried foods are also being determined. Other studies are concerned with the possible formation and stability of N-substituted amides and their N-nitrosated derivatives in processed and cooked food, N-nitrosamine formation catalysis by bacteria, formation and inhibition of N-nitroso compounds in cured meats and the interaction of aldehydes or precursors with amines and amino acids in model and food systems.

A recently initiated study is examining the possible role of dietary nitrite and nitrate in carcinogenesis through formation of N-nitroso compounds in the lower gastrointestinal tract of rats. The nitrite is incorporated in an ion-exchange resin to carry it into the colon. Hen's egg is being used as the source of naturally occurring nitrosatable amines. Using monocontaminated germ free rats, it is also planned to study the effect of specific bacterial species on nitrosation.

Several multidisciplinary program projects are examining various dietary and nutritional influences on mechanisms of carcinognesis. In one, the studies include the isolation and identification of mutagenic substances formed during the cooking of meat and in nitrite and salt-treated fish; the use of colon fragments to study tumor promotion; and investigations of the biochemical effects of medium chain triglycerides.

In another program project, several dietary components, (e.g., protein, carbohydrate, vitamins-A,B_6 and folacin, ethanol and fiber) are being evaluated for their influence on the carcinogenesis process. In preliminary experiments, it was found that the effects of low dietary protein on the promotion phase was much more important than on the initiation phase. Even though a 3-4 fold higher level of aflatoxin-DNA adducts was generated by high dietary protein during initiation, the subsequent feeding of a low protein diet reduced the level of these adducts. An unexpected finding during the search for short-term inducers of ornithine decarboxylase activity in the colon was that starvation plus refeeding produced this induction. The ornithine decarboxylase induction in the colon by sodium deoxycholate and the starvation/refeeding regimen were synergistic.

The role of vitamin A and its synthetic derivatives in the modulation, prevention and treatment of cancer is currently under investigation. Studies include: the role of intracellular retinol and retinoic acid binding proteins, the effect of retinoids on ornithine decarboxylase and transglutaminase, the modulation of RNA polymerases and regulated genes, the effect and alteration of epidermal growth factor receptors, the effect on adriamycin-induced DNA damage, the modulation of human tumor stem cells, the pharmacokinetics of retinoids in humans, an assessment of toxicities of retinoids, and the clinical effects of retinoids on precancerous conditions and as anticancer agents.

A regional center for education in clinical nutrition in the New York-New Jersey metropolitan area is also being supported. The center is developing courses devoted to consideration of the effects of nutrition on cancer and other diseases.

Projections

Several areas of nutritional investigation will be emphasized during the coming year. The first area of emphasis will focus on biochemical epidemiology in order to better characterize the differences in dietary habits which may alter cancer risk. Studies aimed at assessing the "accuracy" of questionnaire derived historic dietary information will also be encouraged.

Additional research areas on which emphasis needs to be placed include 1) the development of short-term tests for identifying inhibitors of carcinogenesis in food and natural products, 2) further exploration of the effects of nutrient interaction on carcinogenesis, and 3) the development of better methods (more quantitative and more specific) for evaluating lipid peroxidation.

An increasing number of chemically diverse substances have been shown in animal systems to inhibit the neoplastic effects of chemical carcinogens. Such inhibitors include vitamins C and E, beta-carotene, selenium, naturally occurring constituents of certain vegetables and fruit, synthetic retinoids, and food additives such as butylated hydroxyanisole and butylated hydroxytoluene. Investigations aimed at determining the range of neoplastic agents inhibited (i.e., complete carcinogens, initiators and promoters) as well as conditions under which inhibition occurs and possible adverse effects of these inhibitors need substantial further investigation to develop the body of information which will be required for considering possible human applications.

DIET AND NUTRITION PROGRAM

CONTRACT INDEX

SRI INTERNATIONAL (NO1-CP8-5620)

Title: Validation and Standardization of In Vitro Techniques to Assess the Effect of Diet/Nutrition on the Mutagenic/Carcinogenic Potential of Human Secretions and Excretions.

Contractor's Project Director: Dr. J. H. Peters

Project Officer (NCI): Dr. A. R. Patel

Objectives: This multidisciplinary project has as its major objectives to: (1) develop and refine techniques for determining the mutagenic potential of various foods and of the body fluids and excretions of subjects consuming these foods; (2) identify, separate and quantitate the compounds responsible for the mutagenic activity in foods and in the body fluids and excretions of subjects consuming these foods; (3) recommend standardize methodologies and techniques for performing the above tasks; (4) assess the applicability of these methodologies and techniques to large scale screening; and (5) determine mean values and ranges for mutagenic potential of certain foods prepared in different ways and for the mutagenic potential of the body fluids and excretions of subjects consuming these foods.

Major Findings: The protocol for screening human urine for mutagens and/or promutagens was extended to provide for a 1000-fold concentration of urinary constituents. In an investigation of one of the possible complexities of screening human urine concentrates (HUCs), it was found that the mutagenic activities of 2-acetylaminofluorene (AAF) and of the promutagens of tryptophan pyrolysis, TRP-P-1 and -2, for Salmonella typhimurium strain TA98 and of 2-anthramine (AN) for strain TA100 were significantly enhanced by HUCs from either cigarette smokers or nonsmokers. The enhancement observed was dose-related either for amount of HUC or promutagen employed. Maximal enhancement of the mutagenic activity of AAF for TA98 was about 10-fold; maximal enhancement of AN for TA100 was about 20-fold. A protocol was developed for testing HUCs for enhancement using 500 ul of a 1000-fold HUC, and three graded amounts of HUC for each of three levels of AAF for TA98 and AN for TA100.

Through the cooperation of Dr. H. E. Sauberlich, Western Human Nutrition Research Center, USDA, at the Presidio of San Francisco, pooled urine samples from each of three weeks were obtained from 11 non-smoking volunteers under strict dietary control. Concentrates (1000-fold) were tested for mutagenic activity using strains TA98 and TA100 as specified in the standard protocol. None of the 33 samples exhibited mutagenic activity for either TA98 or TA100. However, they enhanced the mutagenic activity of promutagen samples.

The mutagenic activities of the tryptophan, the glutamic acid, and the soybean globulin pyrolysis promutagens, TRP-P-1 and -2, GLU-P-1 and -2, and GLOB-P-1 and -2, respectively, was determined. A liquid chromatographic technique was

developed to resolve most of these compounds from one another. Detection employing fluorescence provided sensitivities for quantitation in the picogram range.

Significance to Biomedical Research and the Program of the Institute: Standardized, validated procedures are required for detecting mutagenic activity in human excreta to assess the risk of populations to various potential mutagens and carcinogens. The observation that urines, regardless of source, contain materials that enhance the activities of known promutagens suggests that positive findings of mutgenic activities in HUCs should be interpreted with extreme caution.

Proposed Course: This contract expired on February 28, 1982.

Date Contract Initiated: September 30, 1978

Current Annual Level: 0

WASHINGTON UNIVERSITY (NO1-CP8-5662)

Title: Validation and Standardization of In Vitro Techniques to Assess the Effect of Diet/Nutrition on the Mutagenic/Carcinogenic Potential of Human Secretions and Excretions.

Contractor's Project Director: Dr. Barry Commoner

Project Officer (NCI): Dr. A. R. Patel

Objectives: The objectives of this project have been to: (1) test the effectiveness of the proposed standard protocol for detecting low levels of mutagens in the urine of populations exposed to environmental sources of mutagens, (2) study alternative means of quantifying the mutagenic activity detected as in (1) above; (3) further analyze the interference of urinary toxins on the detection of mutagens; and (4) examine alternatives to the standard protocol to avoid the problems identified in (3) above.

Major Findings: No apparent increase in sensitivity is gained by testing urine extracts over a range of concentrations up to 100 ml. per plate. Using a range up to 50 ml. appears adequate. Thus, smaller urine samples can be used, an important logistical improvement in the protocol. The standard urine protocol proposed in the second year of contract work called for a two-step elution of the XAD-2 columns--first with methylene chloride, and then with acetone. Urine samples from a population exposed to a variety of mutagens (cigarette smoke and other unknown exposures) were analyzed using this protocol. Mutagenic activity appeared with approximately equal frequency in the methylene chloride and acetone fractions prepared from the samples. In a comparison with the protocol in which acetone alone is used for elution, it was found that the two-step elution resulted in fewer toxic samples.

Extracts of nonsmokers' urine enhance the mutagenic activity of the powerful mutagen 2-acetylaminofluorene, but do not enhance the activity of another mutagen, benzo(a)pyrene. This enhancement is largely due to constituents of the urine itself rather than to antifacts of the extraction process. Nonsmokers' urine extracts did not, however, enhance the activity of smokers' urine extract.

Significant increases in the mutagenic activity detectable in a smoker's urine sample can be achieved by a basic wash of the eluate from the XAD-2 column. An in situ wash of the column itself following the application of the urine does not have the same effect.

