Division of

CANCER CAUSE AND PREVENTION

Volume II
October 1, 1982-September 30, 1983

U.S. DEPARTMENT
OF HEALTH
AND HUMAN SERVICES

National
Institutes of
Health

National
Cancer
Institute

National Cancer Institute

ANNUAL REPORT

DIVISION OF CANCER CAUSE AND PREVENTION

NATIONAL CANCER INSTITUTE (U.S.)

October 1, 1982 through September 30, 1983

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ANNUAL REPORT OF

THE FIELD STUDIES AND STATISTICS PROGRAM

NATIONAL CANCER INTSTITUTE

October 1, 1982 through September 30, 1983

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 cancer; coordinates a network of population-based cancer registries for evaluating cancer incidence, mortality, and survival in the United States; analyzes the natural history of cancer and the efficacy of therapeutic and preventive measures; and designs statistical models for clinical and experimental investigations.

Dr. Joseph F. Fraumeni, Jr. continued as the Associate Director for Field Studies and Statistics, while Dr. Robert N. Hoover was appointed this year as Acting Chief of the Environmental Epidemiology Branch. The other components of the program are the Biometry Branch (Chief, Dr. Earl S. Pollack) and the Clinical Epidemiology Branch (Chief, Dr. Robert W. Miller). In May 1983 a site visit was made to the Environmental Epidemiology Branch by the DCCP Board of Scientific Counselors, and in FY 1984 site visits are planned for the Biometry and Clinical Epidemiology Branches. In the annual reports herein the Branch Chiefs have summarized the research and other activities taking place this year. In this report the general orientation, some research highlights, and future direction of the FS&S program are briefly outlined.

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. During the early years of operation of the SEER Program, a network of population-based cancer registries which cover about 10% of the U.S. population, emphasis was placed on the analysis of incidence and mortality statistics. This year, with accumulation of sufficient follow-up information, the first report on cancer patient survival was published from the SEER Program for patients diagnosed from 1973 to 1979 and followed through 1980. For various sites of cancer the observed and relative survival rates were summarized by race, sex, age and time period. The overall five-year relative survival rates for all sites combined were 47% for whites and 35% for blacks. For certain sites the black-white differences in survival were substantial and have stimulated collaborative studies to identify factors responsible for ethnic differences in natural history and prognosis. Because of insufficient coverage of black and Hispanic populations in the SEER program, another registry will soon be added with appropriate ethnic representation. Data from the SEER Program were used extensively this year by the NCI Director to indicate progress as well as problems requiring special attention in cancer research and control. Toward this end, an NCI-wide advisory committee was formed to more fully utilize the SEER resource for various purposes, such as identifying national objectives and target areas for intervention as well as monitoring the impact of such programs.

With the continuing maturation of the SEER Program, systematic and comprehensive analyses of collected data are planned, including detailed survival tables by cancer site, cell type, extent of disease, initial treatment, race, sex, and age. In addition, an analysis of time trends through 1980 in cancer incidence and mortality will be completed for the five areas common to the Second National Cancer Survey (1947-48), the Third Survey (1969-71), and three points in the SEER Program (1975-76, 1977-78, 1979-80). Surveillance of the SEER data for occurrence of multiple primary cancers is also being actively pursued, with emphasis on treatment-induced second cancers and multiple primaries that may share etiologic factors. Since non-melanoma skin cancer is not covered routinely by cancer registries, a special incidence survey was carried out in 1977-78 in various areas of the country, and the results published this year in a monograph. In the past a county-by-county survey of cancer mortality in the United States identified geographic patterns that have provided etiologic clues for more definitive study, and updated maps of cancer mortality are now being completed for the more common tumors.

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 and asbestos exposures during World War II as the explanation for the high rates of lung cancer in several areas along the Atlantic coast. In southern Louisiana, the high rates of lung cancer appeared at least partly due to smoking habits, particularly the heavy use of hand-rolled cigarettes. The use of smokeless tobacco accounted for the elevated rates of oral cancer among women in southern rural areas, and the study also suggested relationships to the use of mouthwash, work in the electronics industry, and dietary deficiencies. A cluster of colorectal cancer in rural Nebraska was linked to a concentration of Czechoslovakian migrants, especially those with high fat diets and familial occurrence of digestive tract diseases. The high rates of renal adenocarcinoma in the north central region appeared related to ethnic factors (especially German ancestry), cigarette smoking, and obesity and dietary habits, particularly in females. A parallel study of renal pelvis cancer in this area indicated a substantial influence of cigarette smoking, plus an assocation with phenacetin-containing analgesic drugs and occupational exposures previously implicated in bladder cancer. A case-control study of nasal cancer in North Carolina and Virginia pointed to the role of smoking, chronic nasal disease, and occupational exposure to wood dusts, chromates, chemicals and textiles. A large case-control study of bladder cancer which previously evaluated the risk of artificial sweeteners is also being analyzed with respect to a number of other potential risk factors. Of special interest is an elevated risk of bladder cancer found among truck drivers, especially those using diesel engines.

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; lung cancer and leukemia among pesticide applicators; leukemia and non-Hodgkin's lymphoma in agricultural workers; pancreas cancer in workers involved in corn wet milling and in press photographers; lung cancer in various groups exposed to asbestos, in steel and foundry workers exposed to polycyclic 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 in contact with formaldehyde during manufacturing and usage is underway. Preliminary surveys of morticians have revealed no excess of nasal cancers as observed in animal bioassay studies of formaldehyde, but have suggested increased mortality from brain tumors, colon cancer, and leukemia. A systematic evaluation of occupational risks of cancer, with adjustment for smoking habits, is being carried out among participants of the Veteran's Followup Study initiated many years ago by Harold Dorn.

Radiation studies received further emphasis in efforts 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 less effective in inducing leukemia than other radiation exposures that have been studied, perhaps related to the cell-killing potential of high-dose radiation to the pelvis. However, a slight risk was found that may be associated with low-dose radiation received by marrow outside the pelvis. A survey of breast cancer among atomic bomb survivors revealed elevated risks among women exposed at younger ages, and for the first time women exposed under the age of 10 showed a dose-related excess risk. A follow-up of patients with non-Hodgkin's lymphoma revealed an elevated risk of acute non-lymphocytic leukemia, strongly correlated with radiation dose to the bone marrow. In a follow-up of children irradiated for enlarged tonsils, preliminary findings suggest a two-fold risk of thyroid nodules and persistent chromosome aberrations in circulating lymphocytes. A case-control study of thyroid cancer has revealed an elevated risk associated with radiotherapy for benign head and neck diseases in childhood, and a study of childhood cancer in twins indicated a two-fold excess risk associated with prenatal x-ray, suggesting that the association is due to radiation rather than the indications for pelvimetry. A survey of children who developed multiple primary cancers revealed dose-response relationships between radiation exposure and the risk of developing second cancers. Among children irradiated for ringworm of the scalp in Israel, preliminary data suggest elevated risks of thyroid cancer, brain tumor and leukemia. In collaborative studies with the Radiation Effects Research Foundation in Japan, current emphasis is being placed on the analysis of case-control interview studies of breast and lung cancers in efforts to evaluate interactions of radiation with other risk factors.

Drug studies were continued to evaluate the effects of estrogenic compounds, which appeared related to the risk of breast cancer in certain high-risk groups, including women with familial predisposition or benign breast disease. No excess risk of breast cancer was associated with thyroid medications or diazepam (Valium) as previously suggested, and patients with Hansen's disease showed no increased risk of cancer that could be linked to the use of dapsone, a carcinogen in animal studies. Among patients with cancer or rheumatologic diseases treated with various alkylating agents, there was a substantial elevated risk of acute non-lymphocytic leukemia. Most recently, methyl-CCNU, a nitrosourea used in cancer chemotherapy, was linked to acute leukemia and pre-leukemia.

Nutritional studies were intensified this year to clarify the role of dietary constituents in cancer etiology. Several studies have utilized 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. 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 in oral cancer. Case-control studies of various cancers are underway to measure the intake of various micronutrients, both by interview about usual dietary patterns and by laboratory assays of serum samples. FS&S investigators have continued to develop and utilize national resources, including HANES I, the first Health and Nutrition Examination Study of the U.S., in an effort to relate dietary habits with the subsequent risk of cancer.

Family and genetic studies, enhanced by collaborative ties with laboratory investigators and a computer-based data resource, have resulted in delineation of familial cancer syndromes and several leads to mechanisms of host susceptibility. For example, the discovering of the dysplastic nevus syndrome has provided a marker of susceptibility to melanoma, enabling early detection and treatment of this potentially lethal cancer. Surveys of neurofibromatosis and other hereditary syndromes have helped clarify the risks of various cancers, and have explored the role of several genetic markers. Studies of a familial disorder featuring sarcomas and other neoplasms have led to the discovery of in vitro cellular radioresistence in this syndrome. The Interinstitute Medical Genetics Clinic, directed by two staff members, provides a multidisciplinary setting for studying families and individuals prone to cancer. The repository of cancer-prone families in the program is now of special interest to experimentalists involved in the identification of human oncogenes.

Environmental pollutants were evaluated through epidemiologic studies that have utilized relevant environmental and body measurements, whenever possible. To test the hypothesis that arsenical air pollution may be related to lung cancer, a case-control interview study was carried out in the vicinity of a large zinc smelter. An elevated risk was found among people living near the smelter and in areas with high soil levels of arsenic, even after controlling for the effects of smoking and occupation. Also underway is a case-control study of bladder cancer to investigate the role of halogenated hydrocarbons in drinking water, a relationship initially suggested by geographic correlation studies.

Infectious agents received substantial attention as the program became heavily involved in investigating the epidemic outbreaks of AIDS (Acquired Immune Deficiency Syndrome), which predisposes to Kaposi's sarcoma and opportunistic infections. Studies have focused on the epidemiologic, immunologic, and virologic characterization of certain high-risk groups, including male homosexuals and patients with hemophilia. In collaboration with the NCI Laboratory of Tumor Cell Biology, a series of investigations has been initiated to evaluate the role of a newly discovered human retrovirus in the origins of T-cell leukemia, which is endemic in certain parts of the world, including Japan and the West Indies. Preliminary evidence also suggests that this retrovirus may also be involved in the development of AIDS. Also conducted this year were studies to clarify the role of the Epstein-Barr virus in Burkitt's lymphoma and nasopharyngeal cancer, and of herpes virus type II and papilloma virus in cervical cancer.

Multidisciplinary projects combining epidemiologic and experimental approaches have been emphasized whenever possible to evaluate the influence of oncogenic viruses, dietary and metabolic factors, host susceptibility, air and water pollutants, and a wide variety of other risk factors that are likely to escape detection until new laboratory probes are integrated with epidemiologic investigations.

Collaborative Activities

Collaborative studies with other Federal agencies continued to receive high priority to (1) evaluate urgent issues including those of immediate regulatory or public policy concern, and (2) stimulate the epidemiologic application of technical and data resources that are used by the Government mainly for other purposes. Although many research and regulatory agencies are concerned with environmental cancer, few have epidemiologic programs and require assistance and support on many issues. Particularly at this time of fiscal constraint, 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. During the year staff members were active in the further development and adaptation of the National Death Index located at the National Center for Health Statistics (NCHS). Record-linkage studies have been planned in efforts to modify and utilize data on occupational exposure and cancer mortality from several agencies, including the Social Security Administration, Internal Revenue Service, Bureau of Census, and NCHS. Staff members also provided advice on modifying the internal revenue code to enlarge opportunities for epidemiologic studies of occupational groups, and on creating an enclave of Federal statistical agencies that could exchange data and ease limitations on the appropriate research uses of individually identifiable records.

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. Feasibility studies have been started in China on cancers of the esophagus, lung and stomach, trophoplastic neoplasms, and T-cell leukemia. In Italy a collaborative case-control study of stomach cancer has been developed to identify reasons for the high rates in the northern part of the country.

Within the Institute, further steps were taken to improve the 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, prepaid health plans, and other resources, FS&S staff became increasingly involved in coordinating case-control and other analytical studies that involve collective approaches with the pooling and sharing of data with outside investigators. In addition, FS&S staff were involved this year with coordinating the preparation of several comprehensive and critical reviews, including reference volumes on cancer epidemiology and prevention, radiation carcinogenesis, cancer mortality patterns, cancer staging, area-wide chemical contamination, and carcinogenic hazards to children.

Biometric Studies

The development of basic and applied statistical methodology in FS&S has contributed greatly to several areas, including epidemiology, carcinogenesis research, therapy trials, and screening programs. Special attention was given to the development and testing of multi-cause and multi-stage models of carcinogenesis. For example, the temporal aspects of lung cancer patterns in a cohort of copper smelter workers suggested that inorganic arsenic acts in the manner of a promoting agent, perhaps explaining why laboratory studies have failed to demonstrate the carcinogenicity of arsenic in the face of positive epidemiologic findings. This year several staff members are contributing to the development of congressionally mandated "radioepidemiology tables" to be used as a guide to the probability that radiation is responsible for cancers which develop among persons exposed to radioactive fallout. There was also 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 and evaluating programs for the screening and early detection of cancer. With the expansion of applied prevention programs in the Division of Resources, Centers, and Community Activities (DRCCA), steps were taken to establish working relationships and some collaborative projects. Emphasis is being given to joint studies in the area of nutrition and chemoprevention, and in the examination and utilization of the SEER Program for a wide variety of projects aimed at cancer etiology, prevention, and control.

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, the FS&S program 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 continuing 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. Guidance into the direction and nature of these programs will be provided after a series of site visits this year by the DCCP Board of Scientific Counselors.

Despite substantial growth and support of the intramural epidemiology program over the past several 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 we need to maintain our capability to gather, process, analyze, and report large-scale descriptive data on cancer statistics such as provided by the SEER Program and the NCHS. It is also clear that more analytical epidemiologic efforts are needed to pursue etiologic clues, and identify the life-style and other environmental factors that are carcinogenic in 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, greater efforts will be made to incorporate biochemical and molecular probes of exposure, response, and mechanisms of action. Studies of cancer-prone families provide exceptional opportunities to apply new molecular techniques, including those indicating the presence of human oncogenes. The AIDS epidemic and the study of T-cell leukemia will continue to receive intensive study by linking epidemiology with immunologic and virologic probes. In addition, more attention will be given to the less common neoplasms, involving collaborative case-control studies in several areas or centers, often utilizing the network of SEER registries. Throughout the program, further efforts will be made to ensure that data from epidemiologic studies are utilized for critical evaluation of methodology and development of more efficient approaches.

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. In this unique function the effectiveness of FS&S depends upon our success in promoting interaction and coordination with other parts of the Institute.

ANNUAL REPORT OF

THE BIOMETRY BRANCH

NATIONAL CANCER INSTITUTE

October 1, 1982 through September 30, 1983

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 sub-groups; 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 following is a brief summary of the program as it developed during the year.

Surveillance, Epidemiology and End Results (SEER) Program

The basic operations of the SEER Program are carried out by Dr. John Young and his Demographic Analysis Section. This 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. During the first few years of operation of the SEER Program emphasis was placed on analysis of cancer incidence data because this information could be made available shortly after the end of the given data year. For survival data, on the other hand, sufficient time must elapse to allow for the accumulation of follow-up information for several years after the date of diagnosis. Thus, the first report on cancer patient survival from the SEER Program was published this year. This revealed that the overall 5-year relative survival rate for all sites combined for white patients was 47% while the corresponding rate for black patients was only 35%. Although part of this difference may be due to a difference between the two races in site distribution, the difference in survival rate for certain sites is substantial. For example, for cancer of the corpus uteri, the 5-year relative survival rate for white patients was 87% compared with only 54% for black patients. The corresponding difference for cancer of the prostate was 64% for white men versus 54% for black men and for urinary bladder, 71% for white patients versus 43% for black patients. These large differences have become the subject of a further study to attempt to identify factors accounting for these differences. This will be a multi-institution collaborative study carried out through contracts. The contract selection process is now proceeding. The paper on survival rates presents data by age and sex for whites and for blacks. A more detailed analysis of cancer patient survival is now being carried out and will be published in the form of a monograph, with the data being made available either in the form of preprints or in unpublished form well in advance of the monograph publication. This will include analysis of survival rates according to extent of disease at time of diagnosis and according to a crude classification of treatment for each specific cancer site. It will also contain data on survival rates for each of the various ethnic groups.

Another registry is being added to the SEER Program to correct for a deficiency in coverage of the black and Hispanic populations. One of the requirements for this registry is that the area it covers must contain at least 300,000 blacks and 300,000 Hispanics. Five proposals have been received in response to the RFP and they are now in the review process.

Data from the SEER Program were used extensively during this past year by the Director of NCI, both for the appropriations hearings and for other purposes including his interaction with the National Cancer Advisory Board. There are now plans to use the SEER mechanism as part of the process of identifying target areas for intervention programs and for monitoring the impact of such programs once they have been put into place. The SEER Program will play a crucial role in this process but its precise nature has not yet been clearly defined.

Clinical and Diagnostic Trials

Dr. David Byar and his staff continue to provide consultation on a number of large clinical trials during the year. These involve assisting the investigators in developing detailed study protocols, in determining the number of patients necessary for the study, in deciding what data should be recorded and at what intervals in time, and in developing forms for recording of the data. Further, they advise on proper methods of analysis of the final data and in many cases undertake these analyses themselves. In situations where appropriate analytical tools are not available, they develop the statistical methodology themselves. Many of these developments are published in the major statistical journals.

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

1) During the year Dr. Byar and Mr. Corle used data on over 4,000 patients with prostate cancer from randomized clinical trials conducted by the Veterans Administration Cooperative Urological Research Group between 1960 and 1975, for an analysis of prognostic variables for prostate cancer using a multivariate survival model. They found that size of primary lesion, histological grade, acid phosphatase level, and use of endocrine treatment were predictive for time until tumor progression for stage I and stage II patients. For stage III and IV patients, on the other hand, they found that size of tumor, histologic grade, acid phosphatase level, presence of ureteral dilatation, the presence of metastases and the use of hormone therapy were prognostic for cancer death.

2) Dr. Green is responsible for the study design and analysis for a nationwide randomized trial for testicular cancer comparing adjuvant chemotherapy following surgery for resectable stage II disease with chemotherapy used only for relapses. Patient accrual is continuing and the data from this study are being used not only to compare treatment approaches but to identify factors that predict which patients will be found to have nodal involvement and to identify patients with increased risk of recurrence.

3) Funding for the Lung Cancer Study Group is in the process of being converted from a grant to a cooperative agreement. Three members of the staff continue to serve as the statistical consultants for this group. If funding is approved, the number of centers will increase from five to eight and the number of protocols will increase to twelve with an accrucal of over 500 lung cancer patients per year. The primary emphasis of these protocols is the study of

adjuvant chemotherapy, radiotherapy or immunotherapy in patients with resected lung cancer.

4) The Section maintains a file on some 3,600 women with breast cancer, many of whom had estrogen receptor assays performed. Mr. Corle and Dr. Byar presented some results of non-randomized but adjusted comparisons at the fall meeting of the Breast Cancer Task Force, indicating that adjuvant therapy was beneficial but that some of the adjuvant studies were so small that they lacked sufficient power to detect important differences. They cautioned, however, that these results cannot be interpreted as though they were derived from randomized trials. Serum is being collected from women with benign breast disease and asymtomatic controls as well as from patients with newly diagnosed breast cancer. Dr. Byar's group is responsible for collection, editing and analysis of all of the data and for providing an updated inventory of material in the serum bank.

5) Dr. Byar and his group continued to be involved in a number of additional studies including work with the Brain Tumor Study Group on the design and analysis of a number of large-scale randomized clinical trials, a study of the possible relationship between personality factors and fibrocystic and malignant breast disease, continuing work on two studies of the Makari skin test, and several other studies.

6) Members of the staff of this section were very active during the year in developing statistical methodology for applications to clinical trials and other related problems. These included the following: logistic regression methods for analyzing data consisting of a sequence of binary responses for each individual that permit adjustment both for initial and for time-dependent covariates; two different approaches to the problem of comparing different staging systems for chronic disease; methods for analyzing survival data appropriate for situations in which censoring mechanisms may not be independent of the death process; methods for dealing with non-independent censoring where the characteristics of patients entering a study change over time and where biased follow-up occurs due to underreporting of death; methods for constructing adjusted survival curves and estimating their variances; estimation of attributable risk for multiple factors; and a number of other methods.

Mathematical Statistics

Dr. John Gart and his Mathematical Statistics and Applied Mathematics Section has continued to provide statistical support and consultation to intramural scientists in the various programs throughout NCI. This involved basic study design as well as data analysis. Using some of the problems that arise out of this consulting work as a stimulus, this section develops basic statistical methodology to deal with these and related problems but in a way that has more general applicability. In many instances this group develops the computer software necessary to apply this methodology.

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

1) Dr. Gart and Mr. Thomas have completed work on a computer program to analyze dose trends in both proportions and lifetable data. This methodology and the

associated computer program has been applied to data from cohort studies in both Norwegian and Hawaiian Japanese populations.

2) The section has developed statistical methodology covering a wide spectrum of problems during the past year. The results of much of this research have led to papers that have already been accepted for publication. Included in this research are analysis of frequency data and distribution-free tests for censored distributions, methods for analyzing survival curves produced by the in-vitro exposure of cell cultures to DNA-damaging agents, efficiency of non-iterative estimators of a common relative risk or a common odds ratio, properties of the relative risk for case-control studies with multiple matched controls, higher order corrections to the mean and higher moments of the logit transformation of binomial proportions and their use in weighted least squares.

3) The section has been active in a wide range of statistical consultation during the year. Some examples of these include the following: collaboration on the two Norwegian studies -- at the University of Bergen and the University of Minnesota -- involving the analysis of cancer incidence and mortality in relation to dietary factors; collaboration on the non-melanoma skin cancer study for the analysis of trends and seasonality in incidence; evaluation of data used to test for the presence of antibodies to the human T-cell leukemia virus; collaboration on the analysis of data on Chinese nasopharyngeal cancer cases in Singapore in relation to Epstein-Barr virus antibody level and survival; estimation of adduct removal rates from data on rat liver repair in animals that have been fed 2-AAF; statistical analysis of mouse skin painting experiments to investigate the mechanisms of conversion of papillomas to squamous cell carcinomas; a study of the relation of dietary fat to pancreatic cancer in animal studies; collaboration on a variety of studies involving cellular biology and on a large number of other studies. Many of these have led to collaborative publications in which the staff of the section provides most of the statistical input.

Analytic Studies

Dr. Max Myers and his Biometrics Research and Analytic Studies Section conduct research in cancer etiology, screening for early detection of cancer, factors related to prognosis and in the statistical methodology related to each of the areas. Considerable emphasis is placed on the application of statistical methodology to the analysis of data from the SEER Program. Several of these analyses have led to hypotheses which in turn have led to the development of specific studies that are now underway. These include the possible adverse effects of specific cancer treatments in producing subsequent cancers, factors associated with large differences in survival following treatment between black and white patients, and a study of hormonally related primary cancers and the subsequent diagnosis of a second hormonally related primary cancer. The following is a brief summary of some of the work carried out in this section during the year:

1) Work has continued during the year on the multi-institution study of morbidity among long-term survivors of childhood cancer and their offspring. The data collection phase was completed during the year and the data are now being processed for detailed analysis. Some preliminary tabulations of the interview data revealed that 28% of the cases indicated they were unable to work due to health problems compared with only 11% of the controls. The proportion

indicating that they had never married due to health problems was 22% for the cases versus 3% for the controls. A somewhat larger proportion of the controls perceived their health to be good to excellent than did the cases -- 88% versus 74%. The detailed analyses will examine differences between cases and controls for a number of possible outcomes.

2) A study of factors associated with large differences in survival between black and white patients was designed during the year. The primary focus of the study will be on cancers of the uterine corpus and the urinary bladder which show the greatest difference in surival between the two races. Also included will be cancers of the colon and breast for which the differences are not as great. Contract proposals for the data collection centers and the coordinating center have been received and are now in the review process. Work on the project will get underway later this year.

3) Analysis of data from the National Bladder Cancer Study revealed that truck drivers and delivery men have a significantly increased risk of bladder cancer. There was a consistently increasing risk of bladder cancer with increasing duration of employment with a peak occurring among those employed 15-24 years and then a lower risk among those employed 25 years or more.

4) Work has continued on a descriptive study of cancers of the stomach, colon and rectum among Puerto Ricans in New York City in collaboration with the Epidemiology Unit at Memorial Sloan Kettering Cancer Center. The basic issue is whether findings from an earlier analysis of mortality data showing essentially no increase in risk of colon cancer mortality among Puerto Rican born migrants to New York City can be substantiated by later mortality data and also by data on cancer incidence both in New York City and Puerto Rico. Work on identification of cancer cases among Puerto Rican born residents of New York City in selected New York City hospitals, based on listings from the Cancer Registry in the New York State Health Department is nearing completion. Preliminary estimates seem to indicate that the registry underestimates the number of new cases by about 20%. Computer tapes for both cancer incidence cases and deaths from Puerto Rico have been received at Memorial Sloan Kettering Cancer Center and linkage of new New York City cases against these files will be carried out to determine the extent to which the same cancer patient is seen in both places. Pending preliminary results from this pilot study, a case-control study of these cancers in New York City and Puerto Rico will be designed.

5) Work has continued on the analysis of trends in cancer incidence and mortality using SEER data in conjunction with those from the earlier national cancer surveys. Five registries, covering areas for which these data were available from the period around 1950 through the late 1970s, were used (Atlanta, Connecticut, Detroit, Iowa and San Francisco). A paper presenting the methodology for this analysis, the rationale for using the five areas, and the application of this approach to seven major cancers has been submitted for publication. A detailed publication of trends for a large number of primary sites is being planned for later this year when revised population data for the 1970s will be available. An analysis of trends in lung cancer incidence from the late 1940s to the late 1970s was carried out and revealed that age-adjusted incidence rates for males had virtually levelled off in recent years while the rate of increase of the rates among white females has declined.

6) Work has continued on the study of multiple primary cancers using historical data from the Connecticut Tumor Registry and more recent data from the total SEER Program. For the latter data, on more than 400,000 patients diagnosed during 1973-1980, significant leukemia excesses were observed following chemotherapy for all first primary sites combined and for breast cancer, ovarian cancer and multiple myeloma in particular. Patients treated with radiation but without chemotherapy were also found to be at increased risk of second leukemias for all sites combined and specifically for cancer of the uterine corpus. A number of additional analyses of these data have been carried out and more are planned during the coming year.

7) Work has begun on a study of hormonally related first primary cancers and a subsequent diagnosis of a second hormonally related cancer. Preliminary findings suggest that females 45-54 years of age at diagnosis of first primary breast cancer have a higher risk of a subsequent primary cancer of the ovary, colon or second breast compared with females of other ages with a first primary breast cancer.

A number of other studies have been carried out during the year including an analysis of the mesothelioma cases reported through the SEER Program, a study of urban versus rural cancer incidence for Iowa and Colorado during 1969-71 and Iowa for the period 1973-78, several analyses aimed at comparing data on patients reported from the SEER Program with those seen in comprehensive cancer centers and reported to the Centralized Cancer Patient Data System, a detailed analysis of patient survival following diagnosis of Hodgkin's disease, further development of statistical theory for evaluation of screening programs for the early detection of cancer, and further work on the study of lung cancer among uranium miners.

International Studies

The contracts for cohort studies in foreign and migrant populations are nearing termination. The Japan-Hawaii Cancer Study will continue as a grant at the end of this year. It is not likely, therefore, that further analyses of the cohort study data will be initiated by the Biometry Branch, but it is conceivable that, as possible hypotheses arise that could be tested using that population, further collaboration might result. Meanwhile, the analysis of the relationship between alcohol consumption and the five most frequently occurring cancers in that population has been completed and the paper presenting the results has been submitted for publication. The findings indicated a strong dose response relationship between beer consumption and rectal cancer and a suggestion of a relationship between other forms of alcohol and lung cancer. Although the financial support for both the cohort study in Norway and the Lutheran Brotherhood Study in Minnesota will be coming to an end shortly, copies of the datasets from both studies will reside in the Biometry Branch where further analyses will be carried out. The relationship between vitamin A and vitamin C and lung cancer was studied in the Lutheran Brotherhood cohort and the finding of a significant negative relationship, after ten years of follow-up, disappeared when follow-up was extended an additional five years, although the suggestion of a negative relationship was still present. A similar negative association was found in the Norwegian cohort study.

Dr. King and Ms. Locke have continued their analyses of cancer incidence and mortality among Chinese populations. These include a number of populations

within the Peoples Republic of China as well as Singapore, Hong Kong and some of the Chinese populations in the United States -- primarily those in Hawaii, San Francisco and Los Angeles. For the first time, it was possible to compute survival rates among the Chinese populations in San Francisco and Hawaii based on the SEER data, using race-specific lifetables to obtain relative survival rates for these populations. In general, the survival rates for the Chinese are somewhat higher than those among whites and the survival rates among the Hawaiian Chinese are higher than those among the San Francisco Chinese. Further analyses of these differences, taking into account other variables such as age and extent of disease at diagnosis, will be carried out during the year.

Skin Cancer

A series of studies on non-melanoma skin cancer in the United States are being carried out by Joseph Scotto and Thomas Fears. An analysis of incidence data over time in two locations (San Francisco-Oakland and Minneapolis-St. Paul) reveals that the incidence of non-melanoma skin cancer is increasing at about 3% per year. The risk for males is about twice that for females and the amount of skin cancer among the non-Caucasian races is negligible. A study of the relationship between psoriasis and skin cancer is being carried out in collaboration with an investigator at Harvard University. The study indicates that those with psoriasis are at increased risk of developing skin cancer even after adjusting for certain host and environmental factors. Recent analyses of the dose-response relationship between ultraviolet radiation and skin cancer indicate that a 1% increase in UV-B may result in somewhat less than 2% increase in skin cancer. Further analyses, taking into account such factors as age, sex, cell type and geographic area, suggest that UV-B may be both a promoter and an initiator. Additional analyses of these data are now being carried out.

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 both by the staff of this section and with the assistance of a large support contract for computer programming and systems analysis. For the most part, this combination is now working quite smoothly after some difficult problems initially with a new contractor. The complex computer system for the Connecticut Tumor Registry, developed primarily by the Computer Science Section staff with some programming help from the contractor, is now in parallel operation in Connecticut and appears to be working quite well. Within a relatively short time, it should be possible to turn off the old system and go over completely to this new system. The Computer Science Section has also been active in providing computer support and consultation to other parts of NCI. In particular, the Division of Resources, Centers and Community Activities has had a number of problems that has required the consultation of this section. These include developing computer software for monitoring some of the ongoing programs of that Division such as the Cancer Centers Program and the Community Clinical Oncology Program. The staff of the section is also called upon to assist various operating units in NCI in selecting computer contractors to help them carry out their missions.

Plans for the Immediate Future

During this coming year, again the primary emphasis of the output from the SEER Program will be on patient survival data. This will include a detailed analysis that will take into account such factors as extent of disease at diagnosis and type of treatment. A preliminary tabulation has just been produced comparing five-year relative survival rates for a number of specific cancers among the various ethnic groups. Substantial differences have been noted for some primary sites. Some of the effort during this coming year will be devoted to attempting to identify factors that might be responsible for some of these differences.

The American College of Surgeons (ACOS) has developed some computer systems for use in individual hospitals to maintain their own tumor registries. The Biometry Branch has begun to work with the ACOS to bring about compatibility between these systems and the SEER Program, particularly where these systems are installed in hospitals that are located in areas with a SEER registry. This effort may also contribute to the potential availability of cancer registry data in areas where SEER registries do not exist.

Recently an advisory committee to the SEER Program was appointed by Dr. DeVita with Dr. Edward Sondik of DRCCA as the Chairman. The first charge to this committee was to identify national goals and targets for intervention programs and to develop measuring devices to monitor the extent to which these goals are being achieved. This is a specific narrow focus. The committee has met several times within a very short period of time to deal with this problem and it is expected that a plan will be ready for implementation within a short time. This is a difficult problem and there is no single obvious solution. After this particular task has been completed, the Biometry Branch expects to use the SEER Advisory Committee as a continuous sounding-board on needs for data that the SEER Program can generate -- both in the area of etiology as well as in the clinical area. Given the increased emphasis on monitoring changes in cancer incidence, survival and mortality, however, a greatly increased emphasis on the surveillance aspect of SEER will be evident in the immediate future.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP04254-09 B

PERIOD COVERED
October 1, 1982 through September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cancer Surveillance, Epidemiology, and End Results Reporting (SEER) Program

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)
John L. Young, Jr., Chief, Demographic Analysis Section, BB, NCI

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 *(Use standard unreduced type. Do not exceed the space provided.)*

Data on cancer patients diagnosed from year of entry into the SEER Program through 1981 (1973-81 for most participants) were submitted to the NCI by the ten participants in December 1980. Nine of the ten participants also submitted up-to-date follow-up information on at least 80% of patients diagnosed 1973-80. Analysis of survival data by primary site and age revealed better survival rates for whites than for blacks and for females than for males. In general survival rates have improved over time when comparison is made of SEER data to data reported from the End Results Program, 1960-73. Currently, data are being analyzed by staging categories of the American Joint Committee on Cancer. A monograph detailing cancer patient survival experience by race, sex, age, stage, anatomic site and histology is being readied for publication. Incidence data for the year 1981 will be available on a preliminary basis and average annual incidence rates for 1978-80 will be published as soon as final 1980 census tapes are made available by the Census Bureau. Mortality data for this same period will also be published if the 1980 mortality data become available from the National Center for Health Statistics in a timely fashion. The program is being expanded to include an additional geographic area with a population of at least 300,000 blacks and 300,000 Hispanics.

PROJECT DESCRIPTION

Names, Titles, Laboratory and Institute Affiliations of Professional Personnel (other than the Principal Investigator) engaged on this Project:

Earl S. Pollack	Chief	BB, NCI
Ardyce J. Asire	Statistician (Health)	BB, NCI
Betty J. Cicero	Medical Record Librarian	BB, NCI
Jack B. Cunningham	Medical Record Librarian	BB, NCI
John W. Horm	Statistician	BB, NCI
Mary A. Kruse	Medical Record Librarian	BB, NCI
William I. Lourie, Jr.	Statistician (Health)	BB, NCI
Constance L. Percy	Statistician	BB, NCI
Lynn A. Ries	Statistician (Health)	BB, NCI
Evelyn M. Shambaugh	Statistician (Health)	BB, NCI

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. Currently a competitive process is under way to add an additional geographic area covering a population of at least 300,000 blacks and 300,000 Hispanics. The areas being considered in the competition are the Miami Consolidated Statistical Area, the City of Chicago, Los Angeles County, Texas Health Regions 5 (Dallas-Fort Worth) and 9 (San Antonio), and the State of New Jersey.

For each case of cancer diagnosed in residents of the participating 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 containing 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 among 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, for the past two years mortality data have not yet become available in machine readable form for 33 months after the end of the calendar year. Thus, mortality data for 1980 will not be available until September 1983. There is every reason to think that similar delays will continue into the future with the result that incidence data for a given calendar year will be available for analysis at least one year prior to mortality data.

Population estimates of the various geographic areas have been obtained from a variety of sources awaiting final population counts from the 1980 census. 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 obtained in July 1983. Problems with 1980 census data are discussed in more detail below.

Major Findings:

Data for cases diagnosed between January 1, 1973 and December 31, 1981 were submitted to NCI in December 1982. 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 1980, data for 1981 may be incomplete. In addition, survival data for patients diagnosed between January 1, 1973 and December 31, 1980 are available through at least December 31, 1981.

During previous years there was considerable variation among the participants with respect to the percent of patients who were actively followed into the most recent calendar year with three of the ten participants having rates which were unacceptably low. As a result, major efforts were undertaken during the past year to improve this deficiency. A goal of having 80% of patients diagnosed 1973-80 followed into 1981-82 was set for each registry. Table 1 shows the results for each registry from the most recent data submission. Two percentages are shown, one based only on persons not known to be dead as of the end of the folow-up period and one counting deaths during the most recent follow-up period (1981-82) as successful follow-up. By either method, with only one exception nine of the ten registries exceeded the SEER goal. Unfortunately, the registry in Puerto Rico has been able to successfully follow fewer than half of the patients included in their data base. As a result, beginning in December 1982 four additional personnel were added to the Puerto Rico SEER contract to make home visits in an attempt to locate patients lost to follow-up. It is hoped that this deficiency can be corrected in time to include data from Puerto Rico in future survival analyses.

Table 1

Percentage of 1973-80 diagnosed cases actively followed into 1981-82 by area, SEER Program

Area	1981-82 Deaths	
	Excluded	Included
San Francisco/Oakland	83	87
Connecticut	82	84
Detroit	85	88
Hawaii	89	91
Iowa	82	84
New Mexico	85	87
Seattle	79	82
Utah	86	88
Atlanta	89	91
All areas excluding Puerto Rico	83	86
Puerto Rico	43	49

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. Since rates for blacks, Hispanics, Chinese, Japanese, Filipinos, Hawaiians, and American Indians are not available these life tables have been constructed by NCI using mortality tapes available for the total United States and appropriate population (census) data. Analyses of survival data for these minorities are currently being undertaken and will be contrasted with survival analyses for whites and blacks just completed.

An area of concern has been the appropriate measure of cancer patient survival. The traditional calculation is that of the relative survival rate in which the observed patient survival rate is "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." 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.

Another concern has been the effect of the percent of patients lost to follow-up on survival rates. It seems much more likely that patients lost to follow-up are alive rather than dead since the registry files are matched against the files of all deaths among residents of the area each year, and the only deaths which should be missed are those for patients who became residents of another

state after diagnosis. In Table 2 the effect of assuming that all patients lost to follow-up lived to the end of the study is shown by comparing the 5-year relative survival rates based on that assumption with the corresponding 5-year relative survival rates as usually computed. These "revised" rates are only slightly higher than the usual rates. Thus, it is likely that the "true" survival rates are very close to the relative survival rates giving further justification for using the relative survival rate as a good measure of survival corrected for normal life expectancy.

Table 2

Comparison of 5-year relative survival rate, with assumption that all patients lost to follow-up were alive at end of study vs. usual 5-year relative survival rate by primary site, SEER Program, patients diagnosed 1973-79

	White patients		Black patients	
Primary site	5-year relative survival rate, %	Revised[a] 5-year survival rate, %	5-year relative survival rate, %	Revised[a] 5-year survival rate, %
All sites	47	50	35	38
Stomach	13	15	14	16
Colon	48	52	44	47
Rectum	46	49	35	39
Pancreas	2	3	4	5
Lung and bronchus	11	13	9	11
Melanoma of the skin	76	79	--	--
Female breast	72	75	60	63
Cervix uteri	66	69	61	65
Corpus uteri	87	89	54	58
Ovary	34	37	35	38
Prostate gland	64	68	54	58
Urinary bladder	71	74	43	47
Kidney	48	51	49	52
Brain	20	22	21	23
Non-Hodgkin's lymphoma	43	45	43	45

-- Number of cases too small.

[a] With assumption that all lost to follow-up lived to end of study, Dec. 1980.

A major area of interest has been trends in relative survival rates over time. Unfortunately, trend data are not available on a consistent population of patients with the exception of those included in the Connecticut Tumor Registry. However, data are available from the End Results Group Program which was largely a hospital-based registration program consisting mainly of large teaching hospitals. Thus data from the two sources are not strictly comparable but do serve as general indicators of change over time. Data from the End Results Program for the time periods 1960-63 and 1970-73 are shown in Table 3 in comparison to

SEER data for the period 1973-79. Rates for Connecticut for the same three time periods are shown in parentheses. In general the trends observed in Connecticut were also observed between the End Results Program and the SEER Program. For most primary sites there has been an improvement in the five-year relative survival rate between 1960-63 and 1973-79, particularly for melanoma, breast, cervix uteri, corpus uteri, prostate, bladder, kidney and non-Hodgkin's lymphomas. For most of these sites the major gains in survival occurred between 1960-63 and 1970-73 with somewhat smaller gains noted between 1970-73 and 1973-79.

While the major emphasis of data analysis during the year has been devoted to survival data and staging schemes for extent of disease at diagnosis, 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 have been calculated by race, sex and anatomic site for each of the geographic areas participating in the Program. These data are felt to be relatively complete. Data are now available for 1981, but these data 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.

Currently, population files based on 1980 census data are being constructed. Unfortunately this task is extremely complex since all data for whites must be corrected due to the fact that many persons of Hispanic origin did not classify themselves as whites in the census questionnaire, but rather indicated their race as "other." Thus, populations for blacks, Orientals and American Indians must be obtained from one set of tapes, Anglos and Hispanics from a second set, and all counts must be adjusted to the total for all races. Finally, intercensal estimates for the years 1973-79 must be developed based on 1970 and 1980 census counts, and projections for 1981 must then be made. It is anticipated that all tapes necessary for developing usable population files will be available from the Census Bureau by mid-July 1983.

Difficulty has also been experienced in obtaining United States mortality data from the NCHS. Data for the calendar year 1980 will not be released to the general public before September 1983. Thus, mortality data are not available on a timely basis, lagging at least two years behind corresponding incidence data.

Significance to Biomedical Research and the Program of the Institute:

Continuous 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

Table 3: Five-Year Relative Survival Rates by Site and Sex, SEER 1973-79 Vs. End Results 1960-63 and 1970-73, and Corresponding Connecticut Rates

SITE	SEX	END RESULTS GROUP 1960-63		1970-73		SEER 1973-79	
		Percent Surviving					
Stomach	M	10	(11)	12	(13)	12	(12)
	F	13	(15)	14	(15)	14	(16)
Colon	M	42	(42)	47	(49)	47	(49)
	F	44	(45)	50	(52)	49	(49)
Rectum	M	36	(38)	43	(46)	44	(45)
	F	41	(42)	48	(48)	47	(48)
Pancreas	M	1	(2)	2	(3)	3	(2)
	F	2	(1)	2	(3)	2	(2)
Lung	M	7	(8)	9	(11)	10	(10)
	F	11	(11)	14	(16)	14	(15)
Melanoma	M	51	(62)	62	(69)	71	(76)
	F	68	(77)	75	(82)	80	(78)
Breast	F	63	(65)	68	(70)	72	(71)
Cervix Uteri		58	(64)	64	(67)	66	(64)
Corpus Uteri		73	(77)	81	(83)	87	(83)
Ovary		32	(35)	36	(38)	34	(32)
Prostate		50	(51)	63	(60)	64	(61)
Testicular		63		72		80	
Bladder	M	53	(56)	61	(71)	72	(72)
	F	53	(59)	60	(69)	69	(70)
Kidney	M	36	(40)	44	(48)	49	(52)
	F	39	(45)	50	(47)	48	(48)
Brain	M	16	(17)	18	(13)	19	(17)
	F	21	(18)	22	(18)	22	(18)
Non-Hodgkin's Lymphoma	M	31	(35)	39	(41)	42	(44)
	F	31	(36)	43	(46)	43	(44)
Hodgkin's	M	34		66		66	
	F	48		69		71	
Leukemias	M	12		21		27	
	F	14		22		30	

( ) Connecticut 5-year relative survival rates

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.

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 Communications, the Demographic Analysis Section received 236 telephone requests for data during the period October 1, 1982 through May 31, 1983 for an average of seven 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, SEER data have been presented at the XIIIth World Cancer Congress, the annual meeting of the International Association of Cancer Registries, the Society for Epidemiologic Research, the American College of Epidemiology, the American Statistical Association, and the American Cancer Society's National Conference on Breast Cancer. SEER data have also been used extensively for planning purposes by the Office of the Director of the NCI as well as by the Division of Resources, Centers and Community Activities.

Proposed Course:

1. By the end of September 1983, a new SEER participant will be selected from among five competitors to augment the coverage of black and Hispanic populations by the Program. Data collection will begin with all cases diagnosed 1983 forward, and if historical data are available for years prior to 1983, these data will be added to the SEER data base.

2. Beginning with all cases diagnosed 1983 forward, detailed data on type of surgery performed will be collected for all patients surgically treated for any of eight leading cancer sites. This will make possible the monitoring of extent of surgery initially received by cancer patients and will allow the study of changes over time.

3. Beginning with all cases diagnosed 1983 forward, the amount of detailed data collected on extent of disease at the time of diagnosis has been reduced. This

should reduce somewhat the amount of coding which each contractor will perform. However, the data submitted to NCI will be sufficently precise to all classification into staging categories of the American Joint Committee on Cancer as well as allowing for compatibility with data collected historically.

4. By the end of June 1983, survival tabulations should be completed for various racial and ethnic groups, including Hispanics, Chinese, Japanese, Filipinos, Hawaiians, and American Indians. Survival for these groups will be compared to that for whites and blacks previously published.

5. By September 1983 detailed survival tables will be available for white and black cases diagnosed through 1980 and followed into 1981 for all cancers by site, cell type, extent of disease, initial treatment, race, sex, and age. Currently, staging categories comparable to those of the American Joint Committee on Cancer are being developed. Once the data are analyzed by stage, the full report will be submitted as an NCI Monograph. Allowing for the usual delays in monograph publication, the material should be published by July 1984. Data will be available in unpublished form or in preprint form prior to that time, however.

6. As soon as the 1980 Census data and intercensal estimates are available and have been analyzed for consistency, and as soon as 1980 mortality data become available from the National Center for Health Statistics (September 1983), cancer incidence and mortality data will be published for the years 1978-80. This will update NCI Monograph No. 57 that covered incidence and mortality data from 1973-77. This report will be less extensive than the monograph, but will contain age-specific rates by cancer site and race.

7. Staff will continue to work with the SEER Steering Committee to use SEER data, as appropriate, to identify geographic areas of the country which are below potential in terms of cancer survival and to monitor future changes in these indices to determine whether progress is being made.

Publications:

Devesa, S. S., Horm, J. W., and Connelly, R. R.: Trends in lung cancer incidence and mortality in the United States. Proceedings of the International Lung Cancer Update Conference, New Orleans, 1983. (In Press)

Horm, J. W. and Asire, A. J.: Changes in lung cancer incidence and mortality rates among Americans: 1969-78. JNCI. 69: 833-837, 1982.

Kruse, M. A.: Self-Instructional Manuals for Tumor Registrars: Antineoplastic Drugs, Book 8. Department of Health and Human Services, Publication No. (NIH) 82-2441, 1983, 68 pp.

Percy, C., Horm, J. W., and Goffman, T. E.: Trends in histological types of lung cancer, SEER, 1973-1981. Proceedings of the International Lung Cancer Update Conference, New Orleans, 1983. (In Press)

Percy, C. L., Horm, J. W., Young, J. L., Jr., and Asire, A. J.: Uterine cancers of unspecified origin - a reassessment. Public Health Reports. 98: 176-180, 1983.

Percy, C., O'Conor, G., Ries, L. G., and Jaffe, E. S.: Non-Hodgkin's lymphomas application of the international classification of diseases for oncology (ICD-O) to the working formulation. Cancer. (In Press)

Percy, C. and Sobin, L.: Surveillance, epidemiology, and end results lung cancer data applied to the World Health Organization's classifications of lung tumors. JNCI. 70: 663-666, 1983.

Ries, L. G., Pollack, E. S., and Young, J. L., Jr.: Cancer patient survival: surveillance, epidemiology, and end results program, 1973-79. JNCI. 70: 693-707.

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. 6: 1-9; 1982.

Swanson, G. M. and Young, J. L., Jr.: Trends in cancer incidence in metropolitan Detroit, 1937-1977: Leads for prevention. Prev. Med. 12: 403-420, 1983.

Young, J. L., Jr.: Cancer and the hispanic elderly. The Hispanic Elderly. Report of a Conference Sponsored by Spanish Heritage Public Health Service Workers, September 16-17, 1981. U.S. Department of Health and Human Services, Bethesda, 1982, pp. 106-111.

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.): Advances in Cancer Control: Research and Development. Alan R. Liss, Inc., New York. 120: 35-53, 1983.

CONTRACTS IN SUPPORT OF THIS PROJECT:

	Current Annual Level	Man Years
CALIFORNIA, UNIVERSITY OF, SAN FRANCISCO (NO1-CP-11004)	$ 252,984	5.15
COMMONWEALTH OF PUERTO RICO (NO1-CP-43386)	55,323	18.6
CONNECTICUT STATE DEPARTMENT OF HEALTH (NO1-CP-61002)	1,106,486	29.5
EMORY UNIVERSITY (NO1-CP-61027)	807,772	24.4
FRED HUTCHINSON CANCER RESEARCH CENTER, SEATTLE (NO1-CP-61059)	637,079	27.4
HAWAII, RESEARCH CORP. OF THE UNIVERSITY OF (NO1-CP-53511)	463,102	15.7
IOWA, UNIVERSITY OF (NO1-CP-43200)	1,477,014	47.0
MICHIGAN CANCER FOUNDATION (NO1-CP-61028)	1,839,339	63.9
NEW MEXICO, UNIVERSITY OF (NO1-CP-33344)	600,000	25.5
NORTHERN CALIFORNIA CANCER PROGRAM (NO1-CP-21025)	1,592,845	50.0
UTAH, UNIVERSITY OF (NO1-CP-43382)	439,420	14.9
YALE UNIVERSITY (NO1-CP-33235)	119,711	1.5

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

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.

Major Contributions: 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. Major analyses, however, are done by NCI staff using the data tapes submitted semi-annually by the contractor.

Proposed Course: The Program is being expanded to add another geographic area which contains a population of at least 300,000 blacks and 300,000 Hispanics. Five geographic areas have submitted responses to a proposal for a new population-based registry - the State of New Jersey, the Miami Consolidated Statistical Area, the City of Chicago, Texas Health Regions 5 and 9 (Dallas-Ft. Worth and San Antonio), and Los Angeles County. The new area will be selected by September 1983. All other contracts will be continued although the level of effort for Yale University will be considerably reduced.

Intensive efforts to assure that the data being submitted to NCI are of the quality and completeness required for an effective cancer surveillance network will be continued. 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 was distributed during the past year. 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 are being updated.

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

Title: Continued Cancer Registration and Selected In-Depth Analyses

Current Annual Level: $137,000

Man Years: 10.1

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 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 Contributions: 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.

Proposed Course: Funding is being planned through March 1984. This should allow sufficient time to complete analyses and to prepare a monograph comparing

data from the Israel Tumor Registry with data from the U.S. SEER Program. 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. Doubtless, this Registry will be forced out of existence once NCI funding is discontinued.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP04257-26 B

PERIOD COVERED

October 1, 1982 through September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies of Etiology, Prognosis and Screening for Early Detection of Cancer

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

M. H. Myers Chief, BRASS, BB, NCI

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:
13.5	10.5	3

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Truck drivers and deliverymen have a significant, 30% increased risk of bladder cancer. Analysis of occupational data from the National Bladder Cancer Study has indicated an increased risk of bladder cancer among painters, regardless of the type of painter. The incidence of pleural mesothelioma is 8 times higher than peritoneal mesothelioma among males but just over twice as high among females. For white males, incidence of pleural mesothelioma increased significantly over the 8 year time period 1973-1980. Among white males, lung cancer incidence rates increased more than 200% from the late 1940s to 1979-80; the corresponding increase among white females was greater than 250%. Rates continue to increase among the older age groups. Among the younger groups, the rates of increase are diminishing and some decreases in rates are apparent among males. Strong significant inverse trends between lung cancer incidence and both income and education were apparent among both white and black males, and the effect of income exceeded that of education. Significant leukemia excesses were observed following chemotherapy for breast cancer, ovarian cancer, and multiple myeloma. Patients treated with radiation, but without chemotherapy, were also found to be at increased risk of second leukemias for uterine corpus cancer. A statistically significant positive association with respect to stage of a first and second breast cancer was found. This finding is consistent with an immunological hypothesis regarding the effect of cell mediated immunity associated with a first breast cancer on second breast cancer behavior. Continued improvement in survival for Hodgkin's disease patients diagnosed in the 1970s in 9 areas covered by the NCI's SEER Program was observed. Between 1973-75 and 1976-77 3-year relative survival rates for whites increased significantly from 71 to 78%. Research in theoretical statistical modeling has led to a characterization of length bias at a prevalence screen in terms of screening parameters and disease natural history.

PROJECT DESCRIPTION

Names, Titles, Laboratory and Institute Affiliations of Professional Personnel (other than the Principal Investigator) engaged on this Project:

S.C. (Steinhorn) Abbott	Statistician (Health)	BB NCI
J.L. Aron	Staff Fellow	BB NCI
A. Baranovsky	Statistician (Health)	BB NCI
R.R. Connelly	Statistician (Health)	BB NCI
R.E. Curtis	Statistician	BB NCI
S.S. Devesa	Statistician (Health)	BB NCI
B.F. Hankey	Mathematical Statistician	BB NCI
H.W. Heise	Statistician (Health)	BB NCI
M.D. Naughton	Computer Systems Analyst	BB NCI
P.C. Prorok	Mathematical Statistician	BB NCI
D.T. Silverman	Statistician (Health)	BB NCI

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. In collaboration with Dr. Robert Hoover and Dr. Thomas Mason of NCI and Dr. G. Marie Swanson of the Michigan Cancer Foundation, the relation between employment in occupations with potential exposure to motor exhaust and the risk of bladder cancer was examined based on data collected during the National Bladder Cancer Study. Of the 2100 white male bladder cancer patients and 3874 white male population controls interviewed to obtain lifetime occupational histories, 1880 subjects had a history of employment in motor exhaust-related occupations. Our findings indicated that truck drivers and deliverymen have a significant, 30% increased risk of bladder cancer. Among truck drivers and deliverymen employed less than 25 years, a consistent trend in risk with increasing duration of employment was also seen ($p < 0.001$). The relative risk for truck drivers and deliverymen peaked at 2.2 (95% CI = 1.4-3.2) for those employed 15-24 years and decreased to 1.1 for those employed 25 years or more. Increased risk was apparent only for truck drivers and deliverymen in study areas located in the northeast and midwest. A nonsignificant excess risk was

seen for taxicab drivers and chauffeurs employed at least 10 years (relative risk = 2.0; 0.9-4.4), but a consistent gradient in risk with increasing duration of employment was not observed. Employment as a bus driver was associated with a slight, nonsignificant elevated risk. The joint effects of 1) latent period and duration of employment on risk and 2) cigarette smoking and occupational exposure on risk were also examined.

2. Analysis of occupational data from the National Bladder Cancer Study has indicated an increased risk of bladder cancer among painters. First, painters in construction and maintenance experienced a significant elevation in bladder cancer risk (relative risk = 1.7; 95% confidence interval (CI) = 1.3-2.4). A significant trend in risk with increasing duration of employment was also apparent; the relative risk for painters in construction and maintenance who were employed at least 10 years was 2.1 (CI = 1.3-3.5). Second, painters of manufactured articles experienced an elevated risk (relative risk = 1.4; CI = 0.9-2.4), but a consistent gradient in risk with increasing duration of employment was not apparent. Third, employment as an artistic painter was associated with a significant excess bladder cancer risk (relative risk = 2.5; CI = 1.1-5.7). A significant trend in risk with increasing duration of employment as an artistic painter was also seen. The relative risk for artistic painters employed 10 years or more was 3.0. This last finding pertaining to artistic painters is being pursued in collaboration with Mr. Barry Miller and Dr. Aaron Blair of NCI. The remaining findings regarding painters are being examined in collaboration with Dr. Robert Hoover of NCI.

3. Work has continued on the descriptive study of stomach and colorectal cancer incidence and mortality among Puerto Ricans in New York City in collaboration with Dr. Earl Pollack 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.

4. Information on 955 mesothelioma cases diagnosed during the years 1973-80 was obtained from the SEER Program. The primary site of origin was recorded as pleura for 776 cases (81%), peritoneum for 146 cases (15%), heart for 9 cases (1%), and other or unknown site for 24 cases (3%). The incidence of pleural mesothelioma is 8 times higher than peritoneal mesothelioma among males but just over twice as high among females. For white males with pleural mesotheliomas, incidence increased significantly over the 8 year time period; no significant

trend was observed for peritoneal mesothelioma among white males or for either pleural or peritoneal mesothelioma among white females. The average annual incidence rate for pleural mesothelioma was 9.4 cases per million among white males during 1973-80 in all SEER areas combined. Significantly higher rates occurred in areas having port cities; 15.4 for Seattle and 13.8 for San Francisco-Oakland while residents of Iowa had a significantly lower rate of 5.5 cases per million. Excess risk to mesothelioma due to occupational exposure to asbestos during shipyard employment has been documented. Comparison of mortality rates for cancer of the pleura among residents of SEER areas with those for the total U.S. suggests that the SEER exposure for mesothelioma is quite representative of that for the entire country. The Median survival time for the series of SEER cases was 8 months and prognosis was similar for pleural and peritoneal mesotheliomas. Females survived significantly longer than males and young age was also an important prognostic variable.

5. Increases observed in lung cancer rates exceed those of any other primary cancer site. Among white males, incidence rates increased more than 200% from the late 1940s to 1979-80; the corresponding increase among white females was greater than 250%. The age-adjusted incidence rates have virtually leveled off in recent years among white males; among white females, the rate of increase has declined. Rates continue to increase among the older age groups; among the younger groups, the rates of increase are diminishing and some decreases in rates are apparent among males. Mortality rates among nonwhite males were lower than those for whites in the past, about equal in the early 1960s, and now are substantially higher. Geographic patterns have changed over time among white males, as rates in the south have increased more rapidly than in other areas of the country.