The "bacterial fluctuation test" was used to analyze several smokers' urine samples. Mutagenic activity was detectable from smaller volumes of urine than in the standard plate test, suggesting that the fluctuation test may be a more sensitive way to detect low levels of mutagens in human urine.

Significance to Biomedical Research and the Program of the Institute: The urinary mutagen assay is becoming an important tool for studying environmental carcinogen exposure. However, before it can gain widespread acceptance, it must be capable of producing consistent, reliable results.

Proposed Course: This contract expired December 30, 1981

Date Contract Initiated: September 30, 1978

Current Annual Level: 0

EPIDEMIOLOGY SUMMARY

Description

The rapid growth of the Epidemiology Program has been prompted by increased concern over environmental, pharmacologic and iatrogenic hazards which appear to be associated with malignancies. General acceptance that most cancers result from the combined effects of multiple exposures in individuals with genetically and idiosyncratically varied susceptibility is demonstrated in the multidisciplinary approaches within the Epidemiology Program. Association of various cancers with occupational, environmental, genetic and/or familial and behavorial risk factors are pursued in terms which can be quantified to permit comparisons among various causes and various effects. Epidemiologic approaches utilize basic descriptive and analytic, retrospective case/control, historical and prospective cohort methodologies.

Of 49 research efforts funded in this fiscal year, 42 are individual investigator initiated grants (R01), three are multifactorial program project grants (P01), two are new investigator awards (R23) and two are conference grants (R13).

Case/control studies investigating relative risk of disease comprise the bulk (66%) of the Special Programs Branch Epidemiology Program. These are retrospective studies in which characteristics and exposures are evaluated in patients already diagnosed with cancer of a specific site. Cancer of the kidney, lung, ovary, skin (malignant melanoma), brain, oral cavity, liver and lymphomas are among the tumors currently studied. Cohort studies, which comprise 10% of the grants, include long term follow-up of hepatitis B virus carriers; alcohol and cigarette users, and a large cohort of nurses.

Descriptive epidemiology studies which seek possible causal clues provided by incidence, prevalence and mortality statistics comprise another 12% of the epidemiology grants.

The effect of life-style and/or specific exposures in relation to cancer is of continuing interest. Two studies investigating malignant melanoma examine risks associated with exposures to chemicals and sunlight. Hypotheses involving viral associations or etiology are addressed in studies of hepatitis B virus (HBV) and primary hepatocellular carcinoma (PHC); herpesvirus II and cervical cancer; Epstein Barr virus (EBV) and Hodgkin's disease. Three studies addressing the assocation of genetic factors, chemical exposure, and analgesic use with kidney cancer indicate a growing concern with this rare (2%) lesion. Two incidence and mortality studies explore the elevated cancer risk of some farmers by type of farm operation. Occupational exposure is addressed in a study of cancer mortality among workers in the meat industry. An investigation focusing on the risk of anal intercourse and possible introduction of sexually transmitted viruses reflects concern with lifestyle and the development of anal cancer.

The importance of prenatal and childhood environmental exposures in the etiology of childhood cancers, particularly of the brain, is receiving attention in several studies. Radiation effects are being investigated in a study which has already demonstrated elevated risk of parotid gland tumors with full-mouth dental x-rays

prior to age 20. Relationships of hormones and cancer of the reproductive organs remain the focus of research in seven studies. While breast cancer remains a major component in two of the three program projects, these research efforts also include cancer of the pancreas, ovary, testes, Hodgkin's disease, and malignant melanoma. The third program project is providing important epidemiologic and laboratory information regarding the association between persistent hepatitis B infection and hepatocellular carcinoma. Vasectomy as a risk factor for testicular cancer is explored in one ongoing study. Another has provided important insights into the cellular development and natural history of neoplasms, and continues to provide information which may be of great value in monitoring the course of therapy in lymphoproliferative diseases. Two additional ongoing studies address the potential protective effects of vitamin A on lung cancer.

The two conference grants have international implications. One which has been ongoing for 21 years organizes study groups which prepare widely distributed technical reports on specific cancers and provides training courses in cancer research to young graduates. The other conference proposes to discuss cancer epidemiology in Latin America and hopefully establish collaborative efforts with U.S. investigators.

Research Accomplishments

Hepatitis B virus (HBV) is a major health problem both in terms of economic impact and disease disability. Several studies are investigating the association between long-term carriage of HBV and primary hepatocellular carcinoma (PHC). The uniqueness of HBV infection from the epidemiologic point of view is indicated by the unusually large variation in the prevalence of HBV markers in various geographic areas and between population groups within specific areas. The cumulative rate of infection (as determined by HBV markers), varies between 7-10% in this country and 60-80% in Southeast Asia and Africa. The antigen persistence rate is 3-7% in most populations, but 11.5% in Chinese. The high rate in Chinese may be related to a genetic predisposition and/or to environmental factors. Primary hepatocellular carcinoma (PHC) is the most common malignant neoplasm in China, much of Asia and in Africa, but is uncommon in the U.S. and Europe, accounting for less than 1% of all malignancies in white males in the U.S.

A prospective study of 22,707 Chinese men in Taiwan has shown that the incidence of PHC among carriers of HBsAg (HBV surface antigen) is much higher than among noncarriers, with a relative risk of about 220 for PHC. Cirrhosis accounted for 54.3% of the 105 deaths among HBsAg carriers, but only 1.5% of the 202 deaths among noncarriers. These findings strongly support the hypothesis that HBV has a role in the etiology of PHC because they establish the temporal sequence of a greatly increased risk of PHC among persons who were HBsAg carriers before they developed PHC (Beasley 1981). Although this study contributes substantially to our understanding of the relation between HBV and PHC, a number of unresolved questions remain, e.g., do HBV carriers of other races and in other places have the same risk of PHC as Chinese men on Taiwan? A study of approximately 20,000 blood donors in the U.S. is addressing this question in a retrospective case/control study. The major resource for this study is an excellent computerized record of those donors found reactive for HBsAg over a 9-10 year period plus a comparable HBsAg negative control group of similar size. If, as a result of this study, excess mortality is documented among HBsAg positive donors in the U.S., a prospective study will be developed.

An ongoing program project studying the various aspects of hepatitis B virus (HBV) and its association with primary hepatocellular carcinoma has been most productive during the past year. Among 247 patients with chronic hepatitis or cirrhosis who are being followed for three or more years in Korea, 26 have developed PHC and 40 have died. Using serum specimens collected over time from these patients, the hypothesis is being tested that alteration in iron metabolism can be used to identify patients at highest risk of developing PHC ((Blumberg (a)). The importance of predicting individuals at greatest risk, or the identification of high risk groups, by use of laboratory tests is an extremely high priority area of investigation for the epidemiology program, as evidenced by the great emphasis currently being placed on biochemical epidemiology.

The issue of whether occupational exposures of parents may result in an increased risk for malignant disease in offspring has been gaining increased attention. Ninety two cases of brain tumor in children, less than 10 years old, were compared for parental occupational histories with 92 controls, matched for age, sex, year of birth (within three years) and social class. Extensive data were obtained on parental work history prior to, during, following pregnancy and at time of diagnosis. Cases were more likely than controls to show (1) maternal occupations involving chemical exposure (2) paternal occupations involving solvents paints in particular and employment in the aircraft industry. These three apparent risk factors were not significantly affected by possible confounding variables such as patterns of food consumption, drug use, alcohol use and smoking habits. Animal and human studies have provided adequate reason for concern about exposure to certain occupational chemicals. In addition, a cancer surveillance program (Henderson) looked at all brain tumors in white men 25-64 years of age diagnosed between 1972 and 1976 in Los Angeles County. This investigator also found an excess over the expected value in men employed in the aircraft industry. Whether a common exposure accounts for both the childhood and adult brain tumors should be further investigated. Two other studies of childhood brain tumors (Nasca, Meadows) and one of adult brain tumors, (Hochberg) which are currently underway are also investigating a wide range of environmental exposures as well as genetic interactions. Information from these four studies should add to the sparse data available on brain tumor etiology.

It has been nine years since the initial study associating clear cell adenocarcinoma of the vagina with intrauterine exposure to diethylstilbesterol (DES). Recent calculations estimated the risk to be on the order of 0.14 to 1.4/per thousand DES-exposed females up to the age of 24 years. A major problem in the calculation of this risk is the lack of precise data on the number of women treated with DES during pregnancy. Currently over 400 cases of clear cell adenocarcinoma of the vagina and cervix have been identified (Registry for Research on Hormonal Transplancental Carcinognesis) of whom about two-thirds are known to have been exposed in utero to DES or similar nonsteroidal estrogens. Although DES is no longer used in this country for the treatment of threatened abortion, it is important to follow both the exposed daughters and their mothers to determine if other malignancies will develop with increased frequency in this population. There has been a reported excess of breast cancer in the mothers as they move into the postmenopausal period (Herbst). Two other studies in the epidemiology program are investigating the effects of in utero DES exposure on male offspring (Kurland, Moss).