6. The association of lung cancer incidence with income and education and the effect of adjustment for socioeconomic (SE) distribution on black-white differences in lung cancer rates were evaluated using data from the Third National Cancer Survey (TNCS). Included in this study were 20,868 cases of lung cancer diagnosed among metropolitan residents of the TNCS during 1969-71. Median family income and median years of education by census tract of residence were used to indicate SE group. Strong significant inverse trends between lung cancer incidence and both income and education were apparent among both white and black males, and the effect of income exceeded that of education. Lung cancer rates among black compared to white males were significantly higher ($p<.001$) before SE adjustment, nonsignificantly higher after adjustment for education, and nonsignificantly lower after adjustment for income. Strong trends in risk with income or education were not observed for lung cancer among females of either race.

7. Patterns in urban and rural cancer incidence were studied based on TNCS data from Iowa and Colorado for 1969-71 and SEER data from Iowa for 1973-78. The TNCS data showed that age and sex are stronger determinants of cancer incidence rates than urban-rural residence but that an urban-rural differential exists with urban rates usually exceeding rural rates. The relative risk for urban residents versus rural residents was significantly elevated for five cancer sites among Iowa and Colorado men: 1) lung, bronchus and trachea; 2) colon excluding rectum; 3) bladder; 4) esophagus; and 5) buccal cavity and pharynx

excluding lip. The risk for prostate cancer was significantly high among urban men in Colorado but not in Iowa. The risk for larynx cancer was significantly high among urban men in Iowa but not in Colorado. Increased risks for male rural residents were encountered infrequently: 1) for lip cancer in Iowa (all ages) and in Colorado at ages 45-54; and 2) for leukemia in Iowa at ages under 25. Among women, significantly elevated relative risks (all ages combined) were observed for urban versus rural residents in Iowa for the following cancers: 1) cervix uteri; 2) lung, bronchus and trachea; 3) bladder; and 4) buccal cavity and pharynx excluding lip. Only for breast cancer was the risk for urban women in Colorado significantly elevated. The only increased risk among female rural residents was for brain cancer at ages under 25 in Iowa. Differences between urban and rural residents in cigarette smoking habits, alcohol consumption, and occupational exposures to carcinogens are likely to account for the excess urban risks for mouth, pharynx, lung, larynx, esophagus, and bladder cancer. The possibility that air pollution contributes substantially to the excess lung cancer risk among urban residents is still uncertain despite considerable research. For several sites (prostate, breast, colon, and corpus), excess urban risks were greater in Colorado than in Iowa; a significant direct association with income (increasing risk with increasing affluence) was found for these sites in Colorado but not in Iowa (Devesa, 1979; Devesa and Diamond, 1980). Socioeconomic status may therefore be an important confounding factor in the urban-rural patterns observed for these sites among Colorado residents. The lack of an association with income among residents of large cities in Iowa may reflect a homogeneity of the population in cultural or nutritional risk factors for these sites. If such homogeneity exists in large cities, it may extend to smaller cities and even to rural areas of Iowa, thus explaining the smaller urban-rural differences for these sites in Iowa compared to Colorado. The significant excess risk for lip cancer among rural men in Iowa undoubtedly reflects an increased risk for this disease among farmers in the state (Burmeister, 1981). Although not statistically significant, leukemia and lymphoma risks among rural men and women in Iowa were also elevated. This might be related to exposure among farmers to fertilizers or pesticides introduced in the last several decades.

8. A comprehensive report of cancer mortality in the white population of the U.S. during 1950-75 has been published. Age-adjusted death rates are presented for residents of each state and the District of Columbia according to cancer site, sex and five-year time period of death. The cancer mortality rates in this report may be used to study trends in rates over time in the states of the country. States are ranked for each site and time period with respect to mortality rates so that it is relatively easy to identify high or low mortality areas.

9. Work has continued on the study of multiple primary cancers utilizing historical data from the Connecticut Tumor Registry and the SEER Program. The leukemia experience following treatment for a first primary cancer was analyzed for more than 400,000 SEER patients diagnosed during 1973-1980. Significant leukemia excesses were observed following chemotherapy for all first primary sites combined and for breast cancer, ovarian cancer, and multiple myeloma. Patients treated with radiation, but without chemotherapy, were also found to be at increased risk of second leukemias for all first sites combined and

specifically for uterine corpus cancer. The association between the stage of two breast cancers diagnosed in the same woman was studied for 1,061 women with at least two nonsimultaneous breast cancers with the second breast cancer occurring in the contralateral breast. A statistically significant positive association was found which is at least consistent with an immunological hypothesis regarding the effect of cell mediated immunity associated with a first breast cancer on second breast cancer behavior. A pathology slide review is currently being planned in an attempt to verify that the positive association in the stage of two independent primary breast cancers in the same women is due to immunological phenomena. Multiple primary cancers in children under age 15 years and in young adults aged 15 through 34 years are beng studied using data on patients diagnosed between 1960 and 1980 from the Connecticut Tumor Registry and between 1973 and 1980 in the SEER areas. Observed and expected numbers of various second cancers will be determined as well as possible associations of second cancer risk with treatment. There have been reports in the literature that lung cancer may occur in excess subsequent to lymphoma. The SEER data and the Connecticut historical data are being used to further investigate this observation in regard to whether lung and lymphoma may have common etiologic factors or whether the excess may be treatment related.

10. Work has begun on a study of hormonally related first primary cancers and the subsequent diagnosis of a second hormonally related primary cancer, with respect to age, stage of disease, and treatment, with some emphasis on treatment with hormones, alone or in combination with other forms of treatment. The cancer sites are female breast, uterine corpus, ovary, colon, and prostate. The data used are those from nine registries participating in the SEER Program, 1973 on. Crude preliminary findings suggest that females 45 to 54 years of age at diagnosis of first primary breast cancer have a higher risk of a subsequent primary cancer of the ovary, colon, or second breast, compared with females ages under 45, 55 to 64, or 65 and older. For males, there seems to be no excess risk for colon following prostate cancer but a suggested increase in the other direction.

11. Work has begun on a collaborative study to compare the characteristics and survival experience of Hodgkin's disease patients diagnosed in the nine SEER areas to newly diagnosed and previously untreated patients admitted to Comprehensive Cancer Centers belonging to the Centralized Cancer Patient Data System (CCPDS). The study will cover the period July 1, 1977 to December 31, 1981. Preliminary findings are as follows: the proportion of all Hodgkin's disease patients with the nodular sclerosing type was greater in the CCPDS group as compared to SEER. There were proportionately fewer patients with the lymphocyte depleted type in CCPDS. The CCPDS patients were younger than the SEER patients - this was true for all histologic types. Within each histologic type no substantial SEER/CCPDS differences were seen in the proportion of cases by race or sex. The sex ratio varied between histologic types - the lymphocyte predominate type is primarily a male disease whereas the numbers with the nodular sclerosing type are approximately the same for both sexes. The analysis will continue with the examination of treatment, stage of disease, and survival comparisons.

12. Significant increases in survival for whites diagnosed with Hodgkin's disease between 1960-63 and 1970-73 were previously documented by the National Cancer Institute and have been attributed to the combination of radiation and multi-agent chemotherapy in the management of these patients. Data now available indicate continued improvements in survival for Hodgkin's disease patients diagnosed in the 1970s in 9 areas covered by the NCI's SEER Program. Between 1973-75 and 1976-77 3-year relative survival rates for whites increased significantly from 71 to 78%. Corresponding to these improvements, Hodgkin's disease incidence for whites in SEER areas fell from 3.53 to 2.93 per 100,000 population from 1973 to 1979, or -2.7% per year, whereas mortality dropped from 1.39 to 0.83 per 100,000, or -8.6% per year. Improvements in survival were observed only for patients under 65 years, a significant increase for those 45-64 years (52 to 71%). Survival for all histologic types showed gains but only those classified as mixed cellularity were significant (66 to 74%). There were no differences by sex and survival for whites exceeded that for blacks.

13. The impact of cancer on persons 65 years of age and older has been assessed by examining incidence rates and survival rates. Even though other publications have presented age-specific incidence or survival rates, the magnitude of the cancer problem for this age group is imbedded among data for all ages plus other age groups and could easily go unnoticed. It is timely to note that for all cancers combined, the incidence rate for males 65 and older (2451.5 per 100,000) is six times the age-adjusted rate for all ages combined (386.6); for elderly females, the incidence rate is five times that for all ages combined (1386.4 versus 301.1). For males, multiples of the order of seven-fold prevail for cancers of the colon, rectum, and urinary bladder, six-fold for lung and eight-fold for cancer of the prostate. For elderly females, the incidence rates for breast cancer, uterine corpus, and lung are four times that for all ages combined, while the multiples for colon and rectum are six and seven times, respectively. Relative survival rates for patients 65 and older are for many cancer sites only a few percentage points lower than rates for all ages combined, suggesting that patients in this age group fare only a little worse than younger patients in escaping the effects of cancer once it has been diagnosed. Exceptions are cancer of the urinary bladder and non-Hodgkin's lymphoma for both men and women and uterine cervix, uterine corpus, and ovary for women. For these sites, survival for older patients is considerably lower than for their younger counterparts.

14. Work has continued on the development of theory for the evaluation of screening programs using stochastic modeling methods. The approach is aimed at investigating age dependence, lead time, length bias and natural history relationships. A model was developed based upon the age at entering the preclinical state, the age at screening, and the duration in the preclinical state. A method was developed for estimation of an approximate lead time distribution at a prevalence screen. A numerical investigation of relationships between length bias, screening parameters, and disease natural history was performed. For a prevalence screen, it was found that certain combinations of correlation between disease free and preclinical state sojourn times and age at screening can result in substantial length bias in either the positive or negative direction. However, use of the randomized trial design and a suitable age range can eliminate most of the extreme or negative length bias effects.

15. The WHO/IARC Working Group on the Evaluation of Screening Programs for Cancer of the Uterine Cervix has continued its efforts to analyze the pap smear screening programs in Iceland, Norway, Sweden, Finland, Denmark, Aberdeen and Canada. An attempt is being made to perform a common analysis of these programs to better evaluate screening impact and frequency. The participating programs are nearing completion of collection of data on cases of cervical cancer, screening histories of individuals and incidence rates. Data analysis will involve examination of incidence rates after sequences of negative screens to estimate the false negative rate and the preclinical state sojourn time density. Completion of a Working Group report is expected in 1984.

16. A study of lung cancer among uranium miners was continued in collaboration with Dr. Thomas Mason of NCI, Dr. Geno Saccommano of Grand Junction, Colorado, and the National Institute of Occupational Safety and Health (NIOSH). 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. Preliminary analyses have indicated that the mild atypia state exhibits a consistent trend in shape of sojourn time distribution and mean duration suggesting a sensitivity to increasing exposure to cigarette smoke. Activities are currently underway to augment the data base to include updated smoking and work histories, as well as clinical and mortality information on lung cancers. The results of this project have potential implications for lung cancer prevention programs and industrial safety regulation legislation.

17. During this year expected survival rates for persons of 8 racial groups other than white or black were developed. This developmental work was undertaken collaboratively with other staff of the Biometry Branch to support future analyses of SEER Program data on cancer patient survival. Expected survival rate tables based on average annual mortality rates for 1969-71 were prepared in a format for use by the Survival System for the following races: American Indian, Chinese, Japanese, Filipino, Hawaiian (white and native), Hispanic (in New Mexico) and Puerto Rican.

18. In collaboration with staff from the Demography Section and the Computer Science Section a system was developed to interactively construct summary coding schemes for the SEER site-specific detailed extent of disease information. The system will enable a non-programmer to accurately enter combinations of codes for any summary scheme that is possible given the extent to which details are provided in the basic detailed code structure. The system has built-in features to assure that all legitimate code combinations are included and that any specific combination can be assigned to only one summary category.

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 research results from this project have produced a number of issues that must be pursued. The finding that truck drivers are at increased risk of developing bladder cancer suggests that a more in-depth study is warranted. Reasons for the incidence and mortality patterns for stomach and colorectal cancer among Puerto Rican migrants to New York City should be sought. Studies to further investigate initial findings regarding subsequent primary cancer risk must be planned. Collaboration arrangements are being made which should permit studies contrasting the survival experience of cancer center patients (CCPDS) with population-based SEER patients. Specific studies or study areas are presented in the following paragraphs.

Biochemical-epidemiologic study of truck drivers that involves mutagenicity testing of urine specimens from diesel- and nondiesel-exposed truck drivers in order to evaluate the potential carcinogenic hazard associated with exposure to motor exhaust, particularly exhaust from diesel engines.

Case-control study of stomach and/or colorectal cancer among Puerto Rican-born residents of New York City and Puerto Rico in order to identify changes in diet and lifestyle after migration that may be responsible for changes in risk of cancer of these sites.

Plans are currently being made to prospectively study Black/White differences in cancer patient survival. Those sites identified as being associated with substantial survival differences between Blacks and Whites are corpus and bladder. Breast and colon cancers will also be studied although the differences in patient survival between Blacks and Whites for these sites are less than for cancers of the corpus and bladder. Patients will be interviewed to obtain information on behavior as related to the diagnosis of their cancer, food consumption patterns, socioeconomic factors, and other factors which might provide insight into Black/White survival differences. Pathology slides for all patients entered into the study will be read by pathology experts to establish the diagnosis and to identify factors that may be of prognostic significance. Initially the data collected from the patient interviews and pathology review will be associated with stage of disease at diagnosis. After a period of time follow-up on the patients will be obtained permitting an in-depth analysis of Black/White cancer patient survival differences.

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, radiotherapy or hormone therapy for the initial cancer. Specific investigations planned include the study of second primaries among those treated for cancer as children or young adults as well as studies of the complexes of smoking related or hormonally related sites.

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.

Research concerning the relationship among cancer incidence, patient survival and mortality will be conducted. Emphasis will be placed on full utilization of data from the SEER Program which provides information on all three measures. The effect on mortality of selective changes in incidence or survival will be explored.

Activities will continue on the development of mathematical models of screening programs. These will focus on disease natural history and the analysis of screening studies. A model for breast cancer screening will be developed which links tumor doubling time to survival. This will be used to investigate the duration and components of benefit relative to subgroups of a screened population, as well as the role of lead time and length biased sampling in the analysis and interpretation of screening data.

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CONTRACT 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

Current Annual Level: $300,000

Man Years: 0.8

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.

Major Contributions: Findings to date strongly suggest the usefulness of annual screening which includes clinical examination plus mammography. Over a 15-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.

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 third 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.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01CP04258-15 B

PERIOD COVERED
October 1, 1982 through September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Studies of Cancer Incidence and Mortality and Related Etiologic Factors

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)
Earl S. Pollack, Chief, Biometry Branch, NCI

COOPERATING UNITS *(if any)*
University of Bergen, Norway; Kuakini Hospital, Honolulu; University of Minnesota; University of Southern California

LAB/BRANCH
Biometry Branch

SECTION
Office of the Chief

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	2.5	0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
- ☒ (a1) Minors
- ☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Research on cancer incidence and mortality continued in a number of populations. Migrant populations from Japan, Norway, China and the South Pacific are being studied to identify possible etiologic factors for specific cancers. An analysis of the updated data on the Hawaiian Japanese confirmed earlier findings of an association between beer consumption and rectal cancer and between consumption of other alcoholic beverages and lung cancer. Among Norwegians there appears to be an inverse association between vitamin A intake and lung cancer. A first analysis of survival rates among Chinese in San Francisco and Hawaii was carried out and revealed survival rates remarkably similar to those of U.S. whites. Analysis of trends in the incidence of specific cancers among U.S. Chinese shows a tendency toward convergence to levels of rates among the white population.

PROJECT DESCRIPTION

Names, Titles, Laboratory and Institute Affiliations of Professional Personnel (other than the Principal Investigator) engaged on this Project:

Haitung King	Senior Research Scientist	BB	NCI
Frances Locke	Statistician (Health)	BB	NCI
Joseph Scotto	Health Services Director	BB	NCI

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 the cancer experience of migrant groups with that in their country of origin.

Methods Employed:

Several projects are reported on here, most of them primarily descriptive epidemiological studies. The Japan-Hawaii Cancer Study is a prospective cohort 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 has then been 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, Hong Kong, 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. Cancer registries are being established in some of the islands in the South Pacific and descriptive data are being analyzed to forumlate etiologic hypotheses in relation to specific cancers.

Major Findings:

Japan-Hawaii Cancer Study:

During the year a reanalysis of the data on the possible relationship between alcohol consumption and the subsequent occurrence of each of the five most frequent cancers in this cohort was carried out by the Biometry Branch Project Officer. This involved the first 14 years of follow-up and confirmed his

earlier findings through 13 years of follow-up -- that there appeared to be a dose-response relationship between alcohol consumption and cancers of the rectum and lung but not for the other three cancers. The major contributor to the relationship with rectal cancer appeared to be high beer consumption and for lung cancer, high wine and whiskey consumption. A paper presenting these results has been submitted for publication. A study of the possible relationship between serum uric acid and specific cancers was carried out and the results were negative. A paper has been submitted for publication.

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 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 in the Biometry Branch in 1981-82 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 the following analyses: a) cancer mortality among Chinese in the United States, Guangdong province, and Hong Kong; 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 Hong Kong indicated that, for all cancers combined, a substantive elevation in risk was shown for idai (foreign-born) and Hong Kong 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 Hong Kong Chinese. Examination of age-specific mortality further noted that, among U.S. and Hong Kong 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 Hong Kong 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 Hong Kong Chinese may be speculated in reference to population composition in the three areas. Take, for example, nasopharyngeal cancer. In Hong Kong, nearly 90 percent of the Chinese population were Cantonese; many of them originated in Canton and Fo-Shan. The Hong Kong rates thus reflected the experience of these high-risk populations. The lower mortality noted for Guangdong than for Hong Kong 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:

Analysis was made of U.S. Chinese cancer rates through use of data for 1973-77 from the SEER Program in San Francisco and Hawaii, in addition to comparable data for those areas for the years around 1970. Data for Chinese was compared to those for whites in each area.

Findings:

1) Chinese rates higher than whites - Chinese rates were higher than for whites for sites of relatively low rate levels, such as liver and nasopharynx, plus esophagus among males and thyroid among females.

2) Chinese rates lower than whites - Conversely, the Chinese have sharply lower rates than whites for the buccal cavity excluding nasopharynx, larynx, lung for males, melanoma, breast, prostate, all other genito-urinary sites except cervix, and lymphomas and leukemia.

3) Leading sites - Despite these differences, the rank order of leading sites for Chinese and whites are similar: lung, colon and prostate for males, and breast, lung, colon and corpus for females.

4) Trends - Chinese rates, high or low, appear to be moving towards the white rates. For example, the lower rates of prostate, breast and corpus are

rising, and the high rates of nasopharynx among males and of cervix, are decreasing.

5) San Francisco vs. Hawaii Chinese - There are differences among the Chinese in these 2 areas, most of them long standing. The rates in San Francisco are significantly higher than in Hawaii for lung, liver and nasopharynx among males. Hawaii shows higher rates for prostate, corpus and thyroid for both sexes. Though these differences are not statistically significant, they were also present in the 1970 time period.

6) Observations on specific sites:

   a) Buccal cavity - Nasopharynx rates are significantly higher for Chinese than whites, but the reverse is true for the remainder of the buccal cavity.

   b) Colon - There appears to be a difference in risk within the colon between Chinese and whites, with Chinese showing lower rates or a smaller proportion of cases in the upper part of the colon (cecum, appendix, ascending) compared to whites than in the lower portion.

   c) Lung - Chinese rates are lower than those for whites among males, but females consistently have shown similar or higher rates.

   d) Cervix - Chinese rates are well below those for whites for all genital sites except cervix, where rates are similar. However, the Chinese rates for in situ cervix are about half that for whites.

7) Survival - Chinese survival was evaluated based on SEER data, using cases diagnosed from 1973-79 and followed through 1980, for Chinese and whites in San Francisco and Hawaii only. For computation of relative survival, a "normal" life expectancy table for Chinese was used based on 1970 Chinese mortality rates, whereas 1975 rates were used for whites. The standard errors for the Chinese survival rates were quite large and precise analysis of site-specific differences is not possible. In general, however, there do not appear to be major differences in survival between Chinese and whites. The differences that were noted were also observed among whites, i.e., large differences in survival by site (poor for pancreas, stomach, lung and better for corpus, melanoma, prostate, breast and bladder), and better survival in Hawaii than San Francisco.

8) Los Angeles incidence - Data for Chinese and whites from 1973-78 were reviewed and compared to findings from the SEER areas. In general, results of Chinese and white comparisons were quite similar, except that there was no difference in thyroid rates between Chinese and whites in Los Angeles. Among the Chinese in the 3 areas, San Francisco remained high for lung, liver and nasopharynx compared to Los Angeles as well as to Hawaii. The rate for corpus was quite low in Los Angeles.

9) Mortality vs. incidence - Mortality is believed to be a poorer indication of cancer levels than incidence because of less accurate diagnosis certification, and because mortality reflects both incidence and

survivorship. Only incidence, with its detailed site specifications, is able to provide leads to the differing risks within the colon and to thyroid differences between Chinese and whites, because thyroid deaths are so few in number. Also, declining mortality rates for corpus among females, and prostate, stomach and lung among males, fail to disclose the apparent rising incidence for the first 3 sites, and the stable or slightly increasing rate of the latter site.

Future plans - These data have proved useful in focusing attention on areas for possible future studies of acculturation practices, particularly with reference to those sites which differ among the various Chinese communities within the U.S. Such studies possibly could be expanded to include the People's Republic of China (PRC), after completion of analyses of data available from the PRC, particularly with reference to homeland area of origin of U.S. Chinese.

Cancer Incidence and Mortality Among U.S. Japanese

Similar to the study of U.S. Chinese, analysis has begun of cancer incidence and mortality of U.S. Japanese and whites in San Francisco and Hawaii, based on SEER data for 1973-77, in addition to comparable data in those areas for the years around 1970. Preliminary results indicate that:

1) Compared to whites in each area, Japanese have higher rates than whites of both sexes only for stomach cancer, and among males, also for esophagus and liver. Other than for the digestive system, the Japanese have much lower rates than whites for almost every site.

2) There appears to be a difference in the distribution of neoplasms within the colon, with the Japanese having a smaller proportion of cases in the upper colon than the whites, a similar situation to that for the Chinese.

3) The leading sites are similar for Japanese and whites, though the levels and order differ. For males, the sites are lung, prostate and colon for both, plus stomach for Japanese but bladder for whites. Among females, though Japanese have much lower breast cancer rates, it is the leading site for them, as it is for whites, followed by colon, stomach and corpus, with lung replacing stomach among whites.

4) Differences exist between San Francisco and Hawaiian Japanese, generally reflected in higher rates in Hawaii than San Francisco, such as for lung for both sexes and stomach, prostate, bladder, thyroid and liver among males. Only for esophagus among males is the rate in San Francisco higher than in Hawaii.

5) There appears to be a rising trend among Japanese in incidence of colon, breast, corpus and of liver among males.

Cancer Mortality in the People's Republic of China

Updating and expanding a paper originally presented at the China Population Conference, 1980, East-West Population Institute, Honolulu, Hawaii, a thorough review of the vital statistics reporting system in the PRC and major causes of

death was prepared for inclusion in China: Facts and Figures Annual. The section on cancer details findings from the 1973-75 national mortality survey, covering the significance of cancer as a leading cause of death, geographical distribution within the PRC, differences among the national minorities, cancer mortality transition among the Chinese, and speculations as to etiologic-interventive clues.

Selected findings:

Cancer, overall, comprises 11% of deaths among males and over 8% among females. Stomach cancer was the leading site for cancer mortality for both sexes, an unexpected finding, followed by esophagus, liver, lung and colon-rectum for males, and cervix, esophagus, liver and lung for females. The average age at death varied by site from 27 for leukemia among males to 65 for bladder among females.

Major differences in the geographical distribution of various cancer sites within the PRC have long been known, but new ones were discovered as a result of the survey. In addition to regional differences, it is interesting to note that great mortality differentials prevail at various sub-provincial levels, as well as urban-rural differences by site. Urban excess was shown for most sites, notably lung, bladder and female breast. However, the reverse was true for esophaghus and genital sites, i.e., penis, cervix and choriocarcinoma. The differing impact on rural and urban residents as to environmental exposure and ways of living seems to be clearly indicated.

Substantial differences in mortality risk were noted among minority groups. Of particular interest was the noticeable mortality difference observed for Kazak and Uighur because the two groups are practically neighbors.

Comparisons were made of mortality among several parts of the PRC, Hong Kong and the U.S. Elevated lung risk was observed for urban Chinese (Shanghai, Hong Kong and the U.S.) versus lower risks for rural areas (PRC, Kwangtung and Taiwan). Generally, those cancers for which Asian Chinese have high risks displayed a downward trend, and conversely, low risk cancers displayed a rise. The reduction in cervix cancer, elevation of colon-rectum, and only moderate rise in breast cancer, were all in keeping with migration experience of other groups.

The geographical variations within the PRC, and site-specific differences by administrative unit, urban-rural residence, and national minority may largely be explained by synergistic effect of environmental exposure and living habits. For example, esophageal cancer was said to be related to the interplay of several factors including fermented and moldy foods, nitrosamines and their precursors contained in some grains, a relative deficiency of certain trace elements in food and drinking water, dietary imbalance, and personal habits. Also, the higher esophageal mortality of the Kazaks than among the nearby Uighurs was said to be related to the large amount of moldy cheese and bread consumed by the former, whereas fresh vegetables were heavily used among the latter.

Norwegian Migrant Studies:

Earlier reports summarized dietary and cancer data for 467 cancer deaths among the Lutheran Brotherhood cohort of 17,818 male respondents (USA, Minnesota), which accrued over an 11 1/2 year follow-up period. Currently a new data set has been developed which provides survival and cause of death information for 15 years of follow-up. There are now 738 deaths due to cancer, an increase of 58 percent available for analytical purposes. On a site specific basis there are now 53, 109, 138, 99 cancers of the stomach, colon or rectum, lung and prostate, respectively. These figures represent percentage increases of 51, 42, 53 and 62, respectively, for these sites over what was available for analysis from the 11 1/2 year follow-up. Utilizing the new data base and new statistical methodology developed in the Biometry Branch (Gart and Thomas), two major observations have been made:

1) Differences in the short-term and long-term effects of diet should be noted and accounted for in future analyses of cohort studies. As an example, using 10-year follow-up data, it was previously reported that high levels of vitamins A and C were negatively associated with lung cancer. A new analysis of the 15-year follow-up data base indicates that vitamin A consumption does not show any statistically significant association with respect to lung cancer mortality. The 15-year analysis for vitamin C, however, still shows a suggestive protective effect but now the statistical level of significance is placed at $P > .10$ (as compared to $P < .03$ for the 10-year analysis). These analyses adjust for urban/rural residency, age and cigarette smoking.

2) The effects of nativity and ethnicity, and perhaps the influence of diet in early life, should be considered and adjusted for in analyses which include a substantial proportion of individuals of foreign stock. An example has been worked out for stomach cancer. The earlier suggestions of a protective effect for high consumption of certain vegetables and vitamin C were not confirmed with the new 15-year data set. The new analyses suggest a positive association for salted fish consumption (not statistically significant). Significant findings were found for nativity and ancestry. Foreign born migrants were at greater risk of developing stomach cancer; and individuals who were first or second generation Norwegian/Swedish were also found to be at greater risk.

With respect to the Norwegian cohort study, a paper written in collaboration with NCI investigators has been published, "Dietary Habits and Lung cancer," Int. J. Cancer 31: 397-408, 1983. This contract expired on December 31, 1982. The Principal Investigator (contractor) summarized his findings in a final report submitted in January, 1983. Negative associations (i.e., protective effects) were reported for the following dietary items and cancer incidence by site: fish--melanoma; vegetables--lung, leukemia; fruits--esophaghus, stomach, kidney; vitamin A--squamous cell carcinoma of the lung; vitamin C--mouth and pharynx, esophagus, stomach; alcoholic beverages--melanoma. Positive associations (i.e., detrimental effects due to high consumption) were found for the following dietary items, by site: fish--thyroid; meats--mouth and pharynx, female breast, cervix; fruits--leukemia; alcoholic beverages--mouth and pharynx, esophagus, colon and rectum, liver, pancreas and larynx. These findings may be

at variance with what may have been reported earlier. The final summary reports also utilize the statistical methods and computer programs developed at NCI (Gart and Thomas). Some of the earlier anticipated results were not reported (e.g., colon cancer (meats)).

South Pacific Studies:

A preliminary analysis of incidence data from Fiji (1979-81), New Caledonia (1977-81) and Papua New Guinea (1972-78) was carried out in the form of proportional incidence rates, using the Los Angeles white population as a standard. The rates for liver cancer were consistently high in all three populations; those for gum and mouth and Burkitt's lymphoma unusually high in Papua New Guinea. The numbers for many cancers were so small that it is difficult to attach significance to them. Preliminary tabulations of incidence cases in American Samoa (1971-80) were prepared but again the numbers were very small.

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 investigators on the Hawaiian Japanese study have successfully applied for a grant to continue their research. Work on the project will continue uninterrupted upon termination of the contract. In the future, the Biometry Branch may contract with this group for specific ad hoc studies based on the cohort if such studies cannot be done under the grant funding. On the Norwegian studies, computer files are being developed at NCI which will facilitate the continued analyses of these dietary studies. We are satisfied with the computer material obtained from the University of Minnesota; however, the data and documentation provided by the University of Bergen was lacking in sufficient detail and was presented in a most confusing manner. In the near future we expect to obtain complete information from the Norwegian contractor. Hopefully we will have a useful analytical data tape for that study. Meanwhile we will continue in-depth analyses of the Lutheran Brotherhood cohort utilizing the new 15-year data base. We suggest that future support service funding be provided to the University of Minnesota which will allow even more complete survival follow-up, perhaps to 20 years. This should be done at a minimal cost of $15,000 per year. The new data base should reside and be maintained at the NCI, while keeping collaborative efforts open with other principal investigators.

Further descriptive studies of Chinese populations will continue based primarily on data that can be made available both in the U.S. and in China at no additional cost. Funding for the cancer registries in the South Pacific will

come to an end this year. Development of these registries will continue, however, through the efforts of the University of Southern California and the Hawaii Tumor Registry. This will make available cancer incidence data for some of the Pacific populations which can be compared with those for Hawaii.

Publications:

Correa, P., Heilbrun, L., MacLennan, R., Newell, G., and Pollack, E. S.: Report of a workshop: Cancers of the colon and rectum. Third Symposium on Epidemiology and Cancer Registries in the Pacific Basin. In Henderson, B. E., Kolonel, L. N., and Donovan, J. T. (Eds.): Third Symposium on Epidemiology and Cancer Registries in the Pacific Basin. NCI Monograph 62: 145-149, 1982.

King, H., and Locke, F. B.: Life style and health risks. In Rothschild, H. (Ed.): Risk Factors for Senescence. New York, Oxford University Press. (In Press)

King, H. and Locke, F. B.: Selected indicators of current health status and causes of death in the People's Republic of China: An historic perspective. In Scherer, L. (Ed.): China: Facts and Figures Annual. Minneapolis, Minnesota, Academic Press. (In Press)

CONTRACTS IN SUPPORT OF THIS PROJECT

KUAKINI MEDICAL CENTER (NO1-CP-61060)

Title: Study of Cancer Among Japanese Migrants

Current Annual Level: $542,193

Man Years: 12.5

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 island 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.

Major Contributions: During the year a reanalysis of the data on the possible relationship between alcohol consumption and the subsequent occurrence of each of the five most frequent cancers in this cohort was carried out by the Biometry Branch Project Officer. This involved the first 14 years of follow-up and confirmed his earlier findings through 13 years of follow-up -- that there appeared to be a dose-response relationship between alcohol consumption and cancers of the rectum and lung but not for the other three cancers. The major contributor to the relationship with rectal cancer appeared to be high beer consumption and for lung cancer, high wine and whiskey consumption. A paper presenting these results has been submitted for publication. A study of the possible relationship between serum uric acid and specific cancers was carried out and the results were negative. A paper has been submitted for publication.

Proposed Course: Since the initiators of this project left NCI several years ago and the involvement of current Biometry Branch staff is minimal, the project staff was urged to apply for a grant to continue the research. The application was successful and work under the grant will commence September 1, 1983 at which time the contract will be terminated.

SOUTHERN CALIFORNIA, UNIVERSITY OF (N01-CP-91027)

Title: Study of Cancer Incidence in the South Pacific

Current Annual Level: $50,382

Man Years: 1

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.

Major Contributions: A preliminary analysis of incidence data from Fiji (1979-81), New Caledonia (1977-81) and Papua New Guinea (1972-78) was carried out in the form of proportional incidence rates, using the Los Angeles white population as a standard. The rates for liver cancer were consistently high in all three populations; those for gum and mouth and Burkitt's lymphoma unusually high in Papua New Guinea. The numbers for many cancers were so small that it is difficult to attach significance to them. Preliminary tabulations of incidence cases in American Samoa (1971-80) were prepared but again the numbers were very small.

Proposed Course: The contract has been extended without additional funds through September 1983 and a request for further such extension has been requested. The purpose of these extensions has been to strengthen the establishment of ongoing registries in the South Pacific and these efforts are beginning to show substantial results.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP04260-23 B

PERIOD COVERED
October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Consultation on Clinical Trials

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)
David P. Byar, Chief, Clinical and Diagnostic Trials Section, 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 and Diagnostic Trials Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.75	3.75	1.0

CHECK APPROPRIATE BOX(ES)
☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither
☒ (a1) Minors
☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to provide consultative services in statistical and epidemiological methodology in the design, interpretation, and evaluation of clinical trials of diagnosis and treatment of cancer and other studies requiring this kind of expertise. For some trials the Section provides full statistical support including development of detailed study plans, assistance in the design of appropriate study forms, supervision of randomization and collection, processing, and editing of data, performance of interim analyses during the progress of the study, preparation of progress reports, final analysis of study data, and collaboration in the preparation of scientific papers. During the last year the Section has provided such full statistical support for several large-scale, multicenter, randomized clinical trials of cancer treatment. At present there is one active protocol for testis cancer, two for brain tumors, and six for lung cancer. In the area of diagnosis, important work includes the evaluation of multiple serum markers in lung cancer, and evaluation of the Makari skin test as a diagnostic test for colorectal, lung, and breast cancer. In another project a large data bank is maintained with information on patients with breast cancer, most of whom have had estrogen receptor measurements performed at least once. In collaboration with the Markers Group of the Breast Cancer Task Force, the Section is responsible for an inventory and information file containing data on over 10,000 women for whom serum samples are available for evaluating serum markers for breast cancer. Additional consultative activity involves collaboration with other scientists in projects such as a study of the geographical variation in death rates from lung cancer in the U.S., an investigation of personality factors in fibrocystic and malignant breast disease, and analyses of prognostic factors and practical staging systems for mycosis fungoides.

PROJECT DESCRIPTION

Names, Titles, Laboratory and Institute Affiliations of Professional Personnel (other than the Principal Investigator) engaged on this Project:

Mitchell H. Gail	Medical Statistical Investigator	BB, NCI
Sylvan B. Green	Medical Researcher	BB, NCI
David L. Levin	Senior Investigator	BB, NCI
Larry R. Muenz	Mathematical Statistician	BB, NCI
Donald K. Corle	Computer Systems Analyst	BB, NCI
Lawrence V. Rubinstein	Staff Fellow	BB, NCI
Steven Piantadosi	Medical Staff Fellow	BB, NCI

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 trials of lung, 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 a study of psychological aspects of breast cancer, analysis of prognostic factors using data from a registry of patients with mycosis fungoides, maintenance of a large data bank containing information on patients with breast cancer, and studies designed to evaluate diagnostic tests and potential tumor markers.

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. Z01CP04409-08 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. They also collaborate in the publication of findings by the medical investigators.

1. Prostate cancer. 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 used these data as the basis for a comprehensive analysis of prognostic variables for prostatic cancer using a

multivariate survival model. For patients with limited disease (stages I and II) they found that the size of the primary lesion, histological grade, acid phosphatase level (even though all values were in the normal range by definition for stage I and II patients), and the use of endocrine treatment (diethylstilbestrol) were predictive for the time until tumor progression. For patients with extended disease (stages III and IV) separate analyses were performed to study factors that predict deaths due to prostatic cancer and deaths from any cause. For cancer deaths the important prognostic variables were the size of the primary tumor, histologic grade, acid phosphatase level, the presence of ureteral dilatation, the presence of metastases, and the use of hormonal therapy (estrogens and/or progestins). For death from all causes the same variables were prognostic, but predictions were improved when information on standardized weight, age, performance level, and hemoglobin were taken into account.

2. Bladder cancer. Dr. Byar helped the Genito-Urinary Group of the European Organization for Research on Treatment of Cancer (EORTC) design a trial comparing placebo and pyridoxine in patients with stage I bladder cancer (recurrent papillomas). 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. For this reason tryptophane load tests are being performed before and during treatment. 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 chemotherapy used only for relapses. Stage I patients are also being followed to determine factors that may predict which tumors will recur. By April 1983, 176 patients had been admitted to the randomized trial, and 192 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. The data from this study are being used not only to compare treatment approaches, but also to identify factors that predict which patients will be found at surgery to have nodal involvement, and to identify patients (both stage I and stage II) with increased risk of recurrence.

4. Data bank for breast cancer studies. The current files contain data on 3600 women with breast cancer, many of whom had estrogen receptor assays performed. A natural history data base of stage II patients who received no adjuvant therapy was created from these files. Non-randomized but adjusted comparisons with patients treated in adjuvant studies indicated that, in general, adjuvant therapy was beneficial but some of the adjuvant studies were so small that they lacked sufficient power to detect important differences. These results along with a "word of caution" about performing these non-randomized comparisons were presented by Mr. Corle and Dr. Byar at the fall meeting of the Breast Cancer Task Force. Serum and background information 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. Serum has been collected from women with benign breast disease and asymptomatic controls as well as from patients with newly diagnosed breast cancer. Most of these women have contributed several yearly samples along with follow-up information. An official announcement of the availability of this valuable resource was made during Fiscal Year 1983. 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. Trials for lung cancer. Drs. Gail, Rubinstein, and Piantadosi are statisticians for the Lung Cancer Study Group, which is comprised of five medical centers. The Lung Cancer Study Group is in a period of transition from support as a contract group to support under a cooperative agreement. If funding is approved, the number of contributing centers will increase to eight, and the activities of the group will expand to include 12 protocols in lung cancer and accrual of over 500 patients per year. The primary emphasis of these protocols is the study of adjuvant chemotherapy, radiotherapy or immunotherapy in patients with resected lung cancer. Some future protocols may evaluate the usefulness of surgery in patients who were formerly deemed inoperable but who were rendered operable through pre-operative radiotherapy and/or chemotherapy.

Dr. Gail is currently responsible for three protocols. The first of those has demonstrated that BCG confers no apparent benefit on patients with resectable stage I non-small cell lung cancer. Prognostic factors have been studied in this carefully staged group of 473 patients. The second 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. The third protocol compares radiotherapy with conventional observation in stage II/III patients with resectable squamous cell carcinoma.

Dr. Rubinstein's future activities with the Lung Cancer Study group will be limited to completion of the three therapeutic protocols described next and to an analysis of natural history for patients with resectable stage I disease. His first protocol compares CAP plus radiotherapy to radiotherapy alone in patients with partially resected non-small cell lung cancer. The second study compares CAP therapy to placebo in the subset of stage I patients with T1N1 or T2NO disease. The third study compares limited resection with lobectomy in patients with peripheral T1NO non-small cell disease. Dr. Rubinstein has contributed to manuscripts on patterns of recurrence and surgical mortality experience, and has written a manuscript describing characteristics of the available patient population for accrual onto future trials.

Drs. Gail and Piantadosi are making preparations for six future protocols. Dr. Piantadosi has reviewed a protocol to study adjuvant surgery in small cell patients who have responded to induction chemotherapy, and he is devising data forms.

6. Lung cancer mortality data. Dr. Piantadosi, in collaboration with Dr. Tom Mason of the Environmental Epidemiology Branch, has been studying the geographical distribution of lung cancer mortality rates in U.S. white males. In this work both standard and new statistical methodology are used to identify

those geographic features which may contribute to variations in the mortality due to lung cancer. Demographic, industrial and other variables at both the state economic area level and county level are being studied.

7. Multiple markers for diagnosing lung cancer. Drs. Gail and Muenz, in collaboration with Dr. Robert McIntire of the NCI Diagnosis Branch, are analyzing a series of serum panels of twelve biochemical assays from patients with benign lung disease and with various stages of lung cancer. Initial analyses show the (anticipated) diagnostic value of CEA (carcinoembryonic antigen) as a marker. Work is proceeding to find other markers which are effective alone as well as in combination. The goal is to combine several tests to produce optimal discrimination between patients with malignant lung disease and benign lung disease. Standard linear discriminant methods will be used to analyze the data. New methods of discrimination based on branching logic ("recursive partitioning algorithm") will also be tried. Separate serum panels will be analyzed to validate the discrimination rules.

8. Brain tumor clinical trials. Drs. Green and Byar have continued to work extensively with the Brain Tumor Study Group (BTSG) on the design and analysis of a number of large-scale randomized clinical trials. They have continued their analysis of three previous phase II trials, as well as two 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. They have also performed interim analyses of the two ongoing studies: the phase III trial, BTSG 80-01, which compares three chemotherapeutic regimens consisting respectively of one, two, and four drugs (BCNU alone, BCNU alternating with procarbazine, and BCNU + hydroxyurea alternating with procarbazine + VM-26); and the phase II trial, BTSG 81-20, which compares AZQ with PCNU. Drs. Green and Byar have also participated in the design of two successor protocols that are under consideration to begin within the next year. In addition, Dr. Green is working with BTSG Pathologist Dr. Peter Burger on an analysis of the survival experience of patients with the specific histopathologic classification of anaplastic astrocytoma, to determine more precisely differences in prognosis for these patients compared to those diagnosed as glioblastoma multiforme and to evaluate the possible importance of additional histological features.

9. Inflammatory breast cancer in Tunisia. Dr. Muenz has continued to analyze further follow-up in a trial of surgery with and without radiotherapy for this disease. There is evidence that initial disease characteristics of patients with this aggressive form of inflammatory breast cancer are predictive of future metastatic site, if any.

10. 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. Research on psychological differences between these groups showed that after statistical adjustment for between-group differences in

demographic factors, women in the fibrocystic group see themselves substantially more often as "type A" than women in the other two groups.

With Dr. Shae Kosch of the University of Connecticut School of Medicine, Dr. Muenz is analyzing further retrospective data concerning the frequency and intensity of stressful life events preceding the development of fibrocystic disease, malignant breast disease, or neither.

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 1983, 250 patients have been entered on the diagnostic protocol. The test has failed to discriminate between patients whose biopsy reveals cancer and those without cancer. Most of the patients have been followed for one year to see if they develop cancer following a diagnosis of benign conditions. Even with this additional year, the test still has not been useful for diagnosis. Analysis of 170 patients on the monitoring protocol will begin later this year.

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). Based on data for 109 patients, differences between hormone subunits for myeloma patients and those with MGUS have been small, but both groups show significant differences from a group of normal controls studied previously.

13. Mycosis fungoides. Drs. Green and Byar have continued their collaboration with Dr. Stanford Lamberg of Johns Hopkins University on analyses of data from patients registered by the Mycosis Fungoides Cooperative Group. Previous analyses of survival have been updated based on additional follow-up information, and alternative staging systems based on clinical information have been constructed and compared with one previously proposed.

14. Radiosensitization of rat intestinal epithelium. With Dr. Agnes Reynaud of the NCI, Dr. Muenz has studied the extent to which various drug and gas exposures lead to increased radiation dose sensitivity of a rat's intestinal epithelium. Since each measured response depends upon the individual experimental animal and a common control group, readings are correlated even between different animals. No change is seen in the dose-response relation according to experimental condition.

15. Hospice demonstration study. With Dr. Ivan Barofsky of the NCI, Dr. Muenz is re-examining data collected during the 1979-80 NCI hospice demonstration project with greater emphasis on patients' responses. Dr. Muenz and Dr. Barofsky are also studying methodologic problems of obtaining and analyzing

data from terminally ill patients who, although alive, are often unavailable or unreliable.

16. Evaluation of the risk of developing breast cancer. Drs. Gail and Byar are collaborating with Dr. John Mulvihill of the Clinical Epidemiology Branch and Dr. Louise Brinton of the Environmental Epidemiology Branch to develop quantitative estimates of the risk of developing breast cancer within the next five or ten years, to be used in counseling patients with a family history of breast cancer.

17. Consultation with the European Organization for Research on Treatment of Cancer. Dr. Green visited the Data Center of the EORTC in Brussels, Belgium, as an invited consultant for five weeks. While there, he reviewed their operations and computer system, formulated design specifications for a cross-check editing program to be used at the Data Center, and assisted in the implementation of computer software for multivariate survival analysis that he had written and that has been in use at the Clinical and Diagnostic Trials Section at NIH. In addition, he participated in the analysis of prognostic factors for malignant brain tumors using data from an EORTC study, and he conducted seminars for Data Center staff on a variety of topics relating to clinical trial design and analysis.

18. Other consultative activities. Drs. Byar and Green have consulted on analysis of a clinical trial conducted by the National Surgical Adjuvant Breast Project concerning chemotherapy with and without tamoxifen in patients with stage II breast cancer. The analysis focused on the finding of the study statistician Dr. Carol Redmond and her colleagues that some types of patients appeared to benefit from tamoxifen whereas tamoxifen appeared to harm others. Drs. Byar and Green generally confirmed these findings in their independent analysis.

Dr. Green has been collaborating with H. and B. Malker from the Swedish Cancer Registry on analyses of survival patterns for cancer patients diagnosed in Sweden from 1960 to 1978. Initial efforts have focused on mesothelioma (of interest because of its relationship to asbestos exposure) and testicular cancer (of interest because of recent improvements in outcome resulting from chemotherapy).

With Dr. Howard Austin of the NIADDK, Dr. Muenz has analyzed measures of lupus activity and chronicity as predictors of time to the development of lupus nephritis, and the role of conventional and electron microscopy of renal lesions in assessing prognosis.

Mr. Corle has been collaborating with Dr. Thomas Dao of Roswell Park to assess the prognostic importance of steroid sulfotransferase in primary breast cancer.

Dr. Levin has continued to serve on the faculties of Georgetown University and the Uniformed Services University of the Health Sciences, teaching biostatistics and programming to medical and postgraduate students.

Dr. Levin has continued to work with Roger Connelly of the Biometric Research Section studying the epidemiology of pancreatic cancer.

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 five 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:

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Austin, H. A., Muenz, L. R., Joyce, K. M., Antonovych, T. A. et al.: Prognostic factors in lupus nephritis: Contribution of renal histology. Am. J. Med. (In Press).

Bharucha, N. E., Schoenberg, B. S., Raven, R. H., Pickle, L. W., Byar, D. P. and Mason, T. J.: Geographic distribution of motor neuron disease and correlation with possible etiologic factors. Neurology (In Press).

Biggar, R. J., Gail, M. H., Banks, R. B., Neequaye, J. and Nkrumah, F. K.: Age and sex as factors influencing remission duration in African Burkitt's lymphoma. Am. J. Epidemiol. 116: 668-677, 1982.

Byar, D. P., Corle, D. K.: Analysis of prognostic factors for prostatic cancer in the VACURG studies. In Denis, L., Murphy, G., Prout, G. and Schroder, F. (Eds.): Clinical Trials in Urology. New York, Raven Press (In Press).

Byar, D. P., and Corle, D. K.: Studies of acid phosphatase in prostatic cancer. In Smith, P. H. and Pavone-Macaluso, M. (Eds.): Cancer of the Prostate and Kidney. New York, Plenum Press (In Press).

Byar, D. P., Green, S. B., and Strike, T. A.: Prognostic factors for malignant glioma. In Walker, M. D. (Ed.): Oncology of the Nervous System. The Hague, Martinus Nijhoff, Vol. I (In Press).

Byar, D. P., and Hovsepian, J.: Pulmonary and osseous metastases in prostatic cancer: methods of quantitative assessment. In Smith, P. H., and Pavone-Macaluso, M. (Eds.): Cancer of the Prostate and Kidney. New York, Plenum Press (In Press).

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Fisher, S. G. and Levin, D. L.: The sexual knowledge and attitudes of professional nurses caring for oncology patients. Cancer Nurs. 6: 55-62, 1983.

Green, S. B., Byar, D. P., Walker, M. D. et. al.: Comparisons of carmustine, procarbazine, and high-dose methylprednisolone as additions to surgery and radiotherapy for the treatment of malignant glioma. Cancer Treat. Rep. 67: 121-132, 1983.

Jansen, M. A. and Muenz, L. R.: A retrospective study of personality variables associated with fibrocystic disease and breast cancer. J. Psychosom. Res. (In Press).

Oldham, R. K., Gail, M. H., Baker, M. A., Forbes, J. T., Heineman, W., Hersh, E., Holmes, E. C., Ritts, R. E., and Wright, P. W.: Immunological studies in a double blind randomized trial comparing intrapleural BCG against placebo in patients with resected stage I non-small cell lung cancer. Immunology and Immunotherapy 13: 164-173, 1982.

Pettrone, F. A., Gail, M. H., Pee, D., Fitzpatrick, T. and Van Herpe, L. B.: Quantitative criteria for the prediction of the results after displaced ankle fractures. J. Bone and Joint Surg. (In Press).

Rosen, S. W., Gail, M. H. and Tormey, D. C.: Use of circulating pregnancy-specific beta-1 glycoprotein as a marker in carcinoma of the breast in women. JNCI 69: 1067-1071, 1982.

Staquet, M. J., Byar, D. P., Green, S. B. and Rozencweig, M.: The clinical predictivity of transplantable tumor systems in the selection of new drugs for solid tumors. Rationale for a three-stage strategy. Cancer Treat. Rep. (In Press).

CONTRACT IN SUPPORT OF THIS PROJECT:

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

Title: Biomedical Computing Software Services

Current Annual Level: $360,000

Man Years: 9

Objectives: The contractor provides system design and computer programming support services for the research projects conducted by the Section (See project numbers Z01CP04409-08 B and Z01CP04260-23B).

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.

Proposed Course:

The contractor has worked with the Section for all of Fiscal Year 1983. A competitive selection was performed during the year to award a new contract for three years continuation of similar support services.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP04265-18-B

PERIOD COVERED
October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Consulting in Statistics and Applied Mathematics

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)
J. J. Gart, Chief, Mathematical Statistics & Applied Mathematics Sect., 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

CHECK APPROPRIATE BOX(ES)
☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

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.

PROJECT DESCRIPTION

Names, Titles, Laboratory and Institute Affiliations of Professional Personnel (other than the Principal Investigator) engaged on this project:

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

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 other institutes of N.I.H., other government agencies, and some contractors. 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 is collaborating with Dr. Thomas Cameron on a paper summarizing survival and growth rates of control animals in carcinogenesis bioassay experiments performed from 1971 to 1978 for the NCI Carcinogenesis Testing Program.

Division of Cancer Cause and Prevention - Field Studies and Statistics

Dr. Gart continues his collaboration on two large prospective studies on the relationship of diet and cancer being done at the University of Bergen in Norway and at the University of Minnesota. The Norwegian study, involving Professor Bjelke and Dr. Kvåle, found that high vitamin A dietary intake was inversely associated with the subsequent incidence of lung cancer, but that this inverse relation was almost entirely due to the effect on those with a high alcohol intake. This study also found that the incidence of

kidney cancer and non-melanoma skin cancer were inversely related to high levels of coffee drinking. Other results in both the Norwegian and U.S. studies, also studied by Professor Schuman, found direct association between pancreatic cancer incidence and mortality and the high intake of alcohol and the use of snuff and chewing tobacco. Dr. Gart has collaborated on these projects with Mr. Scotto who has been project officer of both studies.

Donald Thomas advised Edwin Lisiecki and Joseph Scotto of the Biometry Branch concerning various technical problems in using our program for stratified trend and homogeneity analyses of proportions and life table data on two large prospective studies of the relationship of diet and cancer. He also advised Dr. Pollack and Edwin Lisiecki of the Biometry Branch on technical problems associated with using this program in a prospective study of alcohol consumption and cancer on Japanese men in Hawaii.

Mr. Nam has continued to collaborate with Joseph Scotto of the Biometry Branch on the updated eight year non-melanoma skin cancer survey data on trends and seasonality in incidence.

Dr. Gart and Dr. Tarone are providing statistical assistance to Dr. Blattner and Dr. Blayney of the Environmental Epidemiology Branch and Dr. Saxinger of the Laboratory of Tumor Cell Biology regarding the evaluation of data generated by ELISA assay system used to test for the presence of antibodies to the human T-cell leukemia virus (HTLV). Dr. Gart also advised other members of the Environmental Epidemiology Branch on statistical questions.

Dr. Tarone advised Dr. Howard Hayes of the Environmental Epidemiology Branch regarding statistical issues in the epidemiology of cancer in pet dogs.

Dr. Tarone advised Dr. Thomas Goffman and Mr. William McKay of the Clinical Epidemiology Branch concerning methodologic problems involved in the statistical analysis and mapping of standardized mortality ratios.

Mr. Nam and Dr. Gart have continued to collaborate with Dr. Paul Levin of the Clinical Epidemiology Branch on the Chinese NPC (nasopharyngeal carcinoma) cases in Singapore with regard to Epstein-Barr virus antibody level and survival.

Mr. Nam has given tutorials in statistical methods on the analysis of case-control studies in cancer research to Drs. Xu-Dong Dai of Harbin Medical College and Song Lin Yu of Wuhan Medical College, visitors to the Clinical Epidemiology Branch. He also advised Dr. Yu on statistical analysis of the Chinese dysenteric data.

Mr. Thomas continued to maintain and improve the section's library of computer routines and advised various staff members on their use as well as other technical aspects of computer programming. Again this year numerous researchers throughout the world have requested and received copies of the computer programs developed and used in this section.

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 Field Studies and Statistics Review Group.

Division of Cancer Cause and Prevention - Carcinogenesis Intramural Program

Dr. Pettigrew consulted with Dr. Miriam Poirier of the Laboratory of Experimental Pathology concerning the estimation of adduct removal rates from data on rat liver repair in animals fed 2-AAF.

Dr. Tarone advised Dr. Harry Gelboin and Dr. T. Fujino of the Laboratory of Molecular Carcinogenesis in the statistical evaluation of twin placenta studies designed to study the inhibition of aryl hydrocarbon hydroxylase and 7-ethoxycoumarin deethylase activity by a monoclonal antibody.

Dr. Tarone continued to provide statistical assistance to Dr. Kenneth Kraemer of the Laboratory of Molecular Carcinogenesis in studies of aryl hydrocarbon hydroxylase induction levels in psoriasis patients and of the incidence of internal tumors in patients with xeroderma pigmentosa.

Dr. Tarone continues to design and perform the statistical analysis of experiments 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 elucidate the mechanisms of increased susceptibility to fluorescent light-induced and X-ray-induced chromosome damage in fibroblasts from patients with a variety of cancer-prone disorders.

Dr. Tarone performed the statistical analysis of mouse skin painting experiments performed by Dr. Henry Hennings of the Laboratory of Cellular Carcinogenesis and Tumor Production to investigate the mechanisms of conversion of papillomas to squamous cell carcinomas.

Mr. Nam has collaborated with Dr. Richard Yamamoto of the Laboratory of Carcinogen Metabolism on the relation of dietary fat to pancreatic cancer in animal studies.

Division of Cancer Biology and Diagnosis

Dr. Tarone continues his collaboration with Dr. Jay H. Robbins, Dr. Fujio Otsuka and Ms. Susanna Barrett of the Dermatology Branch and Dr. Dominic Scudiero of the Chemical Carcinogenesis Program of the Frederick Cancer Research Center in their experiments to study the in vitro survival of lymphoblast and fibroblast cell lines from patients with the cancer-prone disease, xeroderma pigmentosa, and with a variety of hereditary primary neuronal degenerations after exposure to DNA-damaging agents such as ultraviolet light, X-rays and MNNG. Dr. Tarone is also analyzing data from experiments performed by Dr. Lana Seguin-Spillman to study chromosomal

aberrations induced by x-irradiation in lymphoblasts from various neurodegenerations and cancer-prone disorders.

Dr. Pettigrew has continued to advise Dr. Pietro Gullino of the Laboratory of Pathophysiology on a variety of topics, most recently concerning experiments to test the effect of magnetic fields on tumor growth and metastasis.

Dr. Tarone continued to provide 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 intra-lesionally administered cell walls of Mycobacterium bovis strain Bacillus Calmette-Guerin.

Various Other Activities

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.

Dr. Gart and Dr. Tarone advised the Commissioner's Office of the Food and Drug Administration on statistical questions in the interpretation of a long-term animal carcinogenesis test of a food additive.

Dr. Pettigrew advised Dr. Floyd Taub of the National Institute of Dental Research on the analysis of autoradiograph data from an experiment on DNA hybridization.

Dr. Pettigrew provided for Dr. Sanford M. Rosenthal, scientist emeritus at the National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, a statistical analysis of mortality data from an experiment designed to determine the effect of sodium chloride on longevity in mice. Dr. Rosenthal's work was published in the American Journal of Physiology. Dr. Pettigrew is collaborating with Dr. Jonathan L. Costa, Clinical Neuropharmacology Branch, National Institute of Mental Health, and others, on the preparation of a paper on changes in P-31 nucleotide resonances.