Excluding skin cancer, carcinoma of the prostate is the second most common cancer among males in most developed countries, and accounts for about 17,000 deaths annually in the United States. Two major etiologic hypotheses have been proposed, one based on chemical exposure and one based on venereal transmission by an infectious agent. Starting with a cohort of 1,432 priests, nearly 31,000 man-years of follow-up were obtained in a cohort study to evaluate the hypothesis that some aspect of sexual contact, possibly a venereally transmitted virus, conveys increased risk of prostatic carcinoma. Cause specific mortality rates for the United States white male population from 1946-1975 were used to compute the expected number of deaths for the cohort. The results did not support the hypothesis that risk of prostate cancer is related to some aspect of sexual contact. In fact, although not statistically significant, a modest excess of prostate cancer deaths over corresponding mortality rates in U.S. white males was observed. Rates for lung and bladder cancer were markedly reduced in this cohort. Catholic priests would not be expected to have significant exposure to industrial chemicals, and as a group, may seek a relatively moderate life style including less cigarette smoking, both risk factors associated with lung and bladder cancer. It seems reasonable to infer that industrial exposure, per se, and sexual contact are not major risk factors for prostatic cancer, since the priests' rates were higher than expected without these exposures, while rates for lung and bladder cancer were markedly decreased, as might be expected (Ross).

An ongoing study in its ninth year continues to have as its goal the combination of epidemiologic and endocrine approaches to identify risk factors in hormone responsive tumors; and to explore the biologic plausibility of such associations. A recent paper which described the interaction between relative weight (wt/ht^2) and risk of breast cancer associated with exogenous estrogen use, found that women with less than average relative weight seemed to exhibit a lower risk for breast cancer for a given level of estrogen use (Sherman). In another case/control study, the relationship between psychoactive drug use and breast cancer was evaluated, since some of these medications alter prolactin secretion. None of the drug categories investigated were significantly associated with breast cancer risk (Wallace).

Farmers sustain a variety of chemical, physical and biological exposures with patterns of exposure which vary substantially among different types of farming. A retrospective cohort study of cancer incidence is utilizing a cohort of 18,000 farmers in a Farm Bureau (dairy, poultry, vegetable and orchard farming). This pilot project should be a valuable step in initiating a systematic investigation of disease occurrence among farmers (Stark).

Unlike most cancers, leukemias occur at elevated rates in rural and farm populations. Previous studies have been unsuccessful in documenting specific risk factors for leukemia attributable to living in rural areas or working in agriculture. A case comparison interview study using incidence data from the Iowa State Health Registry (SEER) has indicated elevated leukemia rates in Iowa which are strongly associated with male sex, rural residence, proximity to high cattle population densities and to dairy herds with bovine lymphosarcoma. The elevated rates and associations noted were primarily associated with acute lymphoid leukemia (ALL). These preliminary data are suggestive that cattle and perhaps bovine lymphosarcoma virus may be factors in the high rates of leukemia seen for ALL among this group of farmers.

A case/control study designed to test for multiple antecedent risk factors in histiocytic lymphomas (HL) and a possibly related disease entity, immunoblastic lymphadenopathy (IBL) includes 100 cases of HL and 50 cases of IBL. In addition to extensive questionnaire information focused on therapeutic history, environmental exposures at work and home, allergic and other past disease history and family history of tumors, blood samples will be obtained on a group of cases and controls to help characterize immune responses and assist in determining the affected cell types of these patients. This study could contribute information relevant to the current epidemic of immunodeficiency syndrome which frequently presents with lymphadenopathy and associated cancers (e.g., Kaposi's sarcoma and lymphomas) (Ross).

A three fold increased risk of hematologic malignancies including multiple myeloma (MM) has been reported in first degree relatives of Alzheimer's disease patients. In a case/control study of 459 multiple myeloma cases and 1,455 matched hospital controls, 16 of the MM cases and 12 of the controls had one one or more first degree relatives with a history of degenerative or demyelinating CNS disease. Separate analyses for component categories of CNS disease yielded relative risks of 5.3 for multiple sclerosis (MS), 2.7 for Parkinson's disease and 16.0 for the residual. In the last category, two of the five "positive" cases had a total of five first degree relatives affected with a similar CNS syndrome of retinitis pigmentosa, optic atrophy and spastic diplegia progressing to quadriplegia. These findings, in conjunction with those for Alzheimer's disease suggest shared etiologic mechanisms for MM and several CNS disorders. The presence of oligoclonal immunoglobulin bands in the cerebrospinal fluid of MS patients suggests an innate aberrant response of the immune system as one possible mechanism.

Knowledge about the origin, development and natural history of human neoplasms has been pursued in an ongoing study in its eighth year which exploits the isoenzymes of glucose-6 phosphate dehydrogenase (G6PD) in heterozygous black females. Numerous papers and reviews have been published. The study has expanded the area of inquiry from the simple evaluation of clonality of various neoplasms to a study of the kinetics of normal and neoplastic cell populations. Extension of the analyses of G6PD enzyme types to hematopoietic progenitors grown in vitro has permitted study of regulation in different myeloproliferative disorders, the delineation of the stem cell levels at which neoplasia begins and how neoplastic progenitors in the marrow influence the regulation of their normal counterpart. Initial findings documented the persistence of normal committed stem cells early in the course of polycythemia vera (PV) and the return of normal hematopoieses in aggressively treated chronic myelogenous leukemia (CML). Current studies suggest that PV is a slowly progressive neoplasm in which growth of normal committed stem cells is suppressed and currently detectable chromosomal abnormalities are not required as the immediate cause of clonal proliferation in the disease (Powell, Fialkow). Recent findings suggest that the primary defect in CML is in the establishment of an abnormal stem cell clone which prevents the expression of normal stem cells. The rapidity with which this occurs stands in contrast to the situation with the slow proliferation in the course of PV (Fialkow, et al, a,b,). Important new findings indicate that acute nonlymphocytic leukemia (ANLL) is heterogeneous. This heterogeneity may reflect differences in causation and might serve as a basis for formulating classifications of ANLL more meaningful for therapy and prognosis than those currently available (Fialkow, C.). The use of G6PD mosaicism as a tool to follow treatment has allowed early diagnosis of

remission in ANLL and the evaluation of remission status after therapy in CML. These studies have provided valuable information about the effects of tumor growth and differentiation.

Three case/control studies focused on renal adenocarcinoma are investigating a wide range of possible etiologic factors including genetic, medical, occupational, diet and tobacco usage (Asal, MacMahan, Ross).

Projections:

The RFA on the Epidemiology of Rare Tumors will be published shortly. Another RFA is planned for release in the near future to stimulate collaborative research between epidemiologists and laboratory scientists in developing and/or applying objective measures useful in studying the etiology of human cancer. An RFA jointly sponsored by DCCP and DCT is being developed to support research on the etiology of the epidemic of acquired immunodeficiency syndrome associated cancers and opportunistic infections. This research should provide an opportunity to investigate the interaction of life style, environment, host immune status and oncogenesis.

Several areas of research will be pursued in newly funded studies: 1) The development and assessment of a viral transformation assay as a procedure to identify individuals at risk in families with neurofibromatosis may prove to be a valuable counseling adjunct. 2) Surveillance for drugs which may be carcinogenic will be attempted in a study utilizing a cohort of over 80,000 persons. Associations discovered between use of a drug and subsequent development of cancer will serve to generate hypotheses for further study. 3) Dietary considerations are being considered in several studies. A cohort study is concerned with the role of estrogens and vitamin A in cancer prevention; and the hypothesis that low levels of selenium are correlated with higher rates of cancer will be investigated in a cohort of 122,000 women.

Additional research on childhood cancer will be encouraged. The existence of cancer in infancy and childhood raises the possibility of identifying prenatal risk factors. Little is known about the role of possible causative agents or genetic and environmental factors.

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PREVENTIVE ONCOLOGY SUMMARY

Description

The Preventive Oncology Academic Award Program grew out of a meeting in 1979 between Deans of Schools of Public Health, Chairman of Epidemiology and Biometry Departments and NCI staff. The sense of this meeting was that a chronic shortage of cancer epidemiologists and biometricians was being exacerbated by the failure of then existing research and training grants to meet certain needs. The delegation thought many of the problems to be unique to epidemiologic and biometric cancer research. They cited lack of institutional financial resources for (1) research planning (2) faculty salaries for teaching cancer epidemiology and prevention (3) curriculum development costs (4) student research stipends and (5) interdepartmental coordination. Several of the National Institutes of Health had already developed academic award programs to deal with specific problem areas. This mechanism was identified as one resource NCI should develop immediately to help alleviate the problems and to stimulate the recruitment of physician epidemiologists.