Mr. Nam advised Dr. Sukwoo Chang of Sandy Hook Laboratory, National Marine Fisheries Service, on statistical analysis concerning an effect of heavy metal ($CuCl_2$) on survival of bay scallops and surf clam from experimental data.

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.

Publications:

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Kvåle, G., Bjelke, E., and Gart, J. J.: Dietary habits and lung cancer risk. Int. J. Cancer 31: 397-405, 1983.

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Parshad, R., Sanford, K. K., Jones, G. M., and Tarone, R. E.: Neoplastic transformation of human cells in culture associated with deficient repair of light-induced chromosomal DNA damage. Int. J. Cancer 30: 153-159, 1982.

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Robbins, J. H., Otsuka, F., Tarone, R. E., Polinsky, R. J., Brumback, R. A., Moshell, A. N., Nee, L. E., Ganges, M. B., and Cayeux, S. J.: Radiosensitivity in Alzheimer disease and Parkinson disease. Lancet 1: 468-469, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP04267-18 B

PERIOD COVERED
October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Research in Mathematical Statistics and Applied Mathematics

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)
J. J. Gart, Chief, Mathematical Statistics & Applied Mathematics Sect., 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

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

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 and HLA data.

PROJECT DESCRIPTION

Names, Titles, Laboratory and Institute Affiliations of Professional Personnel (other than the Principal Investigator) engaged on this Project:

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

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.

John J. Gart and Jun-mo Nam have devised a statistical test for the possible presence of hidden HLA (human leukocyte antigen) alleles when no "double blanks" are found in a population. By this test they have been able to discriminate between the A-locus and the B-locus in the same population as far as the possibility of finding further as yet unidentified antigens is concerned. In related work Jun-mo Nam and John Gart are also investigating the problem of comparing two such groups, particularly in case-control studies, in which neither group has any double-blanks. Jun-mo Nam and John J. Gart are continuing their study on the bias and its reduction when using Bernstein's estimators in such generalized ABO-like genetic systems.

Jun-mo Nam has also continued his work on statistical methods for identifying cyclic trends in epidemiologic data. A paper on this subject has been accepted for publication. He has, in addition, continued research on sample size determination for tests which test for a linear trend in binary data. Together with John J. Gart he is continuing his investigation of the bias of the unconditional estimator of the common slope in the stratified logistic model.

Robert E. Tarone continues his research on the analysis of frequency data and distribution-free tests for censored distributions. Also, ongoing are his investigations of methods for analyzing survival curves produced by the in vitro exposure of cell cultures to DNA-damaging agents. Dr. Tarone is investigating methods for the testing and mapping of standardized mortality ratios. Dr. Tarone and Dr. Gart continued research on the efficiency of various noniterative estimators of a common relative risk or a common odds ratio. Papers on some of these projects have already been accepted for publication.

Donald G. Thomas has developed a more efficient computer algorithm for computing exact randomization tests between two groups. This is exactly the mathematical dual of the exact test for trend in binomial data and is particularly useful in this application. These methods are being extended to the case of multiple strata. Together with John J. Gart, Donald G. Thomas has completed work on a computer program for the analysis of data for prospective and case-control studies. It provides analyses of dose trends in both proportions and life table data and has been usefully applied to several prospective studies of diet and cancer.

Hugh M. Pettigrew is investigating modelling of tumor growth kinetics (by, for example, the Gompertz function) and is also considering the effects various methods of estimating tumor volume from lineal measurements, may have on the process. He is continuing his research in the mathematical theory of epidemics and considering methods to compare linear trends in proportions when the observations are correlated. He is also investigating the properties of various transformations of binomial variates.

Alroy M. Smith provides computer programming support on several of the research projects in the section. In addition, with John J. Gart, she is preparing for publication, a program which implements for the computer Gart's previously published methods for comparing Poissonian incidence rates, their ratios, and ratios of their ratios in stratified exponential models.

John J. Gart has continued his research on several topics in statistical methodology. Among these are the 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. With Hugh M. Pettigrew, he continues the study of the higher order corrections to the mean and higher moments of the logit transformations of binomial proportions and their use in weighted least squares. Donald G. Thomas has done considerable computer programming of the exact calculations of the various moments and of the transformations and the exact bias of various estimators. A joint paper based on the latter work has been submitted for publication.

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 contined, 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:

Gart, J. J., and Nam, J.: Statistical methods for genetic studies of HLA and cancer. In Cornell, R. G. (Ed.): Statistical Methods for Cancer Studies. New York, Marcel Dekker, Inc. (In Press)

Gart, J. J., and Tarone, R. E.: The relation between score tests and approximate UMPU tests in exponential models common in biometry. Biometrics. (In Press)

Nam, J.: Efficient methods for identification of cyclic trends in incidence. Commun. Statist. (In Press)

Tarone, R. E.: The use of historical control information in testing for a trend in Poisson means. Biometrics 38: 457-462, 1982.

Tarone, R. E., Gart, J. J., and Hauck, W. W.: On the asymptotic inefficiency of certain noniterative estimators of a common relative risk or odds ratio. Biometrika. (In Press)

Tarone, R. E., Scudiero, D. A., and Robbins, J. H.: Statistical methods for in vitro cell survival assays. Mutat. Res. (In Press)

Thomas, D. G. and Gart, J. J.: Stratified trend and homogeneity analyses of proportions and life table data. Comput. Biomed. Res. 16: 116-126, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP04269-12 B

PERIOD COVERED
October 1, 1982 through September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Biomedical Computer Systems - Consultation, Research and Development, Service

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)
J. Michael Stump, Acting Chief, Computer Science Section, 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	6	3

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Various aspects of computer science, information technology and data analysis are applied to the resolution of the organizational, operational and logistical constraints related to biometric and epidemiologic investigations. The scope of individual projects range from routine data analysis operations to research and development activities related to developing state-of-the-art complex systems or routines designed to operate in multicenter and multidisciplinary scientific programs and studies in cancer research. Consultation and assistance in scientific studies utilizing computers and related methodology and technology are provided to National Cancer Institute investigators, and as appropriate, to other Government agencies, private institutions, and individual investigators who collaborate with the National Cancer Institute in its mission. The Section also administers and monitors a computer support contract for the Biometry Branch.

PROJECT DESCRIPTION

Names, Titles, Laboratory and Institute Affiliations of Professional Personnel (other than the Principal Investigator) engaged on this Project:

Mary Cusano	Computer Systems Analyst	BB, NCI
Calvin Hollingsworth	Computer Programmer/Analyst	BB, NCI
Vivian Pelham	Computer Specialist	BB, NCI
Valerie Van Holten	Computer Specialist	BB, NCI
Ruth Wolfson	Computer Programmer/Analyst	BB, NCI

Objectives:

To provide consultation and assistance to the scientific and administrative staff of the National Cancer Institute, and as appropriate, to other Government agencies, private institutions, and individual investigators who collaborate with the National Cancer Institute in its mission. Research and development studies are conducted on data processing and computing problems of special interest and priority that arise out of the Computer Science Section's consultation and collaboration in scientific studies. Specific consultations are given on individual investigator studies as well as large-scale multicenter studies involving epidemiologic investigations, cancer surveillance programs, cancer control programs, and information and reporting systems for cancer centers.

Methods Employed:

The Computer Science Section staff applies systems analysis techniques and computer programming expertise to the planning, design and development of information processing systems for scientific 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 Section's primary focus continues to be on the Biometry Branch's scientific programs and studies, particularly the Surveillance, Epidemiology and End Results (SEER) Program. However, members of the Section also routinely consult with other Biometry Branch and Field Studies and Statistics Program investigators on a number of special studies.

The Section has adopted a policy of developing generalized computer routines and systems, whenever possible, in response to requests for assistance from investigators. The resulting methodology and technology where applicable, is then applied to other National Cancer Institute studies. These generalized, user-friendly and table-driven systems usually take somewhat longer to design, develop and test, however, future studies employing the same software package can get started more expeditiously, with less costs and with a proven set of uniform and standardized processing rules. These systems can usually be operated by the researchers themselves thus contributing significantly to the investigators independence from analysts and programmers and freeing the technical staff to address other problems.

Major Findings:

1. The Surveillance, Epidemiology and End Results Program

This year has been a particularly noteworthy one for members of the Section consulting on the SEER Program. A long-standing NCI commitment to the Connecticut Tumor Registry was fulfilled. The NCI was obligated to deliver, implement and test major portions of a data management system to meet SEER as well as local processing requirements. Personnel staffing problems in the Connecticut Registry also necessitated the involvement of Computer Science Section staff in converting the current master file to the form required for processing by the new system.

In another SEER-related project, the Section completed development of a subsystem to generate the match weights used by the Patient Linkage component of the SEER registry processing system. The documentation includes step-by-step procedures to allow generation of registry-specific match thresholds based upon local name distributions, missing values and other characteristics of a local file. In addition to the California and Connecticut SEER registries, the system has been sent to staff at the Illinois Cancer Council, American College of Surgeons, Pennsylvania Department of Health and the Minnesota Department of Health.

2. Other Biometry Branch Consultation

In collaboration with staff of the Biometric Research and Analytic Studies Section, a system was developed to provide investigators the capability to recode multiple Extent of Disease codes into a single code. This generalized software routine facilitates data manipulation for special studies and has been used to assist in the development of new EOD code schemes.

The Computer Science Section staff is responsible for providing Field Study and Statistics investigators access to the 1980 population tapes. The decision to centralize processing of these files will insure that population data are consistent across the entire FS&S Program.

At the request of the Chief of the Biometry Branch, the Computer Science Section conducted a survey of the available data management and statistical software in the Field Studies and Statistics Program (FS&S). All submissions were compiled into a manual that explains the function of the individual routine, the required computer resources and any noted limitations to its use. Copies are routinely distributed to each FS&S organization component and to any other individuals who request a copy. The manual will be updated and re-issued semi-annually.

The Computer Science Section staff participated in a number of other ad hoc consultations with Biometry Branch investigators. Various data management, file processing and documentation activities were completed in support of studies including Financial Cost of Cancer, Cancer in Foreign Born, Skin Melanoma and Third National Cancer Case Lists.

3. Division of Resources, Centers, and Community Activities

The Computer Science Section (CSS) continued to consult with program officials of the Division of Resources, Centers, and Community Activities (DRCCA) on the data management aspects of several studies. Pending the establishment of a DRCCA technical support unit, the Computer Science Section staff participated in two Cancer Centers Program projects, the Community Clinical Oncology Program (CCOP) and the design of a long-term clinical trial protocol. In addition, consultive assistance was provided for contractor selection activities, the development of technical specifications for contracts and the evaluation of data analysis outputs produced by firms under contract to DRCCA.

For the Cancer Centers Program, the CSS has been involved in developing software to produce cancer center profiles. These profiles enumerate the major on-going research and clinical programs, major strengths and special areas of expertise resident at each center. Computer Science Section staff also adapted a portion of the SEER registry system to perform the editing function for another Cancer Centers Program project.

The Community Clinical Oncology Program project involved computerizing data from CCOP grant applications. Various distributions, frequency counts and other analysis products were produced using statistical library routines. All systems that have been developed have been turned over to DRCCA personnel for operation. DRCCA staff are currently exploring the possibility of using the CSS developed SEER system to do the data processing for a long-term clinical trial which is currently under development. CSS staff have briefed program officials on the functions and capabilities of the system. In the event the system is used, all modifications, implementation and testing will be performed by DRCCA staff and/or DRCCA contractors.

4. Contract Selection Committee Activities

Senior members of the Section are routinely requested to serve on contract selection committees for pending data processing related procurements. This year, Computer Science Section staff participated in the evaluation of proposals for the Frederick Cancer Research Facility data processing and library support procurements, a conferencing and data support effort for DRCCA and the Protocol Data Query Cancer Information System for the Office of the Director, NCI.

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.

CONTRACT IN SUPPORT OF THIS PROJECT

Name of Contractor: ORI, INC. (NO1-CP-11025)

Title: Biomedical Computing, Design and Implementation

Current Annual Level: $1,000,000

Man Years: 26

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

Major Contributions: 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. 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.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP04409-08 B

PERIOD COVERED
October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Statistical Methodology Research

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)
David P. Byar, Chief, Clinical and Diagnostic Trials Section, BB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH
Biometry Branch

SECTION
Clinical and Diagnostic Trials Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, MD 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
5.75	4.75	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither
☐ (a1) Minors
☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to conduct research in statistical methods and computer techniques with particular emphasis on those appropriate for analyzing 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 (see Z01CP04260-23B). During the past year methods have been proposed to estimate the total number of oncogenes for a given species based on the number of distinct oncogenes observed in laboratory studies. Logistic regression methods have been developed for analyzing data consisting of a sequence of binary responses for each experimental subject. These methods permit adjustment both for initial and for time-dependent covariates. Members of the Section have taken two different approaches to the difficult statistical problem of comparing different staging systems for chronic disease. One method postulates a proportional hazards model and uses likelihood ratio tests for comparing each model alone with a comprehensive model incorporating variables representing the stages in the separate systems. The other method is non-parametric and relies on bootstrap methods to compare various measures of good prediction. In the area of survival analysis, work has continued on methods of analysis appropriate for situations in which censoring mechanisms may not be independent of the death process and a new measure of censoring dependence has been defined. Two special cases of non-independent censoring have been studied, situations in which characteristics of patients entering a study change over time and where biased follow-up occurs due to under-reporting of deaths. Other work has concerned rules for terminating accrual in clinical trials, comparisons of various methods for setting confidence limits on estimates of median survival, methods for constructing adjusted survival curves and estimating their variances, assessment of diagnostic tests, and estimation of attributable risk for multiple factors.

PROJECT DESCRIPTION

Names, Titles, Laboratory and Institute Affiliations of Professional Personnel (other than the Principal Investigator) engaged on this Project:

Mitchell H. Gail	Medical Statistical Investigator	BB, NCI
Sylvan B. Green	Medical Researcher	BB, NCI
David L. Levin	Senior Investigator	BB, NCI
Larry R. Muenz	Mathematical Statistician	BB, NCI
Donald K. Corle	Computer Systems Analyst	BB, NCI
Lawrence V. Rubinstein	Staff Fellow	BB, NCI
Steven Piantadosi	Medical Staff Fellow	BB, NCI
H. Samuel Wieand	Visiting Fellow	BB, NCI

Objectives:

Many statistical problems arise in the consultative activities of the Section (see Project No. Z01CP04260-23 B). The investigation of these problems, the development of new statistical methods, and adaptation 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:

1. Estimating the number of human oncogenes. Drs. Rubinstein and Gail have developed two methods for estimating the total number of oncogenes for a given species from the number of distinct oncogenes observed in laboratory studies. The first approach is based on likelihood methods and requires considerable computation. The second approach uses a simple approximation more appropriate for laboratory use. Drs. Rubinstein and Gail are collaborating with Dr. Stephen O'Brien of the Laboratory of Viral Carcinogenesis on this project.

2. Logistic regression for serial data. With Dr. Rubinstein, Dr. Muenz has completed work on analysis of binary data in which each patient contributes a sequence of responses, rather than one. Dr. Muenz has generalized this analysis to include time-dependent covariates and, with Dr. Howard Austin of the NIADDK, is analyzing data on relapse and remission in systemic lupus erythematosus. Repeated serologic and renal function measurements provide the time-dependent predictors.

3. Comparing staging systems. Drs. Green and Byar have studied methods for comparing alternative staging systems for a chronic disease. They have used likelihood methods to compare non-nested models. With this approach they have used proportional-hazards survival models to determine whether one staging system provides a significant improvement over another system in predicting survival. They have applied this methodology to staging systems for mycosis fungoides and for stomach cancer.

Dr. Muenz is also analyzing the problem of comparing several staging systems but using non-parametric approaches. Comparisons between systems are made by three criteria: the probability of correctly predicting if a patient in a given disease stage will be alive at a specified future time, measures of the "spread" of stage-specific survival curves at several time points, and an overall test of between-stage difference. Bootstrap methods are used to compare these measures between systems.

4. Rules for terminating accrual in a clinical trial. Drs. Rubinstein and Gail have shown that one can monitor treatment differences to stop accrual without affecting the size or power of a logrank test, provided that test is performed after a fixed number of events have been observed. By separating the decision to stop accrual from the final assessment of treatment effect, one can often avoid ethical problems which arise when treatment differences begin to appear.

5. Sequential monitoring of clinical trials. Dr. Gail is reviewing a variety of frequentist proposals for monitoring clinical trials. These techniques include group sequential tests, stopping based on repeated confidence intervals, stochastically curtailed sampling, and monitoring to stop accrual. This review is to be published in the Handbook of Statistics.

6. Adjusted survival curves. Drs. Gail, Byar, and Green have completed 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.

7. Confidence limits for estimates of median survival time. Drs. Byar and Green have continued collaboration with Dr. Eric Slud of the University of Maryland on comparing the performance of several methods of constructing confidence limits for the median survival time with censored data. They studied small sample behavior using computer simulation, and identified problems with several methods reported in the literature. They concluded that a "reflected" method based on the Greenwood variance applied to the survival curve at the estimated median (or alternatively to a curve on the cumulative hazard scale) was the preferred choice of the methods they studied.

8. Assessing diagnostic tests. Dr. Green, in collaboration with Dr. Maurice Staquet of the European Organization for Research on Treatment of Cancer, is assessing the implications of classification error in the reference test when evaluating a new dichotomous diagnostic test. Under varying assumptions

concerning the relationship of the new test and the reference test, one obtains different estimates of the sensitivity and specificity of the new test.

9. Comparing diagnostic tests via receiver operating curves. Drs. Wieand and Gail have developed non-parametric methods for comparing two diagnostic tests performed on the same patients. This comparison is based on the area under the receiver operating curve, because a large area corresponds to good discrimination. A form of bivariate Wilcoxon statistic is used to compare two such correlated areas, one for each diagnostic test. Small sample Monte Carlo studies and asymptotic efficiency calculations confirm the utility of this procedure.

10. Estimating attributable risk for multiple risk factors. In conjunction with Dr. Paolo Bruzzi of the Instituto Scientifico Tumori in Genoa, Italy and Dr. Louise Brinton of the Environmental Epidemiology Branch, NCI, Drs. Byar and Green have been studying a general formulation of attributable risk for multiple factors which may be calculated from information on the disease cases alone once estimates of relative risk are known. An estimate is obtained of the overall attributable risk for a set of factors while optionally adjusting on another set of factors. In case-control studies the relative risk for the various strata can be estimated by the relative odds, and in this multivariate setting, logistic models can be used to estimate the required odds ratios. This approach was applied to data from a large case-control study of breast cancer.

11. Biases in synthetic retrospective studies. Dr. Gail, with Dr. Jay Lubin of the Environmental Epidemiology Branch, has calculated biases which can arise if one evaluates relative risks in a cohort study by means of a case-control analysis called a "synthetic retrospective study." In particular, quantitative expressions are obtained for biases which result when one excludes cases subsequent to an incident case as controls, when one excludes certain types of controls, and when one requires "pure" controls.

12. Determining optimal treatment for subsets of patients. Drs. Byar and Green have continued previous work on studying treatment-covariate interactions to determine whether different treatments are better for different subsets of patients in a clinical trial. They have compared their analysis of data from clinical trials in prostatic cancer patients with an alternative analytic approach suggested by Peter Thall and John Lachin of George Washington University. This work was presented at a workshop on data analysis at the fourth annual meeting of the Society for Clinical Trials and will be presented again at a meeting of the Multivariate Analysis Group of the Royal Statistical Society where others will be encouraged to analyze the same data using different methods.

13. Theory of survival analysis. Dr. Rubinstein, with Dr. Eric Slud of the University of Maryland, has continued to study methods of analyzing survival data in the presence of censoring mechanisms which may not be independent of survival time for a given patient. They defined a new measure of censoring dependence. Under various models of dependence, they have explored the bias of the standard Kaplan-Meier survival estimator and have developed a useful substitute for this estimator in such cases.

14. Possible biases in estimating survival curves. Estimates of a survival curve at time t based on censored data may be biased if the censoring mechanism is not independent of the death process. Such a situation may occur if the characteristics of patients entering the study change over time or if the status of all patients, alive or dead, is not known at time t and there is selective follow-up. Dr. Rubinstein is studying the extent to which the Kaplan-Meier method either over- or under-estimates the true survival probabilities in cases where patients are either progressively sicker or healthier over the accrual period of a clinical trial. Mr. Corle and Drs. Byar and Piantadosi have investigated the effect of under-reporting of deaths on estimates of survival curves. Such selective reporting may occur during long-term follow-up of patients in clinical trials unless special care is taken to follow every patient. In order to avoid the possibility of bias, it is recommended that one tabulate the percentage of all patients for whom status is known at various points in calendar time and censor all data at the last date for which complete information is available for about 90% of all patients. Further insight into this problem was gained by modeling the clinical trial as an entry and death stochastic process, using differential equations to predict the anticipated percentage of patients alive on various dates and comparing these with the observed percentages. These differential equations may also be useful in planning the volume of data processing in clinical trials.

15. A two-sample test for positive random variables. Dr. Gail has been working with Professor Joseph L. Gastwirth of George Washington University to develop asymptotically distribution-free two-sample tests 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.

16. Tumor growth. Dr. Piantadosi has used mathematical modeling techniques to study the relationship between cell cycle kinetics and overall tumor growth. The aim of this effort is to suggest improved strategies for cancer treatment. Similar techniques have been used to study the implications for treatment strategy of the Goldie-Coldman model of tumor cell mutation.

17. Interactive data analysis programs. Dr. Green has continued to design 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 workers at other centers. Some of these programs have recently been implemented at the statistical office of the National Surgical Adjuvant Breast Project in Pittsburgh and at the Data Center of the European Organization for Research on Treatment of Cancer in Brussels.

18. Interactive graphics. Dr. Levin has worked with Dr. John Mulvihill of the Clinical Epidemiology Branch to develop graphic methods of displaying data on family grouping of cancer. Several aspects of this project have been incorporated into the Division of Computer Research and Technology's program for graphic presentation of data (the MLAB program).

19. Other activities. Dr. Gail has served on the Program Committee and as a member of the Board of Directors of the Society for Clinical Trials for the past two years.

Dr. Gail is serving as a Member of the Program Committee for the March 1984 Biometrics meetings (Eastern North American Region).

Dr. Byar was invited to speak at a special workshop on the "Status and Future of Clinical Trials" at the 25th anniversary meeting of the National Surgical Adjuvant Project for Breast and Bowel Cancers.

Dr. Byar was invited to speak on the topic "Special Problems in Multicenter Studies" at an international symposium held in Cologne, Germany, on "Gastric Cancer: Methodology of Clinical Studies and Therapeutic Attitudes."

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:

Byar, D. P.: Analysis of survival data: Cox and Weibull models with covariates. In Miké, V. and Stanley, K.E. (Eds.): Statistics in Medical Research: Methods and Issues with Applications in Cancer Research. New York, John Wiley & Sons, 1982, pp. 365-401.

Byar, D. P.: Identification of prognostic factors. In Buyse, M., Staquet, M., and Sylvester, R. (Eds.): Cancer Clinical Trials: Design, Practice and Analysis. London, Oxford University Press (In Press).

Day, N. E., Byar, D. P. and Green, S. B.: Letter to the editor: The authors reply. Am. J. Epidemiol. 115: 799-801, 1982.

Gail, M. H.: Comment on heart transplant data. J. Am. Stat. Assoc. (In Press).

Gail, M. H.: Competing risks. In Kotz, S., and Johnson, N. L. (Eds.): Encyclopedia of Statistical Sciences. New York, John Wiley & Sons, 1982, Vol. II, pp. 75-81.

Gail, M. H.: Monitoring and stopping clinical trials. In Miké, V., and Stanley, K. E. (Eds.): Statistics in Medical Research: Methods and Issues with Applications in Cancer Research. New York, John Wiley & Sons, 1982, pp. 455-484.

Gail, M. H., DeMets, D. L., and Slud, E. V.: Simulation studies on increments of the two-sample logrank score test for survival data, with application to group sequential boundaries. In Crowley, J., and Johnson, R. (Eds.): Survival Analysis. California, Institute of Mathematical Statistics, 1982, pp. 287-301.

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Green, S. B.: Patient heterogeneity and the need for randomized clinical trials. Controlled Clin. Trials 3: 189-198, 1982.

Green, S. B.: Statistics, epidemiology, and computers: Implications for the teaching and practice of medicine. In Purcell, E. F. (Ed.): Proceedings of the Macy Conference on Teaching the New Biology. New York, Josiah Macy, Jr. Foundation (In Press).

Green, S. B.: Statistical principles and design of controlled clinical trials. In Javadpour, N. (Ed.): Principles and Management of Urologic Cancer, ed. 2. Baltimore, Williams & Wilkins (In Press).

Lubin, J. H. and Gail, M. H.: Biased selection of controls for case-control analyses of cohort studies. Biometrics (In Press).

Muenz, L. R.: Comparing survival distributions: A survey for non-statisticians. Cancer Invest. (In Press).

Muenz, L. R. and Rubinstein, L. V.: Modeling the covariate dependence of binary sequences. Biometrics (In Press).

Piantadosi, S., Hazelrig, J. B. and Turner, M. E., Jr.: A model of tumor growth based on cell cycle kinetics. Math. Biosci. (In Press).

Rubinstein, L. V. and Gail, M. H.: Monitoring rules for stopping accrual in comparative studies. Controlled Clin. Trials 3: 325-343, 1982.

Slud, E. V., and Rubinstein, L. V.: Dependent competing risks and summary survival curves. Biometrika (In Press).

Slud, E. V.: Consistency and efficiency of inferences with the partial likelihood. Biometrika 69: 547-552, 1982.

Slud, E. V., and Wei, L. J.: Two-sample repeated significance tests based on the modified Wilcoxon statistic. J. Am. Stat. Assoc. 77: 862-868, 1982.

Stanley, K. E., Byar, D. P., Gail, M. H., Gelber, R. D. and Rosen, P. P.: Panel discussion, "Interpretation and presentation of statistical results." In Mikē, V. and Stanley, K. E. (Eds.): Statistics in Medical Research: Methods and Issues with Applications in Cancer Research. New York, John Wiley and & Sons, 1982, pp. 513-541.

Wei, L. J.: Asymptotically distribution-free simultaneous confidence region of treatment differences in a randomized complete block design. J. R. Stat. Soc. [B] 44: 201-208, 1982.

Wei, L. J.: The Friedman's Urn. In Kotz, S., and Johnson, N. L. (Eds.): The Encyclopedia of Statistical Sciences. New York, John Wiley & Sons, 1983, Vol. III, pp. 251.

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

Wei, L. J. and Byar, D. P.: Play-the-winner rules. In Kotz, S. and Johnson, N. (Eds.): Encyclopedia of Statistical Sciences, Vol. V (In Press).

Wei, L. J. and Gail, M. H.: Nonparametric estimation for a scale-change model with censored observations. J. Am. Stat. Assoc. (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. New York, John Wiley & Sons, 1983, Vol. II, pp. 318-320.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP04475-07 B

PERIOD COVERED
October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
National Nonmelanoma Skin Cancer Incidence and Epidemiology Studies

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)
Joseph Scotto, Health Services Director, BB, NCI

COOPERATING UNITS *(if any)*
Interfederal Committee on Stratospheric Ozone Protection (ICSOP), EPA, National Oceanic and Atmospheric Administration, Temple University, National Academy of Sciences, Dermatology Branch (NCI), Harvard University, Census Bureau

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 *(Use standard unreduced type. Do not exceed the space provided.)*

This project, a collaborative effort by the NCI, National Academy of Sciences, National Oceanic and Atmospheric Administration, Environmental Protection Agency and others, was initiated to provide epidemiologic data relative to skin cancer and solar ultraviolet radiation. 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. Various reports from the National Academy of Sciences have suggested that there may be as much as a five to 16% depletion in stratospheric ozone sometime during the next century. Critical reviews must be made of all pertinent information dealing with estimates of increased human skin cancer due to excess amounts of UV 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 studies 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). These data should enhance the progress of other programs dealing with skin cancer prevention, intervention or screening. As nonmelanoma skin cancer (i.e., basal cell and squamous cell carcinoma) information is not being routinely collected by the NCI (Surveillance, Epidemiology, and End Results), final results from this project will also complement our program's task in providing data for all malignancies.