A preventive oncology academic award is made to an institution which sponsors a candidate faculty member. Both the institution and candidate must present plans for improving preventive oncology research and curriculum, building on preexisting expertise and activities. The candidate is required to be committed to research into cancer prevention and to have broad knowledge of clinical oncology, carcinogenesis, cancer epidemiology, biometry, and cancer prevention; an M.D. is not a specified prerequisite. Because few individuals are well versed in all these fields limited additional training for the candidate can be supported by the grant.

Eligible institutions include U.S. schools of medicine, osteopathy, dentistry, public health and NCI-designated comprehensive cancer centers.

The first program announcement was issued in 1980. Since then, nine grants have been awarded; six are made to universities with an NCI-designated comprehensive cancer center. Three other institutions have broadly based, active cancer research programs. Four grantee institutions have schools of public health.

In each case, the candidate awardee has been identified with multi-departmental, multi-institutional or multi-programmatic responsibilities. All had prior doctoral degrees, of which five were M.D.'s and four Ph.D.'s. Two physicians accepted other jobs within the first two years of the 5-year grant period, and the replacement candidates also hold M.D. degrees. Three of the nine awardees proposed formal supplementary education for themselves.

Research Accomplishments

The scope of research activities is broad and varied. One is developing new biometric methods for epidemiologic research; another is involved with occupational carcinogenic risks, particularly for women; a third is engaged in retinoid research and a fourth in epidemiologic studies of low risk populations. In two cancer centers, epidemiologic research and cancer control programs are now directed by the preventive oncologist. This is a new development stimulated by the

grant. At least four institutions have had active teaching programs from inception of the grant; interest in curriculum development is spreading rapidly among the remainder. Thus, the program has retained the flexibility originally planned for it.

At least four awardees have been promoted and given broader administrative responsibilities since their initial award. One candidate concentrating his attention on research has recently been awarded an international research prize which complements the academic award. The recruitment of two awardees to jobs outside their sponsoring institutions may be a further demonstration of the prestige accorded the award.

Projections

A reissuance of the program announcement in the April 23rd NIH Grants and Contracts Guide invites a new round of competition for funding in fiscal year '83. The scope of Preventive Oncology spans etiologic and cancer prevention research. This announcement reiterates interest in fostering skills in epidemiology, biostatistics and human genetics and recognizes the emerging importance of nutrition. The importance of an active research program in preventive oncology as a basis for educational activities is stressed. Evaluation of the ongoing grants continues and will provide data for deciding how much this program should change in the next several years.

Description

Since 1968, the National Cancer Institute's Smoking and Health Program (SHP) has been involved in efforts to understand and mitigate the deleterious effects of smoking on health. Significant past SHP efforts have included development of practical techniques for making and testing less hazardous cigarettes, epidemiology studies seeking means for identifying groups of individuals at high risk to smoking related diseases, and chemical analyses of major whole smoke components and their subsequent metabolic products. Progress has been made to varying extents in each of these areas, the most significant being the evidence to date that low tar, low nicotine cigarette smoke is less harmful to experimental animals than high tar, high nicotine cigarette smoke. These findings are reflected in the current trend to low tar, low nicotine commmerical cigarettes by the consumer. Current program emphasis is focused on epidemiological and pharmacological aspects of the problem. In an attempt to attract more investigator-initiated research in these areas, a Program Announcement was issued in January 1980. Response to date has been insufficient to develop a strong research base to better deal with this very important public health problem. Several new initiatives to accomplish this end have been undertaken with development of Requests for Proposals on specific subjects and Requests for Applications in certain areas of research related to the effects of cigarette smoking.

Research Accomplishments

The Smoking and Health Program continues to support research on identification of carcinogens in cigarette smoke. A reproducible gas chromatography-thermal energy analyzer (GC-TEA) method has been developed and to date four tobacco-specific N-nitrosamines have been identified. These are N'-nitrosonornicotine (NNN), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), N'-nitrosoanatabine (NAT) and N'-nitrosoanabasine (NAB). NNN, NNK and NAB induce benign and malignant tumors of the respiratory tract of mice and rats. It has been shown that NNN and NNK induce tumors in the upper respiratory tract of hamsters and that NNK is the most active of the tobacco specific nitrosamines, also inducing adenoma and adenocarcinoma of the lung of hamsters.

The same method was used for the quantitative determination of N-nitrosodiethanolamine (NDELA) in tobacco, tobacco smoke, and chewing tobaccos. The biologic activity of NDELA was determined in Syrian golden hamsters by skin painting, swabbing of the oral cavity and by subcutaneous injection. Independent of the form of application, NDELA induced carcinomas of the nasal cavity, papillomas of the trachea and tumors of the larynx in some animals. NDELA uptake through the oral cavity in hamsters is presumably greater than through the skin, judging by the higher tumor yield induced by painting of the oral cavity compared to skin painting. Evidence to date indicates that diethanolamine is a major precursor for NDELA in tobacco and tobacco smoke. Diethanolamine is used as a solubilizing agent for maleic hydrazide, the major sucker-growth inhibitor for U.S. tobacco crops.

Chemical analysis of whole smoke from commercial cigarettes has continued in order to assist in the interpretation of results from epidemiology studies.

During the past year over one hundred twenty domestic brands of cigarettes have been processed in the routine manner. Emphasis has been given to the newer low and ultra low "tar" cigarettes which have been characterized for "tar", nicotine, carbon monoxide, hydrogen cyanide, oxides of nitrogen, and acrolein. The profiling of the vapor phase of whole smoke constituents continues to show a quantitative similarity among cigarettes of widely different "tar" deliveries. The one exception was found with a non-tobacco cigarette which yielded a higher carbon monoxide content than tobacco cigarettes.

Histopathology studies are continuing on tissues from dogs exposed to cigarette smoke having different levels of tar and nicotine in inhalation experiments. The findings indicate a quantitative difference in lung tissue damage (greater damage from higher tar cigarettes) under the same conditions of exposure.

Data collection on a retrospective lung cancer case-control study in selected cities in the U.S. is continuing. To date over 2,800 cases have been interviewed along with matched hospital and neighborhood controls. Data analysis is now underway.

A prospective study, utilizing a self-administered questionnaire, is in progress at Kaiser Foundation Research Institute. The study will involve more than 80,000 ambulatory subscribers to a prepaid medical care plan. Preliminary analysis of data does not show a relationship between peptic ulcer and cigarette smoking. Data collection for epidemiology studies on lung cancer and cigarette smoking has been completed in six cities in five European countries. Analysis is now being conducted by the Epidemiology Program, DCCP, NCI.

Projections

The Smoking and Health Program is progressing toward its stated goals. Although many specific projects have been completed, there are still many areas in which work needs to be performed.

The histopathologic evaluation of tissues from inhalation experiments will be completed. These results should yield valuable information on the relationship of whole cigarette smoke and nicotine concentrations to smoking-related diseases.

Data analysis on epidemiologic studies covering selected cities in the United States, six cities in five European countries, and Cuba are to be completed. The vast amount of data relative to smoking habits, type of cigarette smoked, plus a broad medical data base, is expected to yield sufficient data to relate tobacco/cigarette characteristics to smoking habits and to disease incidence.

Identification of individuals at elevated risk of developing tobacco-related disease continues to be of high priority. Continuation of the prospective epidemiologic study provides the potential for profiling characteristics which may contribute to susceptibility (or resistance) to smoking-related illness.

Epidemiology studies utilizing histologically confirmed lung cancer cases in non-smokers, with properly matched controls, has just been initiated and will be supported during the coming year. Additional information in this area should aid in determining the extent of health effects in humans exposed to side stream smoke.

Studies will be initiated in the near future to quantitatively determine the compensation which takes place, if any, in human smokers when switching between high "tar"/nicotine cigarettes and ones providing lower levels of these materials.

Animal studies investigating alterations in body fluids associated with tobacco use and subsequent disease may assist in identifying human smokers at unusually high risk of tobacco-related diseases. A "state-of-the-art" workshop will be held during late 1982 to better define specific areas for further study which have potential for identifying these subgroups of the population.

SMOKING AND HEALTH PROGRAM

CONTRACT INDEX

AGRICULTURE, DEPARTMENT OF (YO1-CP2-0201)

Title: Development, Production, and Evaluation of Low-Yield Reference Cigarettes.

Contractor's Project Director: Dr. T. C. Tso

Project Officer (NCI): Dr. Thomas B. Owen

Objectives: Develop, manufacture and characterize reference low-yield cigarettes as a standard for comparative evaluation of low-yield varieties in smoking and health research.

The 1981 Surgeon General's Report recommended twelve different research needs on low-yield cigarettes. It is not practical to select any commercial cigarette variety currently on the market for study since the "tar" and nicotine yields are constantly changing. It is necessary and desirable to develop and establish a low-yield reference cigarette to serve as a constant base. Any cigarette varieties, whether available now or that will be marketed in the future, can be evaluated in comparison with this "reference". Such a practice has proven to be quite valuable in the case of the Kentucky reference (1R1) for regular cigarettes, which was used world-wide and the Standard Experiment Blend (SEB) series of reference cigarettes used for the evaluation of experimental cigarettes by the Tobacco Working Group.