PROJECT DESCRIPTION

Names, Titles, Laboratory and Institute Affiliations of Professional Personnel (other than the Principal Investigator) engaged on this Project:

Thomas R. Fears	Mathematical Statistician	BB, NCI
Edwin J. Lisiecki	Computer Expert	BB, NCI

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 within the contiguous United States. The geographic areas range in latitude from 47^{0} N to 30^{0} N, and include: Seattle, Minneapolis, New Hampshire/Vermont, Detroit, Utah, San Francisco, New Mexico, Atlanta, San Diego and New Orleans. 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 measures were obtained through the use of Robertson-Berger meters which were installed and maintained at these locations by Temple University and the National Oceanic and Atmospheric Administration (NOAA). NOAA has recently provided raw data from most of the locations which the NCI has available incidence data. However, no UV meter data are available for New Hampshire/Vermont, and best estimates will be derived. New 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:

Each year in the United States there are an estimated 400,000 to 500,000 Caucasian patients being treated for at least one newly diagnosed nonmelanoma skin cancer. Histologically, about 20 percent of these malignancies are squamous cell carcinomas, the more serious form of nonmelanoma skin cancer. Among Caucasians skin cancer is the most frequently occurring cancer for both men and women. As with skin melanoma, increases in incidence have been detected over a six-year study period in two locations: San Francisco-Oakland and Minneapolis-St. Paul. The increase which amounts to about 3 percent per year was noted especially for basal cell carcinomas. The greatest increases were seen for the male trunk and upper extremities. Lip cancer, such as that recently diagnosed for Mrs. Reagan, has been increasing among women, but not men; and eyelid cancer has decreased for both men and women in these two geographic locations. 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 is steeper for squamous cell carcinoma than for basal cell carcinoma. The amount of skin cancer among the non-Caucasian races was quite negligible and also relatively low among Hispanic Caucasians. With respect to specific host factors and environmental exposures, a new monograph is in preparation which will provide complete details for Caucasians only, by age, sex, ethnicity, and geographic location. Our findings identify high risk groups as those individuals with certain host factors, such as "fair" or light skin complexion; light (blue or green) eyes; blond or red hair; sensitivity to sunlight as evidenced by degree of sunburn (erythema) or inability to tan; and, with ancestral links to Scot/Irish, i.e., Celtic ethnic groups. Also we have noted that individuals who have been treated for various skin conditions were also more inclined to develop skin cancer. These conditions include: acne, moles, psoriasis, warts, dry skin and eczema. With respect to environmental exposures high risk groups were identified as those who were outdoors frequently on their principal occupation or held other outdoor jobs (especially those with light skin or light eyes or light hair). Those exposed to radiation (ionizing); coal tar or pitch, oils, and arsenic were also found to be at higher risk.

In collaboration with Dr. Robert Stern of Harvard University, a special in-depth analysis of psoriasis and skin cancer was undertaken. The study indicates that psoriatics are at increased risk of developing skin cancer. These findings persist even when adjusting for certain host and environmental factors. Another special study was done in collaboration with Dr. Kraemer of the Dermatology Branch, NCI. Multiple skin cancers, melanomas and other cancers (such as oral cavity and brain cancer), 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.

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 estimated increases in skin cancer which may result from potential increases in UV-B were found to be greater for squamous cell carcinoma and appear to be double that expected for basal cell carcinoma. 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 UV-B 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 all available skin cancer information as well as new census and UV-B data recently obtained (spring-summer, 1983) from other Federal agencies.

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. Detailed results will provide appropriate benchmark data for use in prevention, detection, intervention, and screening programs.

Proposed Course:

Because other Federal agencies have not provided timely, final, complete reports of UV-B meter data and population data for all locations where skin cancer information was obtained, we must develop the recently acquired raw data for analytical purposes, and ascertain whether additional information is needed. Earlier data did not reveal significant annual trends in the amounts of UV-B radiation reaching the earth's surface. If this is true, it may mean that the increases in skin cancers may be due to lifestyle patterns of outdoor exposure or perhaps other, non-UV related exposures or conditions (e.g., genetics). At this juncture we expect that at least two more years of data will be required. 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.).

It is also proposed that field studies be conducted sometime in the near future which will utilize personal UV-B dosimeters. At present we may estimate the relative amounts of UV-B which may reach the surface of the earth at certain locations, 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 69: 365-370, 1982.

Fears, T. R., and Scotto, J.: Estimating increases in skin cancer morbidity due to increases in ultraviolet radiation exposure. Cancer Invest. 1: 119-126, 1983.

Kraemer, K. H., Lee, M. M., and Scotto, J.: Diseases of environmental-genetic interaction: Preliminary report on a retrospective survey of neoplasia in 268 xeroderma pigmentosum patients. In Sugimura, T., Kondo, S., Takebe, H. (Eds.): Environmental Mutagens and Carcinogens. New York, Alan R. Liss, 1982, pp. 603-612.

Kraemer, K. H., Lee, M. M., and Scotto, J.: DNA repair protects against cutaneous and internal neoplasia: Evidence from studies of XP. J. Invest. Dermatol. (In Press)

Nam, J., and Scotto, J.: Re: Interpretation of Edwards' method in seasonality studies. Am. J. Epidemiol. 116: 194-196, 1982.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05158-03 B

PERIOD COVERED

October 1, 1982 through September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

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

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

M. H. Myers Chief, BRASS, BB, 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 and Clinical Epidemiology Branches

SECTION

Biometric Research and Analytic Studies and Clinical Genetics Sections

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 *(Use standard unreduced type. Do not exceed the space provided.)*

During this year interviewing of cases and sibling controls was completed. All medical record abstracts supporting information recorded by interviews were completed. All materials were submitted to NCI for coding, data entry and editing. The data analysis is beginning as final clean-up is being completed. Preliminary analyses will be completed during FY-83 and the first of a number of reports is expected to be submitted for publication during FY-84.

PROJECT DESCRIPTION

Names, Titles, Laboratory and Institute Affiliations of Professional Personnel (other than the Principal Investigator) engaged on this Project:

John J. Mulvihill	Chief, Clinical Genetics Section	CEB NCI
Sandra C. (Steinhorn) Abbott	Statistician (Health)	BB NCI
Roger R. Connelly	Statistician (Health)	BB NCI
M. Darlene Naughton	Computer Systems Analyst	BB NCI

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:

Preliminary tabulations based on responses to in-person interviews of study subjects or their proxies indicated the following case vs. control relationships: 74% vs. 88% perceived their health as good to excellent, 28% vs. 11% were unable to work due to health problems, 22% vs. 3% never married due to health problems, 50% vs. 56% ever smoked cigarettes and 86% in both groups had at least a high school education. Of subjects who were married 11% of cases saw a doctor because of infertility problems compared with 10% of controls; 15% of cases reported birth defects among offspring vs. 13% for controls. More in-depth analyses will consider confirming information from medical records for subjects whose responses suggested cancer (tumor), infertility or whose offspring had birth defects.

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:

Preliminary examination of the data will be conducted during FY-83 and the first of a series of reports is expected to be submitted for publication during FY-84. 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

Current Annual Level: $1,014,749

Man Years: 6.0

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.

Major Contributions: Preliminary tabulations based on responses to in-person interviews of study subjects or their proxies indicated the following case vs. control relationships: 74% vs. 88% perceived their health as good to excellent, 28% vs. 11% were unable to work due to health problems, 22% vs. 3% never married due to health problems, 50% vs. 56% ever smoked cigarettes and 86% in both groups had at least a high school education. Of subjects who were married 11% of cases saw a doctor because of infertility problems compared with 10% of controls; 15% of cases reported birth defects among offspring vs. 13% for controls. More in-depth analyses will consider confirming information from medical records for subjects whose responses suggested cancer (tumor), infertility or whose offspring had birth defects.

Proposed Course: Preliminary examination of the data will be conducted during FY-83 and the first of a series of reports is expected to be submitted for publication during FY-84.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP05176-03 B

PERIOD COVERED
October 1, 1982 through September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Statistical Methodology - Consultation and Research

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)
Charles C. Brown, Mathematical Statistician, Biometry Branch, 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 *(Use standard unreduced type. Do not exceed the space provided.)*

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.

A new method for the analysis and interpretation of retrospective case-control studies based on a multistage theory of carcinogenesis was developed in collaboration with Dr. Kenneth C. Chu of NIEHS. This extends our work on developing methods for the analysis of cohort studies. We applied our new methods to a synthetic case-control study derived from the occupational study we originally analyzed by our cohort methods. We obtained the same results as we did earlier -- arsenic appears to exert if carcinogenic influence at a late stage in the carcinogenic process.

A statistical method for comparing two or more relative survival curves was accepted for publication. This methodology extends the familiar Cox model approach in that this method operates on the survival function adjusted for "normal" mortality -- thus cause-of-death information is not required for the analysis. This methodology will provide the SEER survival system with the ability to statistically compare two or more relative survival experiences rather than observed survival.

In collaboration with Dr. Jurgen Wahrendorf of the IARC, a new approach is being developed for assessing the interactive effects of exposure to two or more carcinogens. This approach will be applicable to the analysis of retrospective epidemiologic studies and will thus extend our work on interaction in experimental animal studies. In addition, we are also collaborating on examining the utility of the bootstrap method of statistical analysis applied to epidemiologic studies.

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, a series of U.S. life tables for ethnic groups other than White or Black has been developed and added to the SEER Survival System. At the present time these life tables are for Chinese, Japanese, Filipino, Hawaiian, Hispanic in New Mexico and Puerto Rican for the year 1970. These life tables are currently being used with the SEER Survival System to compute relative survival rates for these other ethnic groups.

In response to the needs of our visitors Hans and Birgitta Malker from Sweden, an examination of statistical models for the analysis of registry data over time is being conducted. Without making potentially restrictive assumptions, a product model which incorporates effects of age, birth cohort and calendar period cannot be used to untangle the various time trends. Other models are being examined.

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 Federal regulatory agencies is continuing. The primary work in this area over this time period has been (1) preparation of a chapter on high to low dose extrapolation within a species to appear in the book "Principles for the Evaluation of Toxic Hazards to Human Health;" parts of this material have been presented at scientific meetings and are to be published in their proceedings; (2) collaboration with Dr. Marvin Meistrich of the University of Texas System Cancer Center on the assessment of risk to the human reproductive system; this work has been accepted for publication; (3) consultation with the Environmental Carcinogenesis Subcommittee of the National Cancer Advisory Board; (4) being a member of the Occupational Cancer Risk Subcommittee of the DHHS Committee to Coordinate Environmental and Related Programs; and (5) gerneral consulting with regulatory agency scientists in the area of statistical methodology and data interpretation.

As a consultant to Dr. James Murray of the Low Level Radiation Effects Branch of DCT, a project was undertaken to predict the potential future results of a long-term animal carcinogenesis study in beagle dogs exposed to radiation. The NCI has recently taken over the major funding for this study from the FDA, and the question this project will help to answer is, Should the NCI continue to fund the study?

Consultation with Dr. Jule Lamar of the FDA is continuing. A computer tape containing the results of an animal (mouse and rat) study of asbestos exposure by the IV route has recently been received from the contractor. A detailed analysis of this study is planned.

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.: 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)

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ANNUAL REPORT OF

THE CLINICAL EPIDEMIOLOGY BRANCH

NATIONAL CANCER INSTITUTE

October 1, 1982 through September 30, 1983

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.

Epidemiologic Resource Development

The Branch has long played a role in developing the National Death Index, which greatly simplifies and lessens the cost of follow-up studies. Dr. Gilbert Beebe continues to serve on the NDI advisory group for the National Center for Health Statistics, which collects the mortality data from the states and prepares them in a form that allows them to be matched with identifying information on persons who are being traced to determine causes of death with regard to environmental exposures, treatment or other life experiences. At present, in addition to proposals at large, a surrogate for the decennial census (namely, a large sample from the Current Population Survey in recent years) is being matched against the NDI to study mortality in relation to characteristics of the population, e.g., industry and occupation, for which national mortality statistics exist. This is under the guidance of a special committee on which Dr. Beebe serves. Through a small contract, he obtained the services of Dr. Howard Newcombe, a Canadian expert in record linkage, to provide details for probability matching of the two resources.

Dr. Beebe chairs the NCI Working Group on Epidemiology Data Resources. To this end, an interagency agreement has been made with the Social Security Administration (SSA) to a) update the Continuous Work History Sample (CWHS) with the causes of death as listed on death certificates for 1973-1977 and to seek differences according to industry of employment, and b) compare industry of employment as given on the death certificates with those of the CWHS. Another approach is through the Internal Revenue Service, with which a contract has been made for trial coding of occupation as given on the Form 1040 in the expectation that it may be a useful addition to the CWHS in the years ahead. Another agreement with SSA will allow occupational histories given by the next-of-kin to be compared with the employment history as shown on SSA records. Deaths from mesothelioma and controls are being used as the test sample of 200 cases. The study is part of the larger study of mesothelioma being done under contracts with the VA, New York State, and the University of Southern California.

With Mr. John Fanning of the Office of the Assistant Secretary for Health, DHHS, and representatives of NIOSH, Dr. Beebe has been working on a proposal to modify the Internal Revenue Code a) to allow epidemiologists wider but still restricted access to the IRS address-file when working on suitably approved and sponsored studies, b) to allow the SSA to use its quarterly earnings reports (tax records under IRS control) to generate cohorts of industrial workers for mortality studies, c) to allow transfer of Form 1040 information on occupation to the CWHS file of SSA, and d) to allow the SSA to create employment histories from its quarterly earnings reports -- all this in the interest of epidemiologic research.

Dr. Beebe has also been working with Mr. Hugh O'Neill, the HHS Privacy Officer, on the wording of the OMB proposal for legislation to create an enclave of Federal statistical agencies to allow them to exchange data with one another, and to protect their files from the outside. NCI would not be within the enclave, but could contract with one of the agencies within it, e.g., National Center for Health Statistics, to bring together the files of several agencies for particular projects concerning cancer research. This work has been instrumental in leading Mr. O'Neill, in his annual report concerning the 1974 Privacy Act, to recommend changes in that Act to ease the restrictions on the research uses of individually identifiable records.

Demography

Mr. Frank McKay and Dr. Robert Miller have produced a series of tables and graphs of U.S. cancer mortality under 15 years of age, 1950-79. The data show a steep decline in deaths from leukemia, Hodgkin's disease, bone sarcoma, kidney cancer, and all other cancers combined. The decline appears to be continuing for leukemia, but not for the other cancers. From 1965-79 there were 8100 fewer deaths from leukemia and about 9000 fewer deaths from other cancers than were expected at the 1950 rates. Routine coding of death-certificate diagnoses by NCHS prevents evaluation of certain types of cancer (e.g., neuroblastoma or teratoma) or certain subtypes, such as acute lymphocytic vs. acute myelogenous leukemia. The fall in mortality from 15-19 years of age was much less than at younger ages. There were about 4000 fewer deaths in 1965-79 than expected at the 1950 rates. Dr. Max Myers of the Biometry Branch and Dr. Miller are preparing data on the incidence, mortality and survival from cancer in adolescence, a subject of particular interest to clinicians at this time.

Mr. McKay assisted Dr. Charles E. Land of the Environmental Epidemiology Branch with data for his testimony in court concerning the carcinogenic effects of fallout from nuclear weapons tests in Nevada on people in Utah in the 1950s. The report for publication concludes, "The evidence for a marked increase in childhood leukemia mortality in southern Utah as a result of exposure to radioactive fallout during 1950-58 appears, on closer examination of available data, to be slight or non-existent."

Mr. McKay has also provided Ms. Sandra Abbott and Dr. Myers of the Biometry Branch with data on childhood cancer mortality, 1973-79, for a paper they have prepared for publication on improved survival of children with this neoplasm.

He provided Dr. Paul Levine with maps and graphs of mortality in the U.S. from nasopharyngeal carcinoma, which revealed some unexpectedly high rates along the Gulf Coast among males, and in Florida in both sexes. Also, early age-peaks in mortality were found in blacks of each sex -- this in contrast to whites and Chinese living in the U.S., who had only the age-peak later in life.

Mr. McKay has provided Dr. Frederick Li with mortality data on cervical cancer for a study of trends by region over time. Also, extensive data have been prepared for Dr. Goffman a) to evaluate economic subregions of the country, as defined by the Bureau of the Census, to determine the influence of economic conditions on cancer mortality independent of the usual political boundaries (i.e., state or county), and b) to determine how to avoid the differences in mapping age-adjusted rates that are obtained when different standard populations are used, e.g., for 1960 vs. 1970. Dr. Robert E. Tarone is now working with Mr. McKay on this question. Dr. Susan Devesa has been given data to support her work concerning general cancer mortality statistics.

The data base for cancer mortality has been increased by one year, through 1979. A large number of computer programs were developed to achieve most of the above.

Radiation Effects

Dr. Beebe has concentrated on risk-assessment, especially at low doses. By invitation, he prepared a paper,"Assessment of Health Risks from Exposure to Ionizing Radiation," for a symposium on Environmental Epidemiology: Risk Asessment convened by the Society for Industrial and Applied Mathematics. At the annual meeting of the Health Physics Society, he was invited to speak on a Methodologic Assessment of Radiation Epidemiology Studies, which will be published also. He participated in a symposium held by the Pontifical Academy of Sciences in Rome, concerned with ethical issues in radiation protection, and in another held by the International Atomic Energy Agency in Venice on the Biological Effects of Low Level Radiation. He will give an invited opening speech at a session on cancer epidemiology at the Seventh International Radiation Research Congress in Amsterdam, where he will speak on the Current Status of Human Radioepidemiology.

In addition, he has been an active contributor to the development of the now Congressionally mandated "radioepidemiology tables" to be used as a guide to the probability that persons who develop cancer after exposure to radioactive fallout did so because of that exposure. The work, requested by the Assistant Secretary for Health, DHHS, is being done at NIH with oversight by NAS.

Dr. Beebe has prepared an extensive statement of the carcinogenicity of ionizing radiation in man for a Task Group on the Comparative Carcinogenicity of Radiation and Chemical Pollutants, sponsored by the National Council on Radiological Protection and Measurements. He serves on the NCI Radiation Coordination Committee, and provides technical support for the Inter-Agency Radiation Research Committee, the charge to which is to coordinate federal research on the human health effects of ionizing radiation and to ensure its adequacy.

Dr. Miller serves on the Science Council of the Radiation Effects Research Foundation, which meets annually to advise on research by that organization, which concerns survivors of the atomic bombs in Hiroshima and Nagasaki. He is also a member of NAS' Board on Radiation Effects Research, which serves as a source of ideas and advice to the Academy. During the year he published highlights of two meetings concerning the effects on children of exposure to ionizing radiation. He served on the Epidemiology Advisory Committee for studies of atomic energy workers at Los Alamos National Laboratory, Los Alamos, New Mexico and Hanford Environmental Health Foundation, Richland, Washington. Dr. Beebe was a member of the group that made site visits for the Department of Energy at the Hanford Environmental Health Foundation, Richland, Washington, Oak Ridge National Laboratory, Oak Ridge, Tennessee, Los Alamos National Laboratory, Los Alamos, New Mexico, and Argonne Universities Association, Argonne, Illinois.

Viruses

Several epidemiologic studies investigating the possible role of viruses in the etiology of human cancer were initiated in this past year. A contract was approved to investigate the relationship of hepatitis to liver cancer, which will be developed by Dr. Beebe in collaboration with Dr. Hoofnagle of NIADDK, Dr. Seeff, et al., of the VA Medical Center in Washington, D.C., and Dr. Norman of the Medical Follow-up Agency (MFUA) of National Academy of Sciences, National Research Council. 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.

Epidemiologic support for studies of other possible oncogenic viruses include a collaboration with LTV, DCT, NCI on the epidemiology of HTLV, through which it was found that the incidence of HTLV infection is higher in Ghana than in other parts of Africa, Southeast Asia, or the United States. A study on papovaviruses was initiated to investigate the apparently higher frequency of papovavirus infection in acute lymphocytic leukemia of childhood.

Viral markers are also being applied to studies of cancer families. One of the more important observations was the finding of RAS-oncogene in the bone marrow cells of a patient with acute myelocytic leukemia in the course of a family study. This family initially came to the CEB's attention because the woman has a niece with Hodgkin's disease, two second-degree relatives with cancer, and two brothers with an undefined leukopenia. Her large family makes it possible to see if the detection of the oncogene can be used as a marker for susceptibility

to cancer. Another viral marker of interest is the mouse mammary tumor virus-related antigen identified in breast cancer biopsies. In the past year, it was shown that Tunisian breast cancer patients have a higher frequency of antigen than those in the United States and a study is in progress to determine whether familial breast cancer has a higher frequency of this antigen than non-familial breast cancer.

Among the studies on Epstein-Barr virus completed in the past year was a project demonstrating the distribution and importance of a serum factor inhibiting the lymphocyte response to Epstein-Barr virus in patients with nasopharyngeal carcinoma (NPC). This lymphocyte stimulation inhibitory factor (LSI) was found to be a useful marker of disease activity with a broad distribution, having been detected in patients in Malaysia, Europe, and North America. Epidemiologic studies of NPC demonstrated a previously unrecognized coastal pattern in the United States. Analysis of NPC patients identified by the Surveillance, Epidemiology and End Results Program indicates that environmental factors in Hawaii contribute to a higher incidence of NPC in that state than in any other. Mortality studies confirmed a significant excess of deaths from NPC in young, black Americans as compared to any other racial or ethnic group in the United States.

Well characterized serum samples, biopsies, and other biological materials continue to be collected by the Tumor-Virus Epidemiology Repository, which was consolidated in the past year at the Frederick Cancer Research Facility. Particular attention was given to utilization of samples from Ghana, Malaysia, Hong Kong, Singapore, Tunisia, and Germany in a number of collaborative studies described elsewhere.

Bedside Etiology

The details of a family with the Li-Fraumeni syndrome diagnosed at the University of New Mexico were worked out, and the pedigree published. Two children had double primary cancers, the mother had breast cancer, and her father, brother and the brother's son had brain cancer. An attempt is being made to secure skin biopsies for studies of sensitivity of fibroblasts in vitro to ionizing radiation and certain chemicals.

A New Jersey family with three, and possibly five members affected with pancreatic cancer, the most recent at 32 years of age, will be studied if possible in collaboration with NCI pathologists to determine if an unusual hereditary form of neoplasia is involved. The family is concerned about the risk to the children as they reach young adulthood.

A woman seen at Georgetown University had six primary cancers over a 30-year interval. The array of neoplasms are the same as those in families with Lynch's family cancer syndrome, plus ovarian cancer in this patient and two of her sisters. A report for publication is to be prepared.

A 12-year-old girl seen at the University of Colorado has familial polyposis. Her father, his brother, mother, and grandfather all had the disease. The father and his brother died of brain tumors (Turcot's syndrome?) and the father

had four osteomas (Gardner's syndrome?). Specimens from the girl's polyps will be studied cytogenetically when resection is done in the near future.

Evidence for pre-existing inability to produce IgG antibody following virus infection was apparent prior to the development of Hodgkin's disease in a patient studied by Dr. Levine in collaboration with Dr. Thomas Waldmann.

Genetic Studies

Besides generating leads to understanding the origins of human cancers, studies on familial cancer can provide direct opportunities for cancer control and prevention. In a summary of women at high risk of breast cancer, usually because of family history, 13 women from five families were seen because they had prophylactic mastectomy or sought counseling concerning the magnitude of their breast cancer risk and a means to cope with it. The counseling session included a personalized summary estimate of the woman's individual risk factors, which ranged from a 0.2% to 24% probability of breast cancer within five years. The alternatives of medical surveillance and prophylactic mastectomy -- for those who had not had this surgery -- were discussed. In a 1- to 12-year follow-up, none developed breast cancer, and five elected subcutaneous mastectomy with implantation. None of those who elected surveillance had, in fact, followed a regular screening program. As a model for modifying personal behavior to prevent deaths from familial cancer, many additional questions could be asked. Until appropriate collaborators in behavioral medicine are identified, these breast cancer families have been enrolled in two other investigations. One will evaluate the histopathology and presence of "breast cancer antigens" in familial breast cancer, and the other will seek abnormalities in the diurnal cycles of plasma melatonin in women with high risk for breast cancer, as is seen in women with estrogen receptor-positive breast cancer.

Among a variety of studies concerning pecularities of occurrence of neurofibromatosis (NF), the greatest emphasis has been on a search for the gene locus for the disease. In 11 families with multiple members affected over three generations, blood specimens have been obtained for gene-linkage studies: laboratory analyses that seek evidence that genes with known loci segregate with the occurrence of NF. Five of six informative families showed linkage with GC, just distal to the centromere on chromosome 4q. This suggests that an NF gene is in the vicinity of this marker, but that more than one gene may induce the disease. The results led to winning a grant from the National Neurofibromatosis Foundation to extend the study to include additional families and the new DNA restriction enzyme-length polymorphisms.

In a continually productive contract collaboration with Atomic Energy of Canada, Ltd. (Dr. Malcolm Paterson), three original results were published. 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. The nevoid basal cell carcinoma syndrome, an autosomal dominant disorder with multiple basal cell carcinoma, medulloblastoma, and ovarian fibroma seems sensitive to ionizing radiation, based on results from several fibroblast lines from one individual.

Reports have been published on a man with lung cancer and partial deletion of chromosome 15, who developed fulminant eosinophilia, and on a child with ependymoma treated with chemo- and radiotherapy, who developed glioblastoma multiforme and acute myelogenous leukemia three years later, possibly environmentally induced in a genetically susceptible host -- his maternal grandmother had rectal carcinoma at 45 years, suggesting a link with Turcot's syndrome (colorectal carcinoma and brain tumor in the same patient). Additional case reports included: 1) a seven-year-old boy with Burkitt's lymphoma and an unaffected identical twin; 2) a 48-year-old electrician with renal cell carcinoma and "cancer cachexia" due to treatable mineralocorticoid deficiency from adrenal gland metastases; and 3) a young woman with sarcoidosis which mimicked pulmonary artery embolism until pulmonary arteriography was done.

In addition, melanoma developed in one of a pair of sisters with congenital facial palsy and multiple malformations especially of the head and distal limbs. Chromsomal analysis showed premature separation of centromeres, especially of chromosomes 1, 9, and 16. The report, in preparation, suggests that this chromosomal abnormality, previously rarely detected, may be a new category of cytogenetic defect, related to certain congenital anomalies (and a predisposition to melanoma in this case?).

Finally, all cytogenetic abnormalities associated with human cancer were summarized in our figure that emphasized the known genes assigned near regions affected in cancer, especially the newly described human oncogenes.

Interinstitute Medical Genetics Clinic:

The Interinstitute Medical Genetics Program is a cooperative undertaking involving several clinical branches and research laboratories in six different Institutes. During the fourth year of operation of the program's Genetics Clinic, 22% of patients were seen by members of our Branch. Specimens obtained from many of them are being studied in laboratory investigations concerning the biologic mechanisms of carcinogenesis. Blood from individuals in multi-generation families with neurofibromatosis has been utilized in a linkage analysis whose results tentatively suggest that the proximal region of chromosome 4q may carry a gene for this disease. Peripheral lymphocytes from the probands of families with cancer seen in clinic are examined for the presence of cytogenetic abnormalities; unusual chromosome findings in tumor patients from a Sipple's syndrome family and in a man with oat-cell carcinoma of the lung are being pursued in other family members. Chromosome studies are also being carried out on tumors from clinic patients, including one specimen from a young girl with neurofibromatosis with a massive life-threatening neurofibroma, and another from a young woman with bilateral breast cancer and birth defects whose sister with the same syndrome recently died of hepatocellular carcinoma.

Besides providing an interdisciplinary setting for our research, an additional purpose of the clinic is to provide broad experience in both research and clinical activities in medical genetics for physicians wishing to pursue academic careers in this subspeciality. The Genetics Clinic provides a major focus for these undertakings; for the most part, patients who attend it are seen in the context of on-going research protocols. Fellows working with these

patients become skilled in the diagnosis and management of genetic disease as well as in counseling families with respect to disease risk and prognosis.

Contracts on Genetic Factors and High Risk of Cancer

Since the last annual report, awards have been made for six contracts and one interagency agreement with outstanding laboratory scientists who will utilize their capability to identify genetic and cellular defects predisposing to cancer in patients studied by members of the CEB and the Family Studies Section, Environmental Epidemiology Branch (EEB), NCI. Dr. Dilys Parry is the Project Officer of this set of projects.

During the year, three reports have been published as a result of contract studies on in vitro radiation sensitivity: the laboratory of Dr. Malcolm Paterson, Atomic Energy of Canada, Ltd., has demonstrated 1) an abnormal susceptibility to gamma radiation in members of a family with Gardner's syndrome and in a patient with Turcot's syndrome and radiation-associated acute leukemia, and 2) increased cell killing after treatment with ultraviolet light and 4-nitroquinolone-1-oxide (a radiomimetic chemical) in members of several families with the dysplastic nevus syndrome, which predisposes to melanoma. The contract with UCLA for gene linkage studies (Drs. Robert Sparkes and M. Anne Spence) has resulted in a report which suggests that the gene for neurofibromatosis, a preneoplastic autosomal dominant disorder, may be located on the proximal region of the long arm of chromosome 4. Drs. Spence, Parry and Bader have been awarded a National Neurofibromatosis Foundation grant to pursue linkage studies in additional neurofibromatosis families.

In addition to the contracts with these laboratories, the Branch also made awards for the study of: routinely banded chromosomes (Biotech Research Laboratories, Inc.), banded prophase chromosomes (Yale University), solid tumor chromosomes (Roswell Park Memorial Institute), sister chromatid exchanges (Litton Bionetics), and in vitro sensitivity to ultraviolet light and DNA damaging chemicals (Brookhaven National Laboratory). All of the laboratories are actively engaged in the analysis of specimens provided to them by the staff of CEB and EEB. We are hopeful that new insights into the genetics of cancer causation will be forthcoming from these clinical-laboratory collaborations.

Studies in Boston

Dr. Li and his colleagues in Boston have used clinical observations to generate new clues to causes of human cancer. In the past year, members of his staff have also made rounds and presentations at major university centers to identify unusual patients for etiologic studies.

In following-up on the exceptional finding of an inherited 3;8 chromosome translocation in a family with 10 cases of renal carcinoma, nine new family aggregates of renal cancer affecting two or more relatives have been identified for study. The new cases indicate that familial occurrence of this cancer is more common than previously suspected, but association with the chromosome translocation remains unique in the original family. However, submicroscopic genetic alteration in chromosome 3 or 8 in the remaining families is a possibility, and laboratory techniques to detect such a change (using molecular

probes) are under investigation. Studies of families with renal cancer have also been expanded to include other genitourinary cancers. In one new family, two brothers developed testis cancer in association with a family history of cryptorchism and twinning. In one additional family, three close relatives each had three primary genitourinary cancers including bladder cancer. Examination for the bladder cancer oncogene in the family was unrevealing.

Other research activities in Boston included the follow-up of four families with a cancer family syndrome which showed a 20-fold excess risk of new cancers. The follow-up of survivors of Wilms' tumor revealed that two had offspring with cancer, one with Hodgkin's disease and one with a childhood sarcoma. Fibroblasts from a patient with Turcot's syndrome who developed radiation-associated acute leukemia showed in vitro susceptibility to radiation. Heterogeneous in vitro radiation responsiveness was detected in members of two families with Gardner's syndrome, a disease likely related to Turcot's syndrome. In another study, heterozygotes for Fanconi's anemia were found not to have an increased cancer risk. Not all of the bedside observations made in Boston during the year related to genetic cancers: the finding of anal carcinoma in two male homosexuals suggests a role of sexual practices as a cause of this cancer.

Clinical observation can also reveal late effects of cancer that appear with new treatments that increase survival. Follow-up of nearly 1,000 patients who survived cancer in childhood revealed an increased risk of breast cancer and thyroid tumors after radiation therapy to these tissues. Among women who were treated with abdominal radiation in childhood for Wilms' tumor, 30% of their subsequent pregnancies terminated in the birth of a low weight infant. Dr. Li is currently investigating the possibility of radiation damage to the reproductive organs in these women. In another study, a substantial proportion of patients who had survived brain tumors were found to enjoy high performance status; however, a few patients had major neurologic deficits.

Clinical epidemiology can be applied in diverse settings, including nations that do not have costly laboratory tools. In collaborative studies with Chinese scientists, Dr. Li and others in the Epidemiology Branches showed a geographic correlation between patterns of cervix and penis cancers in China, suggesting shared etiologic factors in these diseases. In addition, an analysis of childhood cancer incidence in Shanghai provided data for comparison with U.S. and other figures.

U.S.-Japan

Dr. Miller served as chairman of the U.S. side for the U.S.-Japan Cooperative Cancer Research Program. The Steering Committee meeting held in Honolulu, June 9-10, 1983, considered a proposal from Prime Minister Nakasone that U.S. scientists engage in cancer research in Japan for a year at a time. Costs would be covered by the Japanese government. Under the current binational program the exchange from the U.S. to Japan is rarely longer than three weeks.

Dr. Miller participated in a symposium on multiple primary cancers convened under the Carcinogenesis Program by Dr. Curtis Harris of NCI and Dr. Keichi Suemasu of the National Cancer Hospital in Tokyo. The meeting was held in Oiso,

Japan, February 16-17, following the annual meeting of the Radiation Effects Research Foundation Science Council attended by Dr. Miller. A main purpose of the Oiso workshop was to stimulate Japanese clinicians to obtain specimens from exceptional patients for study by laboratory scientists at their hospital. One such patient, for example, treated with radiotherapy for acne in adolescence, developed four primary cancers in the radiation field, which may be due to radiosensitivity detectable by studies of the survival of skin fibroblasts in culture after exposure to various doses of radiation.

Ideas formulated at meetings of the Interdisciplinary Group of the U.S.-Japan Program were advanced during the year. In 1981 a workshop on differences between the two countries in the frequency of lymphocytic diseases fostered interest in this subject, an exchange of several scientists, and a follow-up meeting in September 1982 in Seattle, in conjunction with the International Cancer Congress. At that meeting it was learned that necrotizing lymphadenitis, which rarely occurs elsewhere, is almost epidemic among women 20-35 years old in northern Japan. Participants from Europe, the U.S. and Japan had all noted the disease in nurses, which suggests that the source of the ailment is in hospitals. Another disorder, which the Japanese call Takatsuki's disease (plasma cell dyscrasia, endocrine disorders and polyneuropathy), thought in 1981 to be rare in the U.S., was reconsidered in 1982 and thought never to have been seen here. The reciprocal relation of certain autoimmune diseases (high freqencies in Japan) and lymphoproliferative disorders (low in Japan) continues to be of interest.

In 1982 the Interdisciplinary Group's workshop concerned tumors derived from the neural crest. In the ensuing year a hypothesis has been prepared for publication on the relationship between these tumors and certain genetically determined disorders. The principal authors are R. Neil Schimke, Alfred G. Knudson, Jr., Harukazu Nakamura of Hiroshima University, and Dr. Robert W. Miller.

Dr. Beebe also participated in meetings at RERF of its Science Council and Board of Directors, as well as a special workshop on the new dosimetry (based on the recent realization that contrary to previous belief, neutron radiation from the Hiroshima bomb was negligible).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01CP04325-20 CEB

PERIOD COVERED
October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Planning and Development in Cancer Epidemiology

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)
Robert W. Miller, Chief, CEB, NCI

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Clinical Epidemiology Branch

SECTION

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 *(Use standard unreduced type. Do not exceed the space provided.)*

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

Name, Title, Laboratory and Institute Affiliations of Professional Personnel (other than the Principal Investigator) engaged on this Project:

F. P. Li	Chief, Clinical Studies Section	CEB, NCI
J. J. Mulvihill	Chief, Clinical Genetics Section	CEB, NCI
G. W. Beebe	Expert, Biostatistics	CEB, NCI

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:

The main developments are given this year under the Project Description (Epidemiologic Resource Development Z01CP04325-20 CEB); and establishment of a series of contracts for clinical studies of somatic cell genetics in relation to

cancer (Z01CP04377-12 CEB). In addition, under the U.S.-Japan Cooperative Cancer Research Program, a new perspective of neural crest tumors has been developed, which is ready to be submitted for publication. The Childhood Cancer Etiology Newsletter, issued about ten times a year by the Branch, has gained circulation among pediatric pathologists in consequence of the Sidney Farber Lecture given by Dr. Miller at the annual meeting of the Pediatric Pathology Club, at which the Newsletter was called to attention during the introduction of the lecture period.

A U.S.-China symposium held by the AAAS and organized by Drs. Henry S. Kaplan, Robert W. Miller and Wu Min, revealed that domestic animal models for cancer epidemics in China offer exceptional opportunities for clarifying mechanisms of carcinogenesis. This was well demonstrated by maternal-fetal transmission of hepatitis virus, which causes chronic hepatitis in the offspring and can progress to hepatocellular carcinoma. The work was done using Peking ducks by investigators at the Institute for Cancer Research in Fox Chase. Other opportunities include comparisons of cancer rates in national minority groups in China as described by Dr. Frederick P. Li.

Significance to Biomedical Research and the Program of the Institute:

Progress in cancer research through epidemiology depends among other things, on 1) new resources for collecting data and for linking them with laboratory studies, 2) new thinking about carcinogenic processes in people, 3) setting examples that will increase interest and activity in cancer epidemiology, and 4) speeding the communications of new findings.

Proposed Course:

In developing epidemiologic resources, tests will continue to be made of their usefulness through record-linkage as described under the Project Description on this subject. Reports need to be published concerning individuals or families with unusual findings revealed through contracts for laboratory research developed by the Branch. Binational meetings and other workshops will be organized in an attempt to develop new thinking about cancer etiology/ epidemiology. The Newsletter will continue as a stimulus to such etiologic thinking and is a means for recruiting interesting cases for study.

Publications

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP04377-12 CEB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Familial, Congenital, and Genetic Factors in Malignancy

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

John J. Mulvihill, Chief, Clinical Genetics Section, CEB, NCI

COOPERATING UNITS *(if any)*

EEB, POB, NCI; Atomic Energy of Canada, Ltd.; Georgetown University; UCLA

LAB/BRANCH

Clinical Epidemiology Branch

SECTION

Clinical Genetics Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
4.1	2.9	1.2

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Study of genetic diseases with neoplastic manifestations and detailed investigations of families at high risk of cancer may help detect environmental and genetic influences in carcinogenesis, especially when appropriate laboratory assays are used, and may lead to important opportunities for cancer control. Thirteen women from five families had prophylactic mastectomy or sought counseling because of their high risk for breast cancer, usually because of a family history; a counseling strategy was devised that provided personalized summary estimates of risk and presented the options for preventing cancer deaths by medical surveillance or prophylactic mastectomy. Neurofibromatosis, an autosomal dominant disorder with a large predisposition to cancer, was linked to the serum protein marker, GC, on chromosome 4, by classical genetic linkage analysis in five families with a lod score of 2.2; because a sixth family gave a negative lod score, genetic heterogeneity may be present in this common but understudied preneoplastic disorder. A similar condition, the nevoid basal cell carcinoma syndrome, was found to have abnormal in vitro sensitivity to gamma-radiation, paralleling known in vivo radiosensitivity. Cells from patients with the hereditary dysplastic nevus syndrome had ultraviolet (but not gamma) radiosensitivity. Five case reports illustrated important lessons in cancer diagnosis and etiology, and suggested leads for definitive studies. For example, fulminant fatal eosinophilia in a man with lung cancer, several benign neoplasms, and a positive family history of cancer, was diagnosed as a rare eosinophilic leukemia because a new chromosomal abnormality, 15q-, was found. All cytogenetic abnormalities associated with human cancer were summarized in a figure that emphasized the genes assigned to specific chromosomes, including the newly described human oncogenes. Other literature reviews, guest lectures, and committee activities were done to stimulate similar research in the metropolitan Washington area and worldwide.

PROJECT DESCRIPTION

Name, Title, Laboratory and Institute Affiliations of Professional Personnel (other than the Principal Investigator) engaged in this Project:

D.M. Parry	Geneticist	CEB, NCI
T.E. Goffman	Medical Staff Fellow	CEB, NCI
A.E. Bale	Medical Staff Fellow	CEB, NCI
S.Z. Doyle	Nurse Epidemiologist	CEB, NCI
P.H. Levine	Clinical Investigator	CEB, NCI

Objectives:

To identify genetic factors and disorders associated with human cancer and to promote similar studies worldwide. To document patterns of familial aggregation of neoplasms; to study selected disorders and families by genetic and laboratory investigations in an effort to elucidate carcinogenetic 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 similarly study patients with birth defects and other heritable disorders that may predispose to malignancy.

Methods Employed:

Interviews of patients with cancer or other diseases to ascertain familial occurrences of cancer and birth defects, as well as prior medical and environmental history; documentation of history by reviewing appropriate vital and medical records; collection and distribution of biological specimens from such families. 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 the project comprise eight reports of original research [concerning clinical studies (1), laboratory findings in cancer families (2), and case reports (5)], eight reviews, and seven abstracts for national meetings. Research reports involved 21 co-authors from the Environmental Epidemiology, Medicine, Pathology, Pediatric Oncology, and NCI/VA Medical Oncology Branches of the National Cancer Institute, Atomic Energy of Canada Ltd., University of California at Los Angeles, and Georgetown University.

Several years of study culminated in the report of our experience in counseling women at hish risk of breast cancer, usually because of family history. In brief, 13 women from five families were seen (the number is now 45 women), because they had prophylactic mastectomy or sought counseling concerning the

magnitude of their breast cancer risk and a means to cope with it. A counseling session was designed that included a personalized summary estimate of the woman's individual risk factors, which ranged from 0.2% to 24% probability of breast cancer within the subsequent five years. The alternatives of medical surveillance and prophylactic mastectomy -- for those who had not had this surgery -- were discussed. In a 1- to 12-year follow-up, none had developed breast cancer, and five elected subcutaneous mastectomy with implantation. None of those who elected surveillance had, in fact, followed a regular screening program. As a model for modifying personal behavior to prevent deaths from familial cancer, many additional questions could be asked. Appropriate collaborations are not now available to pursue this type of study. Presently, these breast cancer families are enrolled in two other investigations: one to evaluate the histopathology and presence of "breast cancer antigens" in familial breast cancer, and the second to search for abnormalities in the diurnal cycles of plasma melatonin in women with high risk for breast cancer, as is seen in women with estrogen receptor-positive breast cancer.

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. Analysis excluded linkage with 15 loci, including HLA. Five families gave a lod score of +2.2 (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 0.9, suggesting genetic heterogeneity of neurofibromatosis. Additional families are being enrolled and plans developed to study polymorphisms of restriction enzyme fragment lengths of DNA, partially under a grant from the National Neurofibromatosis Foundation.

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. The nevoid basal cell carcinoma syndrome, an autosomal dominant disorder with multiple basal cell carcinoma, medulloblastoma, and ovarian fibroma, seems sensitive to ionizing radiation, based on results from several fibroblasts lines from one individual.

Clinical Observations. Bedside observations of etiology and diseases associated with peculiar occurrences of cancer continue to yield insights into the origins of neoplasia and to provide useful leads for follow-up studies.

Five cases reports were published:

1) 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 his 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 other men with prostatic cancer, a common tumor rarely reported to occur in familial aggregations.

2) A seven-year-old with minor dysmorphisms and abdominal Burkitt's 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 can be used when appropriate tests are agreed upon.

3. The three years after multimodality therapy for ependymoma, a young boy developed glioblastoma multiforme and acute myeloblastic leukemia. The array of tumors seemed to suggest ecogenetic origins, for the cancer therapy was intense and the family history remarkable for a 45-year-old grandmother with rectal cancer.

4. 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.

5. A similarly important clinic observation was made when all signs, symptoms and tests indicated pulmonary embolism in a young woman who was finally shown to have sarcoidosis by pulmonary arteriography. The therapy was, obviously, corticosteroids, and not anticoagulation.

Synthesis. 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. Two book chapters and a rapporteur's report on cancer genetics were published in two proceedings: one of a symposium on host factors in human carcinogenesis (cosponsored by the International Agency for Research on Cancer), and the other, of an International Workshop on Mutagenesis, Carcinogenesis, and Teratogenesis held in Shanghai. Four additional book chapters provided comprehensive reviews of genetic factors in lung cancer, the estimated frequency of hereditary large bowel cancer, the fetal alcohol syndrome, and the interrelationship of human cancer, radiosensitivity and faulty DNA repair.

Resources. Seven major contracts were renewed or initiated to provide nationally recognized laboratory expertise for our collaborations on cytogenetic and radiosensitivity mechanisms of carcinogenesis. (See contract narratives below).

Consultations, Committees, and Lectures. In an effort to recruit junior staff and to promote clinical and laboratory collaboration, teaching responsibilities were carried out in the NIH Medical Genetics Training Program, the Pediatric Branch of the National Cancer Institute, George Washington University School of Medicine, and the Uniformed Services University of the Health Sciences.

In the Interinstitute Genetics Clinic and Consultation Service, Branch members counseled 5 women at high risk for breast cancer, evaluated 25 individuals referred with possible neurofibromatosis and 10 with malformations syndromes, and diagnosed and obtained biological specimens from 3 patients with urogenital cancer and 9 families with familial cancer.

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 and the U.S.-Japan Joint Panel on Environmental Mutagenesis and Carcinogenesis of the U.S.-Japan Cooperative Medical Science Program.

Critical reviews of manuscripts were prepared for the The Journal of the National Cancer Institute, Cancer Genetics and Cytogenetics, American Journal of Human Genetics, and Teratology.

Finally, invited lectures were given within the metropolitan area and worldwide. Dr. Parry presented the genetic linkage paper on neurofibromatosis at the American Society of Human Genetics Meeting (Detroit). Dr. Mulvihill presented at Grand Rounds of the NICHD, the Clinical Oncology Program of the National Cancer Institute, the Medical Genetics Metropolitan Area Conference, and Howard University's Cancer Center; at Research Seminars of the Pediatric Oncology Branch, the Field Studies and Statistics Program (with Dr. Parry), the NIH Library, the National Capital Area Cytogenetics Association (with Dr. Parry), the Pediatric Genetics Unit of the Johns Hopkins Hospital (with Dr. Miller), and the Department of Epidemiology of the University of Maryland. Student lectures were given in the NIH Medical Genetics Elective Course and the FAES Graduate School. Overviews were presented on request at the International Conference on Lung Cancer (New Orleans), the Symposium on Cancer Genetics (Tromsø), and the International Workshop on Environmental Mutagenesis, Carcinogenesis, and Teratogenesis (Shanghai). Dr. Mulvihill was elected a Fellow of the American College of Epidemiology and Director of the NIH Interinstitute Medical Genetics Program, with Dr. Parry continuing as co-Director.

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 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, 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 cancers, a patient with the newly delineated Kabuki make-up syndrome, and a life table analysis of a Danish cohort of neurofibromatosis patients. A contract for support services for the enlarging program of the Section is due for award before the new fiscal year.

Publications

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Mulvihill, J. J.: Clinical genetics of human cancer. In Bartsch, H. and Davis, W. (Eds.): Host Factors in Human Carcinogenesis. Lyon, International Agency for Research on Cancer, 1982, pp. 107-117.

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Mulvihill, J. J. and Robinette, S. M.: Neoplasia of man (Homo sapiens). In O'Brien, S. J. (Ed.): Genetic Maps, Bethesda, Frank Gumpert Printing, 1982, Vol. 2, pp. 356-359.

Mulvihill, J. J., Sayfer, A. W. and Bening, J. K.: Prevention in familial breast cancer: Counseling and prophylactic mastectomy. Prev. Med. 11: 500-511, 1982.

Paterson, M. C., Bech-Hansen, N. T., Smith, P. J. and Mulvihill, J. J.: Radiogenic neoplasia, cellular radiosensitivity and faulty DNA repair. In Boice, J. D., Jr. and Fraumeni, J. F., Jr. (Eds.): Radiation Carcinogenesis: Epidemiology and Biological Signficance. New York, Raven Press. (In Press)

Smith, P. J., Green, M. H., Devlin, D. A., McKeen, E. A. and Paterson, 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 30: 39-45, 1982.

Spence, M. A., Bader, J. L., Parry, D. M., Field, L. L., Funderburk, S. J., Rubenstein, A. E., Gilman, P. A. and Sparkes, R. S.: Linkage analysis of neurofibromatosis (von Recklinghausen disease). J. Med. Genet. (In Press)

BIOTECH RESEARCH LABORATORIES, INC. (NO1-CP-21031)

Title: Genetic Factors in Patients at High Risk of Cancer -- Routine Chromosome Analysis.

Current Annual Level: $74,896

Man Years: 1.5

Objectives:

To determine if persons who have had cancer or who are at risk of cancer because of their personal or family history have chromosome abnormalities detectable by standard cytogenetic techniques.

Major Contributions:

This year, 86 specimens have been submitted under this contract: lymphocytes = 65, lymphoblastoid lines = 8, fibroblast lines = 7, tumors = 4 and bone marrow = 2. Results have been received on 51 specimens; in the great majority of these, no cytogenetic abnormalities have been detected. Unusual results in two families are being pursued: In one family with the autosomal dominant disorder of Sipple's syndrome, constitutional changes involving chromosomes 14 and 21 have been detected in one person with medullary thyroid carcinoma and in another person with pheochromocytoma, respectively. Additional family members, both with and without tumors, will be studied to determine if either cytogenetic finding is etiologically related to tumor development. In a second family, the proband with oat cell carcinoma of the lung had an inversion of 9H. Lymphocytes of his brother, who also has the same tumor, and of unaffected family members will be studied to determine the segregation pattern of this abnormality.

Proposed Course:

We will continue to submit specimens from patients with cancer, or at high risk of cancer, for routine cytogenetic analysis. Abnormalities found in individuals with tumors will be looked for in other family members and in unrelated individuals with the same tumor type. This contract will end on June 29, 1987.

YALE UNIVERSITY (NO1-CP-21037)

Title: Genetic Factors in Patients at High Risk of Cancer -- Prophase Chromosome Analysis.

Current Annual Level: $128,515

Man Years: 1.2

Objectives:

To determine, by studying banded prophase chromosomes, if persons with cancer or at high risk of cancer because of family history, environmental exposure or prexisting disease, have cytogenetic abnormalities likely to be relevant in tumor development. When cytogenetic abnormalities are found that involve rearrangements of chromosome material, to localize the breakpoints through the use of additional assays including: 1) spectrophotometry, 2) radioactive substrates, 3) specific antibodies and immunoprecipitation techniques, and 4) electrophoresis.

Major Contributions:

This laboratory has determined that in one patient with the dysplastic nevus syndrome and melanoma, there are no detectable cytogenetic abnormalities on either chromosome 1p or 6q, regions previously suggested by genetic linkage studies to possibly be the site(s) of the genetic trait responsible for this syndrome. Cytogenetic studies of a 69-year-old male with familial renal cell carcinoma, hypospadias and an atrophic testicle were normal. A terminal deletion of 8 had been suggested by previous studies. The excess heterochromatin in both of the number 16 chromosomes from a girl with Wilms' tumor and ipsilateral hemihypertrophy were determined to be normal variants. The banding patterns of all other chromosomes, including number 11, were normal. The laboratory is currently studying prophase chromosomes from fresh and previously frozen lymphocytes from a member of a cancer family who has a familial translocation involving chromosomes 13 and 18. Obtaining preparations from previously frozen tissue that are suitable for prophase chromosome analysis is difficult. The laboratory is hoping to be able to modify their culture techniques to facilitate the cytogenetic analysis of stored frozen specimens. Additional frozen lymphocytes on this patient will be sent to them to assist in this venture.

Proposed Course:

We will continue to send the laboratory specimens from cancer patients or high risk individuals for detailed analysis of abnormal cytogenetic findings. The contract expires on September 29, 1987.

HEALTH RESEARCH INC., ROSWELL PARK MEMORIAL INSTITUTE (NO1-CP-21033)

Title: Genetic Factors in Patients at High Risk of Cancer -- Tumor Chromosome Analysis.

Current Annual Level: $41,526

Man Years: 1.45

Objectives: To determine if tumors from persons with cancer have cytogenetic abnormalities which may ultimately be important in tumor etiology.

Major Contributions:

To date, tumor specimens from 17 persons have been studied. The specimens received include: Kaposi sarcoma (6), breast cancer (2), liposarcoma (1), neuroblastoma (1), neurofibromatosis (1), familial renal cell carcinoma (1), mesothelioma (1), familial oat cell tumor (1), Hodgkin's disease (1), and brain tumor (1). Most studies are still in progress but major findings to date are:

1. A translocation involving chromosomes 3 and 8 -- t(3;8)(p21;q24) -- was identified in all studied cells from a pleural effusion from a patient with familial renal cell carcinoma. This same translocation had been found in all somatic cells of all available tumor patients in this family in which 10 members in three generations had renal cell carcinoma.

2. Two translocations involving chromosomes 3 and 7, and 9 and 11 respectively, and an interstitial deletion of chromosome 13 were found in all examined cells from a mesothelioma. These abnormalities may be important in the etiology of this tumor type and we are planning to obtain specimens from additional mesothelioma patients to see if these chromosome changes are specific to this disease.

Proposed Course:

Tumor specimens will continue to be submitted to this laboratory as they are available. Efforts will be made to obtain several different tumor specimens from patients with any tumors that appear to have unique and possibly characteristic chromosome changes. The current contract will continue until July 20, 1987.

LITTON BIONETICS, INC. (NO1-CP-21035)

Title: Genetic Factors in Patients at High Risk of Cancer -- Sister Chromatid Exchange Analysis.

Current Annual Level: $184,575

Man Years: 1.69

Objectives:

To determine if lymphocytes, and in some cases fibroblasts, from persons with cancer, or considered to be at risk of cancer, have abnormal levels of sister chromatid exchanges -- in baseline studies -- after exposure to chemical mutagens, or both.

Major Contributions:

1. Because we want to be able to use frozen specimens (lymphocytes) from appropriate cancer patients seen in past years, as well as fresh specimens, considerable effort has been expended in determining the effects of cell separation on the measurement of sister chromatid exchanges (SCE). Dr. Galloway has demonstrated that baseline SCE are consistently higher in purified lymphocytes than in whole blood cultures. The addition of red blood cells back to the lymphocyte cultures returned the SCE frequency to normal baseline values. SCE induced by mitomycin C were also higher in purified lymphocytes than in whole blood cultures, but the relative increases over baseline were essentially the same in the parallel sets of experiments.

2. Baseline and induced SCE were examined in whole blood cultures from a patient with the dysplastic nevus syndrome, an age-matched control, and the patient's mother. The dysplastic nevus syndrome is an autosomal dominant disorder that predisposes to melanoma. Fibroblasts from patients with this disorder have demonstrated increased cell killing following exposure to UV light and 4-nitroquinoline-1-oxide (4NQO), a UV-mimetic chemical. These SCE studies did not reveal any differences between the patient and control in baseline SCE or SCE induced by 4NQO or mitomycin C. We are now planning to repeat this study using fibroblasts cultures, since this is the tissue in the syndrome in which the deleterious effects of UV light have been shown.

Proposed Course:

Protocols have been developed to examine the effects of freezing, with and without lymphocyte purification, on baseline SCE and on SCE induced in cell cultures treated with mutagens, and in blood samples from patients undergoing treatment with chemotherapeutic drugs. When these initial essential studies have been completed, specimens from patients under study will be sent to this laboratory on a regular basis. The current contract expires on September 8, 1987.

UNIVERSITY OF CALIFORNIA AT LOS ANGELES (NO1-CP-21032)

Title: Genetic Factors in Patients at High Risk of Cancer -- Genetic Markers for Linkage Analysis.

Current Annual Level: $37,725

Man Years: 0.45

Objectives:

The major goal of this contract is to determine the chromosomal location of genes known to cause cancer in humans. This involves: 1) determining the phenotypes of some 32 red blood cell enzymes, antigens and serum proteins in individuals from three generation families in which a gene predisposing to cancer may be segregating, 2) undertaking segregation analysis of the pattern of occurrence of cancer (or a predisposing disease) in these families to determine if it can be attributed to a single gene, and 3) if the cancer (or the disease) can be shown to result from a single gene defect, undertaking linkage analysis to determine if the cancer gene co-segregates with any of the assayed polymorphic markers.

Major Contributions:

During the year, the laboratory has received and processed 97 specimens from three neurofibromatosis (NF) families (39 specimens), one family with a platelet disorder and cancer (47 specimens), two ovarian cancer families (6 specimens), and one other cancer family (7 specimens). Earlier work done in collaboration with this laboratory suggested the possible linkage of the gene for neurofibromatosis to the proximal portion of the long arm of chromosome 4. Linkage studies are now being carried out on the recently assayed NF families and the lod scores will be added to those obtained earlier.

2. Segregation analysis of the pattern of transmission of the clinical/laboratory phenotypes in the family with the platelet disorder has begun. If it can be identified as being a single gene trait, linkage analysis will be undertaken.

3. The laboratory has assayed fresh blood and blood previously frozen for up to two and one-half months from the same individual for the array of polymorphic markers. Both specimens gave identical results. This is encouraging since it had been thought that red cell antigens, in particular, were destroyed by the freezing - thawing process. If these results are verified in further studies, then blood can be collected from members of cancer families and frozen, to be studied later, if segregation analysis reveals that the familial cancer predisposition is attributable to a single gene trait.

Proposed Course:

The laboratory will continue to receive specimens from appropriate families for polymorphic marker studies and segregation and linkage analysis. They will examine the suitability of blood frozen for more extended times for the analysis of the red cell markers. The contract expires July 6, 1987.

ATOMIC ENERGY OF CANADA, LTD (NO1-CP-21029)

Title: In Vitro Radiosensitivity and DNA Repair in Genetic Syndromes and Families at High Risk of Malignancy.

Current Annual Level: $497,285

Man Years: 7.34

Objectives:

To determine if persons with increased susceptibility to cancer, e.g., members of cancer families, individuals with multiple primary tumors, radiogenic tumors or genetic disorders predisposing to cancer, have abnormal repair of gamma radiation or chemically induced DNA damage, and when repair defects are found, to identify the repair pathways involved and the cellular cause of the repair defects.