Major Findings: Specifications for the reference cigarette have been developed. Sources are now being sought for the procurement of raw products and manufacture of the finished product.

Significance to Biomedical Research and the Program of the Institute: Yields of tar and nicotine are one of the most, if not the most, important factors in the study of toxicity and pharmacology of smoking. The reference cigarette will provide basic information for the evaluation of low-yield cigarettes, and a valuable reference for all those concerned with the smoking and health problem.

Proposed Course: Continue with procurement and quality control testing of the cigarettes.

Date Interagency Agreement Initiated: April 1, 1982

Current Annual Level: $200,000

AMERICAN HEALTH FOUNDATION (NO1-CPO-5684)

Title: Epidemiology of Smoking-Related Diseases.

Contractor's Project Directors: Dr. Ernst L. Wynder
Dr. Margaret H. Mushinski

Project Officer (NCI): Dr. Thomas B. Owen

Objectives: The major objectives of this retrospective study include (1) measuring the effects of smoking cigarettes with varying levels of tar and nicotine on the risk of developing lung, bladder, pancreas and upper respiratory tract cancers plus first episode myocardial infarction, and (2) monitoring the use of the newer low tar cigarettes through hospital cases, with proper hospital and neighborhood controls.

Major Findings: Occupational level and smoking history were found to be significantly related ($p<.001$) among the white males interviewed to date. More professionals and men in technical occupations were found in the never smoked and exsmoking categories; and more men employed in blue-collar positions were long-term smokers, smoked high tar filtered or non-filtered cigarettes, and began smoking earlier than men in white-collar positions.

Risk of developing lung or larynx cancer continues to be decreased among long-term (10+years) filter cigarette smokers when compared to long-term non-filter cigarette smokers. Risk of developing oral cavity cancer is associated with excessive alcohol consumption among cigarette smokers.

Ongoing analysis of male and female bladder cancer patients continues to support earlier findings that risk of this disease is not associated with saccharin or coffee consumption.

The smoking habits of the women in the study are becoming more similar to those of men. Proportionately more women choose filtered cigarettes, in general, and very low tar cigarettes (1.0 mg. tar), in particular, than men, and women begin the cigarette habit approximately three years later than their male counterparts.

Significance to Biomedical Research and the Program of the Institute: This epidemiological study is a necessary companion to the chemical and biological studies which try to identify the tumorigenic components in tobacco and tobacco smoke condensate. This study provides the Smoking and Health Program of the National Cancer Institute with data to (1) determine the nature of the low tar cigarette and identify the constituents which may differentially affect various tobacco-related diseses, (2) encourage further development in the area of the low tar, low nicotine cigarette, and (3) determine the effect of health education on smoking habits of a segment of the general population.

Proposed Course: Data collection and analyses of the following aspects of smoking and health will be continued: the tar content of cigarettes in relation to the risk of developing certain cancers and myocardial infarction, coffee consumption in relation to the risk of pancreas and bladder cancer, mouthwash use and the risk of oral cavity cancer, smoking habits of men and women in a effort to predict future disease patterns, and changes in quantity smoked after switching from a high tar to a low tar cigarette.

Date Contract Initiated: July 1, 1980

Current Annual Level: $482,316

ENERGY, DEPARTMENT OF (Y01-CP6-0206)

Title: Collection, Separation, and Elucidation of the Components of Cigarette Smoke and Smoke Condensate.

Contractor's Project Directors: Dr. Michael R. Guerin
Dr. Roger A. Jenkins
Dr. Wayne H. Griest

Project Officer (NCI): Dr. Thomas B. Owen

Objectives: Activities carried out under this contract constitute physical and chemical resources to the National Cancer Institute and the Smoking and Health Program. This involves (a) providing quality-assured data on the deliveries of selected chemical constituents by commercial and experimental cigarettes, (b) providing validated methods for the quantitative determination of additional smoke constituents and for the assessment of smoke composition, (c) providing sampling and monitoring services to define exposures accompanying tobacco smoke inhalation exposure experiments, and (d) providing methods and data to establish the relationship of exposures and smoking conditions to the resulting dose of smoke constituents experienced by the smoker.

Major Findings: In one series of analyses, a total of 61 samples of 45 domestic commercial low and ultra-low tar cigarette brands were tested for smoke deliveries of tar, nicotine, carbon monoxide, and carbon dioxide. Forty eight of these received additional characterization including measurement of acrolein, oxides of nitrogen, or hydrogen cyanide.

The tar deliveries of these cigarette brands ranged from 6.7 mg/cig to "undetectable" (<0.1 mg/cig) by conventional analytical procedures. Lot-to-lot variation in tar deliveries was found for some brands, e.g., the Barclay King samples ranged from 0.5 to 1.0 mg tar/cig, and the Barclay 100, 1.0 to 3.7 mg tar/cig. There appears to be more cigarette-to-cigarette and lot-to-lot variation (particularly among early production runs) for the ultra-low tar cigarette brands than for higher tar delivery brands. This may result from a lack of uniformity in the ventilated filter dilution factor. Cigarettes are selected for smoking on the basis of cigarette weight and resistance-to-draw. Measurement of an additional cigarette physical parameter, ventilated filter dilution factor, could further define the characteristics of the cigarettes being tested, and may indicate if these lot-to-lot differences are attributable to differences in filter dilution factor.

One relatively new cigarette product (Free) was tested under routine procedures and delivered 6.7 mg tar/cig, and had no nicotine detectable by conventional methods. However, the carbon monoxide, carbon dioxide, acrolein, and hydrogen cyanide (but not oxides of nitrogen) deliveries were considerably higher than those for tobacco cigarettes of comparable tar delivery.

Five variants of an experimental cigarette containing a chitin or chitosan tobacco extender were received from the manufacturer. Forty to 45 percent of the tobacco from the standard experimental blend (SEB-IV) had been replaced with chitin (N-acetylglucosamine, the structural support material of the crustacean exoskeleton), or chitosan (deacetylated chitin) or other modifiers. Analyses

were conducted on the smoke deliveries of tar, nicotine, carbon monoxide, carbon dioxide, hydrogen cyanide and oxides of nitrogen.

Three main observations were noted. First, the use of any of the modifiers (chitin, chitosan, or carboxymethyl cellulose) appears to have increased the CO:tar ratio is greater than 1.0, as opposed to 0.7 for the standard blend alone. The higher ratio is more characteristic of filtered cigarettes than unfiltered varieties. Also, the substitution of chitin, or especially chitosan, for SEB-IV considerably increased the HCN deliveries. However, the substitution of chitin or chitosan did reduce the per cigarette deliveries of tar, nicotine, carbon monoxide, carbon dioxide, and oxides of nitrogen, as compared to the straight SEB-IV tobacco cigarette.

The data generated from the analysis of these cigarette smokes and others are being examined from the standpoint of smoke characteristics as a function of tar delivery to address the question of the comparative composition of smokes from conventional, low, and ultra-low tar delivery cigarettes. Basically, as tar delivery decreases going from the "normal" tar to the low tar and ultra-low tar brands, the deliveries of gas phase and whole smoke constituents also decrease. Because the cigarette puff numbers do not decrease, the per puff deliveries of these constituents also decrease. However, it is interesting to examine the constituent deliveries relative to tar. It is found that deliveries of nicotine and some gas phase constituents relative to tar generally increase as tar delivery decreases. Interestingly, hydrogen cyanide remains fairly constant relative to tar. The former effect is caused by the tar delivery decreasing more rapidly than that of the gas phase constituents. It appears to result in low and ultra-low tar cigarette smoke being relatively more enriched in gas phase constituents as opposed to tar. This effect is being examined further at this time.

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 that might be taken to reduce that potential. Chemical studies provide the methods and data by which biological activity may be related to constituents of the smoke and biological testing may be standardized. No other laboratory is engaged in general analytical chemistry and instrumental support of NCI tobacco smoke related studies.

Proposed Course: The contractor will continue to act as a tobacco smoke characterization and inhalation exposure facility for the National Cancer Institute by maintaining a core activity of analytical support services. Research activities are directed toward the development of a more cost-effective characterization of cigarette smokes and the identification and quantitative determination of smoke constituents and/or metabolites in physiological materials for tobacco smoke dosimetry in human beings.

Date Interagency Agreement Initiated: Aril 1, 1980.

Current Annual Level: $500,000

KAISER FOUNDATION RESEARCH INSTITUTE (NO1-CPO-5681)

Title: Surveillance of the Health Effects of Tobacco Products.

Contractor's Project Director: Dr. Gary D. Friedman

Project Officer (NCI): Dr. Thomas B. Owen

Objectives: This project is a prospective, epidemiologic study of the health effects of possibly less hazardous cigarettes. The contractor has continued to collect questionnaire information on past and current tobacco use, making progress toward their goal of enrolling into the study 80,000 ambulatory subscribers to a pre-paid medical care plan. The occurrence of hospitalized illness in members of this study population will eventually be ascertained in order to calculate the incidence rates of cancers and other serious illnesses in relation to the tar and nicotine content of the cigarette smoked at the time the questionnaire was completed. In order to obtain a ten percent random sample of the study population, information from the review of outpatient medical records is being retrieved. It will be used to study the relation of tar and nicotine level to the occurrence of conditions for which medical care is sought, but does not lead to hospitalization. A mailed, resurvey of a 10 percent random sample of last year's questionnaire respondents is in progress. This information will be used to assess the stability of smoking habits and cigarette brand preference in members of the study population.

As a separate project performed under this contract, a retrospective cohort analysis comparing mortality in persistent cigarette smokers with that in cigarette smokers who have quit smoking is being conducted.

Major Findings: Analysis of smoking questionnaire data showed that the mean number of cigarettes smoked per day was higher in smokers of low-yield cigarettes (tar<1.0 mg. per cigarette) than in smokers of high yield cigarettes at all ages and in both sexes. No differences in mean duration of cigarettes smoking or in age at starting to smoke were observed. These observations provide evidence for "compensation" in smokers of low-yield cigarettes.

Data analysis to date has shown the risk of dying from coronary heart disease among persistent smokers was 2.2 times that of smokers who quit ($p=0.004$), even after adjustment for baseline difference in cardiovascular risk. Adjustment for the higher baseline risk of persistent smokers, who smoked more cigarettes and reported more cardiovascular symptoms, was more than compensated by adjusting for the lower baseline risk since they were thinner and drank more alcohol. After similar adjustment, the risk of death from any cause in persistent smokers was 1.6 times that in quitters ($p<.001$).

In an analysis of data from health questionnaires completed at the same time that the smoking questionnaires were completed, there was no significant association of a history of peptic ulcer disease with tar or nicotine content of currently smoked cigarettes.

Significance to Biomedical Research and the Program of the Institute: This study addresses two program goals: (1) identification of individuals at high risk to tobacco-related diseases and (2) identification of toxic constituents of smoking products.

Proposed Course: Continue the collection and analysis of data from human subjects through the use of the self-administered questionnaire.

Date Contract Initiated: October 1, 1980.

Current Annual Level: $190,000

VETERANS ADMINISTRATION MEDICAL CENTER (Y01-CP8-0201)

Title: Inhalation Bioassay of Cigarette Smoke in Male Beagle Dogs.

Contractor's Project Director: Dr. Oscar Auerbach

Project Officer (NCI): Dr. Thomas B. Owen

Objectives: The objective of this study is to determine the extent of changes in the tracheobronchial tree, lung parenchyma and other organs of the body produced by smoking cigarettes of varying tar and nicotine content, whether these changes are less when cigarettes of progressively lower levels of tar and nicotine are smoked and whether there is a point to which tar and nicotine levels can be reduced so that only minimal changes result.

Major Findings: All animals have been sacrificed and histologic evaluation is now in progress. The preliminary findings do not indicate that any lung tumors have been produced by exposure to smoke. There are definite changes in lung tissue which can be correlated directly with the type of cigarette smoked by the individual animal. The least extensive changes occurred in those groups smoking cigarettes of lower tar and nicotine content, and a progressive increase in these changes seems to occur in those groups smoking cigarettes of higher tar and nicotine content.

Significance to Biomedical Research and the Program of the Institute: The endpoint of this study is not the production of lung cancer, but rather the study of developing pathology in various organs of the experimental animals to determine the effects of cigarettes of varying tar and nicotine content in the development of pulmonary and cardiovascular diseases. The contractor is attempting to determine the point to which tar and nicotine levels can be reduced so that only minimal changes will result from smoking cigarettes in the animal.

Proposed Course: Continue the histologic evaluation to determine the degree of change in the body systems of the dogs.

Date Contract Initiated: October 1, 1980.

Current Annual Level: $325,669

SPECIAL PROGRAMS BRANCH

GRANTS ACTIVE DURING FY 82

BIOMETRY PROGRAM

Investigator/Institution/Grant Number	Title
ACTON, Ronald T. University of Alabama 5 R01 CA 30467-02	Genetic Analysis of Melanoma
BARRON, Bruce A.* Columbia University 5 R01 CA 19145-06	Optimization of Screening for Cervix Cancer
BLOIS, Marsden S* University of California 5 R01 CA 26655-03	Natural Language Access to Clinical Data Bases
CECH, Irina* University of Texas Health Science Center 5 R01 CA 24138-02	Relation of Cancer Rates and Source of Drinking Water
CORREA, Pelayo Louisiana State University 5 P01 CA 28842-02	Etiologic Studies of Gastric Carcinoma
DUDEWICZ, Edward J.* Ohio State University 5 R01 CA 26254-03	A Model for Medical Treatments Evaluation
ELSTON, Robert C. Louisiana State University 5 R01 CA 28198-03	Statistical Genetic Analysis for Cancer Families
KLOTZ, Jerome H. University of Wisconsin 5 R01 CA 18332-08	Statistical Problems in Clinical Cancer Research
KNUDSON, Alfred G. Jr. Institute for Cancer Research 5 R01 CA 22780-05	Biomathematical Approaches to Cancer
KOZIOL, James A.* University of California, San Diego 5 R01 CA 26666-03	Topics in Biostatistics
LACHENBRUCH, Peter A.* University of Iowa 5 R01 CA 24089-05	Estimation of Prognosis Using SEER Data

LYNCH, Henry T. Creighton University 5 ROl CA 27831-03	Epidemiologic-Biologic Study of Colon Cancer Families
MACK, Thomas M. University of Southern California 1 ROl CA 32262-01	Determinants of Cancer Within Disease-Discordant Twins
MANTEL, Nathan American University 5 ROl CA 30205-02	Cancer Research: Statistical Methods
MIKE, Valerie* Memorial Sloan-Kettering Cancer Center 1 R13 CA 29801-01	Conference on Biostatistics in Clinical Oncology
MILLER, Kenneth J. Rensselaer Polytechnic Institute 5 ROl CA 28924-02	Computer Assisted Analyses of Carcinogenicity
MOOLGAVKAR, Suresh H.* Institute for Cancer Research 5 ROl CA 25588-02	Temporal Evolution of Cancer
MOOLGAVKAR, Suresh H. Institute for Cancer Research 5 ROl CA 30671-02	Malignant Melanoma Multifactorial and Stochastic Models
MYERS, George C. Duke University 5 ROl CA 23399-05	Certification of Cancer Related Deaths
NEEL, James V. University of Michigan 5 POl CA 26803-03	Program Project: The Study of Human Mutation Rates
PAFFENBARGER, Ralph S. Stanford University 2 ROl CA 25264-04	Early Predictors of Site-Specific Cancers
PAGANO, Marcello Sidney Farber Cancer Institute 5 ROl CA 28066-03	Statistical Computing and Clinical Trials of Cancer
PHILLIPS, Roland L. Loma Linda University 5 ROl CA 14703-09	Epidemiology of Cancer in Adventists - A Low Risk Group
PIERCE, Donald A. Oregon State University 5 ROl CA 27532-03	Statistical Methodology for Response-Time Data

PUNNETT, Hope H.* St. Christopher's Hospital for Children 5 ROl CA 19834-04	Genetic Constitution and Cancer Predisposition
SCHNEIDER, Robert University of California, Davis 5 ROl CA 14916-09	Animal Neoplasm Registry
SCHOENFELD, David A.* Sidney Farber Cancer Center 5 R23 CA 25162-03	Regression Analyses Techniques for Cancer Research
SKOLNICK, Mark H. University of Utah 5 ROl CA 28854-02	Genetic Epidemiology of Cancer in Utah Genealogies
STRONG, Louise C. University of Texas 5 ROl CA 27925-02	Genetic Etiology and Consequences of Childhood Cancer
STRONG, Louise C. University of Texas 1 ROl CA 32064-01	Identification of Genes Predisposing to Childhood Cancer
SWIFT, Michael R. University of North Carolina 5 ROl CA 14235-10	Neoplasia-Predisposing Genes of Man
TARTER, Michael E. West Coast Cancer Foundation 5 ROl CA 28142-02	Modern Functional Representation in Cancer Research
TSUTAKAWA, Robert K. University of Missouri 5 ROl CA 29765-02	Statistical Analysis of Cancer Mortality Rates
WEISS, Kenneth M. University of Texas Health Science Center 5 ROl CA 19311-06	Genetic Epidemiology of Cancer
WHITE, Colin Yale University 5 ROl CA 30931-02	Systematic Analysis -- Connecticut Cancer Incidence Trends
WHITTEMORE, Alice S. Stanford University 2 ROl CA 23214-04	Effects of Multiple Exposures-Quantitative Aspects
ZELEN, Marvin Sidney Farber Cancer Institute 5 ROl CA 23415-05	Statistical Models of Biomedical Phenomena

ZIMMERMAN, Stuart O.*
M.D. Anderson Hospital
and Tumor Institute
5 R01 CA 11430-17

Biomathematics and Computing
in a Cancer Institute

*Grants Active During FY 82 but Funded with Previous Year's Funds

SPECIAL PROGRAMS BRANCH

GRANTS ACTIVE DURING FY 82

EPIDEMIOLOGY PROGRAM

Investigator/Institution/Grant Number	Title
AMSEL, Jonathan* University of Pennsylvania 2 R01 CA 23544-03	Case-Control Study of Malignant Melanoma
ASAL, Nabih R. University of Oklahoma 5 R01 CA 31059-02	Risk Factors in Kidney Cancer
BEASLEY, R. Palmer University of Washington 5 R01 CA 25327-03	Hepatocellular Carcinoma Risk in Hepatitis B Carriers
BLUMBERG, Baruch Institute for Cancer Research 5 P01 CA 06551-19	Cancer Clinical Research at the Fox Chase Center
COMSTOCK, George W. Johns Hopkins University 5 R01 CA 24758-04	Cancer Studies in Washington County, Maryland
DALING, Janet R. University of Washington 1 R01 CA 32010-01	Epidemiology of Anal Cancer
DAVIS, Scott Fred Hutchinson Cancer Center 5 R23 CA 29395-02	A Case-Control Study of Hodgkin's Disease
DELAFRESNAYE, John F. International Union Against Cancer 5 R13 CA 05096-21	Program of the International Union Against Cancer
DODD, Roger American Red Cross 5 R01 CA 31002-02	Hepatitis B Surface Antigen as a Risk for Hepatocellular Carcinoma
DONHAM, Kelley J. University of Iowa 5 R01 CA 28626-03	The Epidemiology of Leukemia in Rural Iowa

EVANS, Alfred S. Yale University 1 ROl CA 30433-01	Epidemiological Studies of EBV in Hodgkin's Disease
FIALKOW, Philip J. University of Washington 5 ROl CA 16448-08	Human Cancer--Origin and Genetic Markers
FISCHMAN, Harvey R. Johns Hopkins University 5 ROl CA/OH 30410-02	Cancer Mortality Among Workers in the Meat Industry
FRANKEL, Jack W. Life Sciences Biomedical Research Institute, Inc. 7 ROl CA 32953-01	Neurofibromatosis: Study of Prenatal Diagnostic Test
FRIEDMAN, Gary D. Kaiser Foundation Research Institute 2 ROl CA 19939-06	Surveillance for Drugs that may be Carcinogenic
GRUFFERMAN, Seymour* Duke University 2 ROl CA 22104-04	The Epidemiology of Multiple Myeloma
GUTENSOHN, Nancy M. Harvard School of Public Health 1 ROl CA 31747-01	Hodgkin's Disease and Pre-Diagnostic EBV-Antibody Status
HENDERSON, Brian E. University of Southern California 2 POl CA 17054-07	Cancer Center Epidemiology and Biostatistics Support
HENDERSON, Brian E. University of Southern California 1 ROl CA 32197-01	The Role of Estrogens and Vitamin A in Disease Prevention
HERBST, Arthur L. University of Chicago 5 ROl CA 20084-06	Exogenous Maternal Hormones and Cancer in Daughters
HINDS, M. Ward University of Hawaii 5 ROl CA 30119-02	A 50,000 Member Cohort Study of Alcohol and Cancer
HOCHBERG, Fred H. Massachusetts General Hospital 5 ROl CA 22533-05	Epidemiology of Brain Tumors
HUTCHISON, George B. Harvard University 5 ROl CA 22849-05	Second Cancers in Patients with Hodgkin's Disease

HUTCHISON, George B.* Harvard University 5 ROl CA 24209-03	An Epidemiologic Study of Cancer of the Ovary
JOHNSON, Carl J. Medical Care & Research Foundation 3 ROl CA 32565-01S1	Evaluation of Low-Level Plutonium and Cancer
KESSLER, Irving I. University of Maryland 3 ROl CA 25019-04S1	Male Role in Cervical Cancer
KURLAND, Leonard T.* Mayo Foundation 5 ROl CA 25441-03	Study of Males Exposed in Utero to Diethylstilbestrol
LAWRENCE, Charles E.* New York State Dept. of Health 5 ROl CA 24367-03	Endometrial Cancer Epidemiology and Control
LITVAK, Jorge* Pan American Health Organization 1 R13 CA 30307-01	Cancer Epidemiology in Latin America (Meeting)
LOPEZ-S, Arthur* Louisiana State University 3 ROl CA 23205-03S1	Lung Cancer and Vitamin A
LYNCH, Henry T. Creighton University 5 ROl CA 27831-03	Epidemiologic-Biologic Study of Colon Cancer Families
MACK, Thomas M. University of Southern California 5 ROl CA 23927-03	Case-Control Study of Malignant Melanoma
MACMAHON, Brian Harvard University 5 POl CA 06373-21	Cancer Epidemiology and Prevention Research Center
MACMAHON, Brian Harvard University 5 ROl CA 29723-02	An Epidemiological Study of Renal Adenocarcinoma
MASHBERG, Arthur V.A. Medical Center, New Jersey Medical School 5 ROl CA 29214-02	Role of Alcohol as Primary Risk Factor in Oral Cancer

MEADOWS, Anna Children's Hospital of Philadelphia 5 ROl CA 29275-02	Heredity and Environment in Childhood Cancer
MOSS, Andrew R. Northern California Cancer Program 5 ROl CA 27752-02	Testicular Cancer and Prenatal DES Exposure
NASCA, Philip C. New York State Dept. of Health 5 ROl CA 26194-03	Epidemiologic Study of Childhood Gliomas
PRESTON-MARTIN, Susan University of Southern California 2 ROl CA 28215-03	Epidemiology of Tumors of the Parotid Gland
REEVES, William C.* Gorgas Memorial Institute of Tropical and Preventive Medicine 5 ROl CA 25419-03	Cervical Cancer Epidemiology in Panama
ROSS, Ronald K.* University of Southern California 5 ROl CA 24082-04	Immunoblastic Lymphadenopathy and Histiocytic Lymphoma
ROSS, Ronald K. University of Southern California 5 ROl CA 25669-04	Epidemiology of Cancer of the Renal Pelvis and Ureters
SAMET, Jonathan M. University of New Mexico 5 ROl CA 27187-03	Lung Cancer Etiology in New Mexico's Hispanics and Anglos
SPEIZER, Frank E.* Peter Bent Brigham Hospital 5 ROl CA 23645-05	A Prospective Cohort for Risks in Breast Cancer
STARK, Alice New York State Dept. of Health 5 R23 CA 29713-02	Cancer Incidence and Death from all Causes in Farmers
SZKLO, Moyses Johns Hopkins University 5 ROl CA 24757-04	Epidemiology of Aplastic Anemia in Baltimore
SZKLO, Moyses Johns Hopkins University 5 ROl CA 26500-04	Epidemiological and HLA Study of Leukemia
WALLACE, Robert B. University of Iowa 5 ROl CA 15104-09	Anovulation and Epidemiology of Hormone-Responsive Tumors

WEISS, Noel S.
Fred Hutchinson Cancer Research Center
5 R01 CA 23350-04

Epidemiology of Myeloma and Lymphocytic Leukemia

WEISS, Noel S.
University of Washington
5 R01 CA 30279-02

Vasectomy as a Risk Factor for Testicular Cancer

*Grants Active During FY 82 but Funded with Previous Year's Funds.

SPECIAL PROGRAMS BRANCH

GRANTS ACTIVE DURING FY 82

SMOKING AND HEALTH PROGRAM

Investigator/Institution/Grant Number	Title
CASTONGUAY, Andre American Health Foundation 1 R01 CA 32391-01	Tobacco-Specific Nitrosamine: RIA for DANA-Adducts
COLE, Philip University of Alabama 5 R01 CA 29968-02	Hepatocellular Carcinoma and Cigarette Smoking
HECHT, Stephen S. American Health Foundation 5 R01 CA 21393-06	Metabolism of the Carcinogen Nitrosonornicotine
HOFFMAN, Dietrich American Health Foundation 5 P01 CA 29580-02	Experimental Tobacco Carcinogenesis
JANERICH, Dwight T. New York State Dept. of Health 1 R01 CA 32088-01	Epidemiology of Lung Cancer in Nonsmokers
MCCOY, George D. Case Western Reserve University 1 R01 CA 32126-01	Role of Ethanol in the Etiology of Head and Neck Cancer

SPECIAL PROGRAMS BRANCH

GRANTS ACTIVE DURING FY 82

DIET/NUTRITION PROGRAM

Investigator/Institution/Grant Number	Title
ABRAHAM, Samuel Children's Hospital Medical Center 5 R01 CA 29767-02	Effect of Dietary Fat on Mammary Neoplasia
ASANO, Tomoaki University of Notre Dame 5 R01 CA 28276-02	Experimental Carcinogenesis by Dietary Nitrite
BIRT, Diane F.* University of Nebraska 5 R01 CA 24549-03	Influence of Dietary Selenium on Pancreatic Cancer
BLACK, Homer S. Baylor College of Medicine 2 R01 CA 20907-03A1	Effects of Dietary Factors on UVL-Carcinogenesis
CAMPBELL, T. Colin Cornell University 5 P01 CA 26755-03	Nutrition and Cancer
CHA, Young-Nam Johns Hopkins University 5 R01 CA 27594-03	Mechanism of Antimutagenesis by Anticarcinogens
CORWIN, Laurence M.* Boston University 5 R01 CA 26604-03	Effect of Vitamin E and Lipids on Tumorigenicity
DAO, Thomas L. Roswell Park Memorial Institute 5 R01 CA 26597-03	Dietary Fat and Mammary Carcinogenesis
DRAPER, Harold H. Univesity of Guelph 5 R01 CA 28242-03	Toxicity and Metabolism of Malondialdehyde
GRAHAM, Saxon State University of New York 5 P01 CA 11535-12	Social Epidemiology and Control of Cancer

GRAY, James I. Michigan State University 5 ROl CA 26576-03	Formation of N-Nitroso Compounds in Processed Food
HAMILTON, Stanley R. Johns Hopkins University 5 ROl CA 29714-02	Role of Beer and Ethanol in Experimental Colon Cancer
HAWRYLEWICZ, Ervin J.* Mercy Hospital and Medical Center 5 ROl CA 26547-02S1	Effect of Diet on the Hypothalamus and Breast Tumors
HEINIGER, Hans-Jorg Jackson Laboratory 5 ROl CA 19305-06	Cholesterol in Normal and Malignant Lymphocytes
HSIEH, Dennis P. University of California, Davis 5 ROl CA 27426-03	Comparative Toxicology of Carcinogenic Mycotoxins
IP, Clement C. Roswell Park Memorial Institute 5 ROl CA 27706-03	Selenium Supplement and Dietary Fat in Breast Cancer
JANGHORBANI, Morteza Massachusetts Institute of Technology 5 ROl CA 27917-03	Dietary Bioavailability of Selenium in Man
KING, M. Margaret Oklahoma Medical Research Foundation 8 ROl CA 34143-05	Dietary Fat and Mammary Carcinogenesis
KOLONEL, Laurence N. University of Hawaii 3 ROl CA 26515-03S1	Case-Control Study of Lung Cancer and Dietary Vitamin A
LE MAISTRE, Charles A.* University of Texas 1 R13 CA 28905-01	1981 Annual Symposium on Fundamental Cancer Research
MACKENZIE, Cosmo G. University of Colorado 5 ROl CA 27861-03	A Nutritional Control of Cancer
MEYSKENS, Frank L. University of Arizona 5 POl CA 27502-03	Vitamin A Program Project
MILNER, John A. University of Illinois 5 ROl CA 29462-02	Dietary Arginine and Tumor Growth and Development

MILNER, Max American Institute of Nutrition 1 R13 CA 32669-01	Research Opportunities in Diet and Disease (Symposium)
MUSEY, Paul I.* Emory University 5 R01 CA 24616-03	The Effect of Diet on Estrogen Biosynthesis and Metabolism
NEWBERNE, Paul M.* Massachusetts Institute of Technology 5 R01 CA 25382-03	Zinc, Nitrosamine, and Esophageal Cancer
NEWBERNE, Paul M. Massachusetts Institute of Technology 5 R01 CA 26917-03	Dietary Fat in Colon Carcinogenesis
PARIZA, Michael W. University of Wisconsin 5 R01 CA 29618-02	Structure and Origin of Mutagens in Fried Beef
PAULING, Linus C. Linus Pauling Institute of Science 5 R01 CA 26541-02	Diet and Breast Cancer in Mice
PAWLOWSKI, Norman E. Oregon State University 5 R01 CA 25766-04	Mechanisms for Biological Activity of Cyclopropenes
PETHICA, Brian A.* Clarkson College of Technology 5 R01 CA 26379-03	Dietary Fiber--the Physical Chemistry of Lignins
ROEBUCK, Bill D. Dartmouth College 5 R01 CA 26594-03	Modulation of Pancreatic Carcinogenesis by Diet
ROGERS, Adrianne E. Massachusetts Institute of Technology 3 R01 CA 25538-03S1	Dietary Fat, Prolactin and Mammary Cancer
ROSS, Morris H. Institute for Cancer Research 5 R01 CA 16442-08	Regulation of Tumor Susceptibility
ROTHMAN, Kenneth J. Harvard University 5 R01 CA 29666-02	Case-Control Study of Laryngeal-Hypopharyngeal Cancer
RUDOLPH, Frederick B. Rice University 5 R01 CA 14030-10	Regulation of Metabolism by Purine Interconversions

SARKAR, Nurul H. Sloan-Kettering Institute for Cancer Research 5 ROl CA 25679-03	Effect of Diet on Murine Mammary Tumorigenesis
SCANLAN, Richard A. Oregon State University 5 ROl CA 25002-12	Nitrosamines in Foods
SELIVONCHICK, Daniel P. Oregon State University 5 ROl CA 30087-02	Membrane Protein Composition: Cyclopropanoid Fatty Acid
SHILS, Maurice E. New York Academy of Medicine 5 ROl CA 32241-02	NY/NJ Regional Center for Clinical Nutrition Education
SHINOZUKA, Hisashi University of Pittsburgh 5 ROl CA 26556-03	Diet Modification and Promotion of Liver Carcinogenesis
SIDRANSKY, Herschel George Washington University 5 ROl CA 26557-03	Nutritional Influence on Chemical Carcinogenesis
SINNHUBER, Russell O. Oregon State University 5 ROl CA 20990-05	Protein Effects of Aflatoxin Carcinogenesis in Trout
SMITH, George S. University of California, Los Angeles 5 ROl CA 26164-03	Dietary Restriction, Cancer and Immune Functions
SPEIZER, Frank E. Channing Laboratory 2 ROl CA 26560-03	Prospective Study of Diet and Cancer in Women
TAMBURRO, Carlo* University of Louisville 5 ROl CA 25602-03	Dietary Modifications, Exercise and Tumor Growth
THOMPSON, Henry J. University of New Hampshire 5 ROl CA 28109-03	Nutrition and Mammary Carcinogenesis
TROLL, Walter New York University Medical Center 5 ROl CA 16060-11	Inhibition of Tumor Promotion by Protease Inhibitors
VISEK, Willard J. University of Illinois 5 ROl CA 28629-03	Hormones, Dietary Fat and Mammary Carcinogenesis

WADE, Adelbert E.* University of Georgia 5 R01 CA 29583-02	Effect of Dietary Fat Type on Chemical Carcinogenesis
WARREN, Guylyn R. Montana State University 5 R01 CA 26647-03	Mutagenic/Carcinogenic Agents in Body Fluids of Children
WEISBURGER, John H. American Health Foundation 5 P01 CA 29602-02	Nutritional Carcinogenesis
WEST, Dee W. University of Utah 5 R01 CA 25580-03	Diet and Colon Cancer in Man: The Effects of Fiber
ZAMIR, Lolita O. State University of New York 5 R01 CA 32131-02	Biochemical Intermediates of Aflatoxin Biosynthesis

*Grants Active During FY 82 but Funded with Previous Year's Funds.

SPECIAL PROGRAMS BRANCH

GRANTS ACTIVE DURING FY 82

PREVENTIVE ONCOLOGY ACADEMIC AWARD PROGRAM

Investigator/Institution/Grant Number	Title
ALBERTINI, Richard J. University of Vermont 5 K07 CA 00743-03	Preventive Oncology Academic Award
BRESLOW, Norman E. University of Washington 5 K07 CA 00723-03	Preventive Oncology Academic Award
ENSTROM, James E. University of California, Los Angeles 5 K07 CA 00748-02	Preventive Oncology Academic Award
GRUFFERMAN, Seymour Duke University Medical Center 5 K07 CA 00726-03	Preventive Oncology Academic Award
LOVE, Richard R. University of Wisconsin 5 K07 CA 00721-03	Preventive Oncology Academic Award
RUSSELL, Diane H. University of Arizona Health Sciences Center 5 K07 CA 00732-03	Preventive Oncology Academic Award
SANDLER, Robert S. University of North Carolina 5 K07 CA 00722-03	Preventive Oncology Academic Award
SCHOTTENFELD, David Memorial Hospital for Cancer 5 K07 CA 00727-03	Preventive Oncology Academic Award
STELLMAN, Jeanne M. Columbia University 5 K07 CA 00730-03	Preventive Oncology Academic Award

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