Major Contributions:

The colony formation assay used to detect altered growth of fibroblast cultures after in vitro gamma radiation or radiomimetic or other chemicals demonstrated abnormalities in 7/11 fibroblast cell cultures submitted at the start of this fiscal year. The unusual phenotype of radioresistance was observed in the cell line from a key person in the sarcoma family known to have this trait. The cells of another relative in this family were sensitive to both oxic gamma radiation and far UV. Abnormal resistance to gamma radiation was also present in fibroblasts from a man with familial bladder cancer. Fibroblasts from a young woman with rhabdomyosarcoma, whose sister also had a sarcoma, were sensitive to both gamma radiation and mitomycin C. This chemical is a DNA interstrand cross-linking agent, and is highly recombinogenic in a number of organisms. Fibroblasts from two other patients, a woman with breast lymphoma and a child with hepatoblastoma and pancreatic cancer, were also abnormally sensitive to this chemical.

Proposed Course:

Work is in progress to identify the cellular mechanisms of these abnormal findings and to study high risk relatives from the same or similar families to determine the mode of inheritance of these defects in DNA repair. Ten additional fibroblast lines have been submitted for study and another 5-10 are pending for submission during this fiscal year. The current contract will continue until June 29, 1985.

DEPARTMENT OF ENERGY, BROOKHAVEN NATIONAL LABORATORY (Y01-CP-20518)

Title: In Vitro Radiosensitivity and DNA Repair in Genetic Syndromes and Families at High Risk of Malignancy.

Current Annual Level: $250,000

Man Years: 4.0

Objectives:

To determine if persons with increased susceptibility to cancer, e.g., members of cancer families, individuals with multiple primary tumors, radiogenic tumors or genetic disorders predisposing to cancer, have abnormal repair of UV light or chemically induced DNA damage, and when repair defects are found, to identify the repair pathways involved, and the cellular cause of the repair defects.

Major Contributions:

To date, this laboratory has received 14 fibroblast lines from the NCI inventory and it is in the process of completing a series of cell survival curves to determine the effects on cell growth of a variety of DNA damaging agents: 254 nm UV light, 308-360 nm UV light, N-methyl-N'-nitro-N-nitrosoguanidine (MNNG), and mitomycin C. Because of the laboratory's special interest in the repair of DNA damage following UV radiation, it is initiating a series of experiments to further study cell lines from patients with the dysplastic nevus syndrome, an autosomal dominant disorder that predisposes to melanoma. Recent studies at Atomic Energy of Canada, Ltd., have shown that non-tumor fibroblasts lines from some persons with this syndrome demonstrate increased cell killing in response to exposure to 254 nm UV light and also to 4-nitroquinoline-1-oxide (4NQO), a UV-mimetic chemical. Preliminary studies are now underway at Brookhaven to study the response of these fibroblast lines to sunlamp radiation (308-360 nm UV light) which produces wavelengths most commonly found in sunlight, as well as to MNNG, an alkylating agent which, like 4NQO, requires intracellular activation to produce its effect, and also to determine the ability of these cells to remove O^6-methylguanine from DNA enriched with this substituted purine. This latter type of repair of alkylation-damaged DNA has been studied by this laboratory in a variety of cell types; lymphocytes from patients with chronic lymphocytic leukemia (CLL - a B cell disease) showed an increased ability to remove O^6-methylguanine from DNA compared to normal T cells and lymphocytes from unaffected relatives and spouses. Frozen lymphocytes from 24 individuals, including those with familial and nonfamilial CLL as well as unaffected family members, were sent by NCI to Brookhaven to be tested for O^6-methylguanine removal activity. All CLL cultures gave responses that were lower than those detected in fresh lymphocyte samples, and they did not differ from the unaffected controls. The laboratory is currently investigating this result to determine whether it can be attributed to the freezing and storage of the cells over long periods of time.

Proposed Course:

The studies described above will be completed and cell lines that exhibit consistent abnormalities will be studied in detail to determine the cellular mechanisms responsible for the abnormal findings. Additional cell lines from cancer patients and high risk individuals will be submitted for study as appropriate. This interagency agreement will end September 27, 1985.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP04400-19 CEB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Clinical Epidemiology of Cancer

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Frederick P. Li, Head, Clinical Studies Section, CEB, NCI

COOPERATING UNITS *(if any)*

None

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 *(Use standard unreduced type. Do not exceed the space provided.)*

Persons who have exceptionally high risk of developing cancer are studied to find explanations for their susceptibility. These unusual individuals are identified through referral by practitioners or self-referral, and through clinical observations at the bedside. With informed consent, epidemiologic inquiries are made to identify predisposing host and environmental factors, and concurrent laboratory studies help to clarify biologic mechanisms of cancer susceptibility. Results show that carriers of cancer genes develop cancer at very high rates in a few tissues. Early cancer detection has been achieved through screening of high-risk persons, and counseling has been provided to appropriate patients. High-risk patients also tend to develop multiple primary cancers in childhood, and nearly 1000 patients are under prospective observation for second cancers through the Registry of Survivors of Childhood Cancer in Boston.

PROJECT DESCRIPTION

Names, Titles, Laboratory and Institute Affiliations of Professional Personnel (other than the Principal Investigator) engaged on this Project:

R. W. Miller	Branch Chief	CEB, NCI
J. J. Mulvihill	Section Head	CEB, NCI
J. Tu	Visiting Scientist	CEB, NCI

Objectives:

To employ clinical observations at the bedside to find causes of human cancers. Susceptibility factors in the development of cancer are identified, and high risk subgroups in the population are examined with new laboratory techniques to uncover biologic mechanisms of predisposition to cancer. In addition, counseling and consultation regarding appropriate medical management are provided to these cancer-prone persons.

Methods Employed:

Patients admitted for cancer therapy at the Dana-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. In the past year, several 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. In addition, a registry has been established of nearly 1000 patients who have survived childhood cancer for at least five 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. Prospective studies are in progress to confirm predictions of high risk of cancers in individuals, families, and other groups. Recently, the re-establishment of relationships with China has paved the way for binational studies to compare risk factors for cancer in China and the United States.

Major Findings:

The unusual finding of a family with an inherited chromosomal translocation and renal cancer in 10 relatives prompted additional studies of hereditary genito-urinary cancers. Nine new family aggregates of renal cancer were found to indicate a higher frequency of familial clusters than reported previously, but none had the chromosome translocation. In another family, testis cancer in two brothers was associated with familial cryptorchism and twinning. One additional family included three close relatives, each of whom has three primary cancers of the genito-urinary tract. In other studies based on bedside observations, anal cancer was found in two homosexual males, a group shown in recent years to develop AIDS (Acquired Immunodeficiency Syndrome) with high frequency. Studies of children with cancer and blood diseases showed marked

excess of second cancers among patients irradiated for Wilms' tumor. In addition, relatives of children with Fanconi's anemia had no excess of cancers, indicating that cancer susceptibility is limited to homozygotes. The collaborative studies of childhood cancer in China showed excess liver and nasopharyngeal cancer in Shanghai, and relatively low rates of acute lymphocytic leukemia.

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 worldwide and clues to causes of certain prevalent neoplams in the United States.

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 to receive counseling and supportive care. Also, pilot studies are in progress to examine new approaches to mapping cancer mortality in the United States. Time trends in mortality of curable and preventable cancers (e.g., cervix) will be examined by geographic area to identify localities which might lag in cancer education, detection, and treatment services.

Publications

Anderson, K. C., Li, F. P. and Marchetto, D. J.: Dizygotic twinning, cryptorchism, and seminoma in a sibship. Cancer (In Press)

Costanza, M. E., Li, F. P., Greene, H. L. and Patterson, W. B.: Cancer prevention and detection: Strategy for the office practice. In Cady, B. (Ed.): Cancer: A Manual for Practitioners, ed. 6. American Cancer Society, Massachusetts Division, Inc., 1982, pp. 1-22.

Green, D. M., Fine, W. E. and Li, F. P.: The offspring of patients treated for unilateral Wilms' tumor in childhood. Cancer 49: 2285-2288, 1982.

Kaplan, M. M., Garnick, M. B., Gelber, R., Li, F. P., Cassady, J. R., Sallan, S. E., Fine, W. E. and Sack, M. J.: Risk factors for thyroid abnormalities after neck irradiation for childhood cancer. Am. J. Med. 74: 272-280, 1983.

Kinsella, T. J., Little, J. B., Nove, J., Weichselbaum, R. R., Li, F. P., Mayer, R. J., Marchetto, D. J. and Paterson, M. C.: Heterogeneous response to X-ray and UV light irradiation of cultured skin fibroblasts in two families with Gardner's syndrome. JNCI 68: 697-701, 1982.

Li, F. P.: The chronic leukemias: Etiology and epidemiology. In Wiernik, P. H., Canellos, G. P., Kyle, R. A. and Schiffer, C. A. (Eds.): Neoplastic Diseases of the Blood. (In Press)

Li, F. P., Corkery, J., Vawter, G., Fine, W. and Sallan, S. E.: Breast carcinoma after cancer therapy in childhood. Cancer 51: 521-523, 1983.

Li, F. P. and Fraumeni, J. F., Jr.: Family cancer syndrome: In reply. JAMA 247: 2692-2694, 1982.

Li, F. P. and Fraumeni, J. F., Jr.: Prospective study of a family cancer syndrome. JAMA 248: 1972, 1982.

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. 74: 343-348, 1983.

Li, F. P. and Marchetto, D. J.: Familial renal carcinoma. Cancer Genet. Cytogenet. 7: 271-275, 1982.

Li, F. P., Osborn, D. and Cronin, C. M.: Anorectal squamous carcinoma in two homosexual men. Lancet 2: 391, 1982.

Li, F. P., Winston, K. and Gimbrere, K.: Follow-up of children with brain tumors. Cancer. (In Press)

Li, J. Y., Li, F. P., Blot, W. J., Miller, R. W. and Fraumeni, J. F., Jr.: Correlation between cancers of the uterine cervix and penis in China. JNCI 69: 1063-1065, 1982.

Marchetto, D. J., Li, F. P. and Henson, D. E.: Familial carcinoma of the ureters and other genitourinary organs. J. Urol. (In Press)

Meadows, A. T. and Li, F. P.: The practicing etiologist. In Newell, G., (Ed.): Cancer Prevention and the Clinician. New York, Raven Press. (In Press)

Pastore, G., Antonelli, R., Fine, W., Li, F. P. and Sallan, S. E.: Late effects of treatment of cancer in infancy. Med. Pediatr. Oncol. 10: 369-375, 1982.

Potter, N. U., Sarmousakis, C. and Li, F. P.: Cancer in relatives of patients with aplastic anemia. Cancer Genet. Cytogenet. 9: 61-66, 1983.

Tu, J. T. and Li, F. P.: Incidence of childhood tumors in Shanghai, 1973-1977. JNCI 70: 589-592, 1983.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP04822-13 CEB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Multi-Disciplinary Studies on EBV-Associated Tumors

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Paul H. Levine, Senior Investigator, CEB, NCI

COOPERATING UNITS *(if any)* LCMB, NCI; University of Ghana, Accra, Ghana; AFIP, Washington, D.C.; Institute Malpighi, Bologna, Italy; Faculte de Medicine Broussais-Hotel Dieu, Paris, France; University of Malaysia, Kuala Lumpur, Malaysia; Mayo Clinic, Rochester, MN; IDB, NINCDS

LAB/BRANCH

Clinical Epidemiology Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has integrated several disciplines in an attempt to understand the pathogenesis and improve the control of Epstein-Barr virus (EBV)-associated tumors. Studies of EBV serology in the diagnosis and monitoring of patients with nasopharyngeal carcinoma (NPC), Burkitt's lymphoma (BL), and other EBV-related diseases as well as other laboratory-oriented studies were completed in this fiscal year.

A factor inhibiting the stimulation of lymphocytes by EBV was found in the serum of North American, German and Malaysian patients with NPC and correlated very closely with the activity of disease. EBV-related antibodies proved to be very useful in the diagnosis of NPC in North American patients. Both EBV-related and non-EBV-related markers were noted to correlate with central nervous system disease in African BL patients and may prove to be of diagnostic value.

PROJECT DESCRIPTION

Name, Title, Laboratory and Institute Affiliations of Professional Personnel (other than the Principal Investigator) engaged on this Project:

None

Objectives:

To utilize immunologic techniques in studies on the etiology and control of Epstein-Barr virus (EBV)-associated diseases, with particular emphasis on nasopharyngeal carcinoma (NPC) and Burkitt's lymphoma (BL).

Methods Employed:

A battery of serologic tests were used to monitor patients with BL, NPC, and other EBV-associated diseases. Marmosets and rhesus monkeys were inoculated with EBV and Herpesvirus saimiri (HVS) and monitored by humoral and cell-mediated immunity (CMI) assays. Transfer factor was prepared from immune leukocytes and the transfer of specific immunity to EBV and HVS antigens was evaluated.

Major Findings:

1. A serum protein with activity inhibiting the ability of EBV to stimulate sensitized lymphocytes was discovered in the serum of NPC patients with active disease. This serum factor proved to be more reliable than any EBV-related antibody in the monitoring of NPC patients, and was detected in NPC patients in North America, Germany, and Malaysia.

2. The IgA antibody to EBV viral capsid antigen (VCA) appeared to provide a useful diagnostic test for NPC. Either high titers or absence of IgA antibody to EBV VCA were of great help in assisting in the diagnosis of patients with head and neck cancer and, on occasion, determined the diagnostic work-up of the patient. The finding of elevated EBV antibodies and/or oligoclonal antibodies in the cerebrospinal fluid of African BL patients indicated the presence of active disease in the central nervous system.

3. Studies on EBV and HVS in vitro and in vivo assisted in the evaluation of antiviral agents in the treatment of BL and NPC. Transfer factor was prepared from rhesus monkeys immunized with HVS. The transfer factor was replicated in vitro using the LDV/7 lymphoblastoid cell line. Successful transfer of immunity to HVS was observed in vivo in both rhesus and owl monkeys.

Significance to Biomedical Research and the Program of the Institute:

The availability of laboratory assays measuring immunity to EBV provides an opportunity to define the factors modifying the effect of EBV in different individuals. Additional diagnostic tools and predictors of morbidity may be

developed from these new assays. The detection of a serum factor present in NPC patients with active disease may have great importance in the monitoring and treatment of NPC patients, while the detection of immunologic markers of active CNS disease in African Burkitt's lymphoma patients may facilitate the treatment and improve the survival of patients with this disease.

The IgA antibody to EBV has proven to be an effective diagnostic test for NPC and is now being used frequently for patients in the Washington area. Progress in the in vitro and in vivo studies of EBV and HVS will permit the testing of materials potentially useful in the control of EBV-associated tumors (Z01CP05319-01 CEB).

Proposed Course:

The laboratory studies for this project have been completed and no further studies are anticipated. All epidemiologically oriented studies have been transferred to a new project on the epidemiology of virus-associated tumors.

Publications

Ablashi, D. V., Baron, S., Armstrong, G., Faggioni, A., Viza, D., Levine, P. H. and Pizza, G.: Spontaneous production of high levels of leucocyte (alpha) interferon by a human lymphoblastoid B-cell line (LDV/7). Proc. Soc. Exp. Biol. Med. 171: 114-119, 1982.

Bertram, G., Pearson, G. R., Faggioni, A., Krueger, G. R. F., Sesterhenn, K., Ablashi, D. V. and Levine, P. H.: A long term study of EBV and non-EBV related tests and their correlation with the clinical course of nasopharyngeal carcinoma. In Prasad, U., Ablashi, D. V., Levine, P. H. and Pearson, G. R. (Eds.): Nasopharyngeal Carcinoma: Current Concepts. Kuala Lumpur, University of Malaysia Press. (In Press)

Hewetson, J. F., Levine, P. H., Neubauer, R. H. and Rabin, H.: Discordant Epstein-Barr virus nuclear antigen (EBNA) antibody patterns in nasopharyngeal carcinoma. Int. J. Cancer 30: 581-585, 1982.

Kamaraju, L. S., Levine, P. H., Sundar, S. K., Ablashi, D. V., Faggioni, A., Armstrong, G. R., Bertram, G. and Krueger, G. R. F.: Epstein-Barr virus-related lymphocyte stimulation inhibitor: A possible prognostic tool for undifferentiated nasopharyngeal carcinoma. JNCI 70: 643-647, 1983.

Kamaraju, L. S., Levine, P. H., Sundar, S. K., Ablashi, D. V., Faggioni, A., Bertram, G. and Krueger, G. R. F: Epstein-Barr virus related lymphocyte stimulation inhibitor in Malaysian, European, and North American patients with nasopharyngeal carcinoma. In Prasad, U., Ablashi, D. V., Levine, P. H. and Pearson, G. R. (Eds.): Nasopharyngeal Carcinoma: Current Concepts. Kuala Lumpur, University of Malaysia Press. (In Press)

Levine, P. H.: Cell-mediated immunity and genetics in nasopharyngeal carcinoma: An overview. In Prasad, U., Ablashi, D. V., Levine, P. H. and Pearson, G. R. (Eds.): Nasopharyngeal Carcinoma: Current Concepts. Kuala Lumpur, University of Malaysia Press. (In Press)

Levine, P. H., Nkrumah, F., Ablashi, D. V., Pearson, G. R., Faggioni, A., Viza D., Lvovsky, E., de Vinci, C. and Pizza, G.: Clinical and experimental data on the effect of antiviral agents against oncogenic herpesviruses: Implications for the treatment of nasopharyngeal carcinoma. In Prasad, U., Ablashi, D. V., Levine, P. H. and Pearson, G. R. (Eds.): Nasopharyngeal Carcinoma: Current Concepts. Kuala Lumpur, University of Malaysia Press. (In Press)

Lvovsky, E., Levine, P. H., Bengali, Z., Leiseca, S. A., Cicmanec, J. L., Robinson, J. E., Bautro, N., Levy, H. B. and Scott, R. M.: Stimulation of hematopoietic stem cells by interferon inducer in nonhuman primates receiving fractionated total body irradiation. Int. J. Radiat. Oncol. Biol. Phys. 8: 1721-1726, 1982.

Pearson, G. R., Weiland, L. H., Neel, H. B., Taylor, W., Earle, J., Mulroney, S., Goepfert, H., Lanier, A., Pilch, B., Goodman, M., Huang, A., Levine, P. H., Hyams, V., Moran, E., Henle, G. and Henle, W.: Application of Epstein-Barr virus (EBV) serology to the diagnosis of North American nasopharyngeal carcinoma. Cancer 51: 260-268, 1983.

Pizza, G., Levine, P. H., Ablashi, D. V., Armstrong, G., Bengali, Z. and Cannon, G. B.: Variations in the immune response to Herpesvirus saimiri in squirrel and rhesus monkeys. Comp. Immunol. Microbiol. Infect. Dis. 5: 437-466, 1982.

Sundar, S. K., Menezes, J., Levine, P. H., Ablashi, D. V., Kamaraju, L. S., Faggioni, A. and Prasad, U.: Relationship of IgA to an EBV-specific lymphocyte stimulation inhibitor (LSI) present in the sera of patients with undifferentiated nasopharyngeal carcinoma. In Prasad, U., Ablashi, D. V., Levine, P. H. and Pearson, G. R. (Eds.): Nasopharyngeal Carcinoma: Current Concepts. Kuala Lumpur, University of Malaysia Press. (In Press)

Viza, D., Boucheix, C., Cesarine, J. P., Ablashi, D. V., Armstrong, G., Levine, P. H. and Pizza, G.: Characterization of a human lymphoblastoid cell line, LDV/7, used to replicate transfer factor and immune RNA. Biol. Cell 46: 1-10, 1982.

Wallen, W. C., Biggar, R. J., Levine, P. H. and Iivanainen, M. V.: Oligoclonal IgG in cerebrospinal fluid of patients with African Burkitt's lymphoma. Arch. Neurol. 40: 11-13, 1983.

Wallen, W. C., Biggar, R. J., Levine, P. H., Neequaye, J. and Nkrumah, F.: Cerebrospinal fluid markers in African Burkitt's lymphoma with central nervous system involvement. JNCI 69: 787-792, 1982.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05137-04 CEB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Bedside Etiologic Consultative Program

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Robert W. Miller, Chief, CEB, NCI

COOPERATING UNITS *(if any)*

Georgetown University Medical Center, Washington, D.C.

LAB/BRANCH

Clinical Epidemiology Branch

SECTION

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 *(Use standard unreduced type. Do not exceed the space provided.)*

By participation of the staff at area 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

Names, Titles, Laboratory and Institute Affiliations of Professional Personnel (other than the Principal Investigator) engaged on this Project:

P. A. Gilman	Expert	CEB, NCI
T. E. Goffman	Guest Researcher	CEB, NCI

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:

Cases of interest were ascertained on ward-rounds, or referred by mail or telephone from physicians and others outside NIH. The cases are evaluated by the appropriate members of the CEB staff, referred to Environmental Epidemiology Branch if of particular interest to a scientist in that group, or to Centers for Disease Control if a geographic cluster is involved. If the cases are of etiologic interest, the diagnoses are confirmed as necessary by review of hospital records and/or pathology specimens. Arrangements are made for special laboratory studies, when indicated, to search for new subclinical evidence of high risk. Letters are written to the referring physician, patient, family member, or concerned citizen to explain the situation as we understand it from experience and a knowledge of the literature. When new information is developed from these studies, the cases are reported in the medical literature.

Major Findings:

1. The details of a family with the Li-Fraumeni syndrome diagnosed at the University of New Mexico were worked out, and the pedigree published. Two children had double primary cancers, the mother had breast cancer, and her father, brother and the brother's son had brain cancer. An attempt is being made to secure skin biopsies for studies of sensitivity of fibroblasts in vitro to ionizing radiation and certain chemicals.

2. A patient at the National Cancer Center in Tokyo developed four primary cancers in the field of radiation, during exposure about 10-15 years earlier as treatment for acne. Skin biopsy has been requested for the studies described above as a measure of unusual radiosensitivity.

3. A New Jersey family with three, and possibly five, members affected with pancreatic cancer, the most recent at 32 years of age, will be studied if possible in collaboration with NCI pathologists to determine if an unusual hereditary form of neoplasia is involved. The family is concerned about the risk to the children as they reach young adulthood.

4. A woman seen at Georgetown University had six primary cancers over a 30-year interval. The array of neoplasms are the same as those in families with Lynch's family cancer syndrome, plus ovarian cancer in this patient and two of her sisters. A report for publication is to be prepared.

5. A 12-year-old girl seen at the University of Colorado has familial polyposis. Her father, his brother, mother, and grandfather all had the disease. The father and his brother died of brain tumors (Turcot's syndrome?) and the father had four osteomas (Gardner's syndrome?). Specimens from the girl's polyps will be studied cytogenetically when resection is done in the near future.

Significance to Biomedical Research and the Program of the Institute:

From rarities of cancer occurrence new understanding of cancer biology can be developed through laboratory research. Chromosome mapping is but one example, which has important implications with regard to the burgeoning information concerning oncogenes and how they act.

Proposed Course:

Continue to try to make the most in research or referrals and our own clinical observations in Bethesda and at the Dana-Farber Cancer Center in Boston (see also Project Number Z01C004400-18 CEB).

Publications

Duncan, M. H. and Miller, R. W.: Another family with the Li-Fraumeni cancer syndrome. JAMA 249: 195, 1983.

Gilman, P. A.: Epidemiology of human teratomas. In Damjanov, I., Knowles, B. B. and Solter, D. (Eds.): The Biology of Human Teratomas. Clifton, New Jersey, Humana Press, 1983, pp. 81-104.

Gilman, P. A. and Holtzman, N. A.: Acute leukemia in a patient receiving pencillamine for Wilson's disease. JAMA 248: 467-468, 1982.

Goffman, T. E., Mulvihill, J. J., Carney, D. N., Triche, T. J. and Whang-Peng, J.: Fatal hypereosinophilia with chromosome 15q- in a patient with multiple primary and familial neoplasms. Cancer Genet. Cytogenet. 8: 197-202, 1983.

Goffman, T. E., Woo, S. Y., Manz, H., McCullough, D., Sinks, L. F., Chun, B. K. and Miller, R. W.: Ependymoma, glioblastoma and acute leukemia in a child. Med. Pediatr. Oncol. 11: 130-133, 1983.

Miller, R. W.: Genetic and familial factors. In Calabresi, P., Schein, P. S. and Rosenberg, S. A. (Eds.): Medical Oncology. (In Press)

Miller, R. W., Maron, L. and Mulvihill, J. J.: Burkitt's lymphoma and dry eyes in an identical twin -- etiologic consultation. Cancer Bull. 34: 111-112, 1982.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05139-04 CEB

PERIOD COVERED
October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
NIH Interinstitute Medical Genetics Program: The Genetics Clinic

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)
Dilys M. Parry, Geneticist, CEB, NCI

COOPERATING UNITS *(if any)*

CC, NEI, NIADDK, NICHD, NIDR, NINCDS

LAB/BRANCH
Clinical Epidemiology Branch

SECTION
Clinical Genetics Section

INSTITUTE AND LOCATION
NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.90	.80	.10

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

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 fourth year comprised 250 individuals representing some 60 different diagnostic categories. Of these, 56 patients (22%) 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 an 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

Names, Titles, Laboratory and Institute Affiliations of Professional Personnel (other than the Principal Investigator) engaged on this Project:

J. J. Mulvihill	Chief, Clinical Genetics Section	CEB, NCI
A. E. Bale	Medical Staff Fellow	CEB, NCI
T. E. Goffman	Medical Staff Fellow	CEB, NCI
S. Z. Doyle	Research Nurse Epidemiologist	CEB, NCI

Objectives:

1. To provide a multidisciplinary setting in which patients with cancer or at high risk of cancer can be studied through clinical and laboratory collaboration to identify host or environmental factors for increased cancer risk.

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

3. 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. For research studies, specified categories of patients 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.

Clinic Patients Seen by Members of the Clinical Epidemiology Branch

Neurofibromatosis	25
Birth defect syndromes	7
Women at high risk of breast cancer	5
Testicular cancer	3
Familial leukemia	2
Other cancer families	3
Birth defects and cancer	3
Gardner's syndrome	2
Oat cell carcinoma	1

Nasopharyngeal carcinoma	1
Other diagnoses	4
Total	56

Major Findings:

1. Published reports on counseling for prevention in familial breast cancer and on the possible linkage of the gene for neurofibromatosis to chromosome 4 are described in detail in Project Z01CP04377-12 CEB (Congenital, Genetic and Familial Factors in Human Cancer).

Other studies in progress include the following:

2. In a Sipple's syndrome family, two different chromosome abnormalities, one involving chromosome 14, and the other, chromosome 21, have been found in individuals with medullary thyroid carcinoma or pheochromocytoma. Additional genetically informative family members are being studied and will have blood drawn for chromosome analysis to determine whether either or both chromosome abnormalities are related to the development of the tumors.

3. Similarly, a marker chromosome has been found to be present in lymphocytes from a man with oat cell carcinoma. Blood from his brother, who also had the same type of tumor, and from unaffected family members is being studied to determine if the marker chromosome is etiologically related to tumor development.

4. Although patients with von Hippel-Lindau disease have not been seen in our clinic, our Fellows have seen them on the consultation service. This autosomal dominant disorder predisposes to pheochromocytoma, hypernephroma and brain tumors. Its cardinal features (angiomata of the retina and hemangioblastoma of the cerebellum) are highly penetrant. We have ascertained an affected three generation family on whom we will do genetic linkage studies. Other von Hippel-Lindau disease families have been seen at NIH and we are making a concerted effort to seek them out to pursue linkage studies.

5. In past years in the clinic we have seen two multiple generation families with Cowden's disease, also referred to as multiple hamartoma syndrome. This autosomal dominant disorder predisposes to multiple hamartomatous lesions of the skin, mucous membranes, breast and thyroid. The proband in one of our families had seven primary neoplasms. A medical student who saw the members of the other family during his three months in our Branch while completing medical school electives, has reviewed the world's literature on Cowden's disease and has drafted an article defining its cardinal diagnostic criteria and neoplastic sequelae. Working with an NCI dermatologist, he has also developed a protocol to study the effects of 13-cis retinoic acid on the skin lesions of persons with this disorder.

6. Not all patients seen in the clinic have cancer. A 13-year-old black male with mental retardation, progressive short stature, peculiar facies characterized by long palpebral fissures and eversion of the lateral third of the lower eyelids, and abnormal dermatoglyphics probably has the Kabuki make-up syndrome, a disorder of unknown etiology which has previously only been reported in Japanese children. We are preparing a report on this child, as well as another report on a child with features of the fetal aminopterin syndrome, a pattern of severe growth retardation seen in offspring exposed to folate antagonists in vivo.

7. The gene causing some forms of spinocerebellar ataxia is known to be located on the sixth chromosome. In collaboration with researchers from NINCDS, we are studying a new type of spinocerebellar ataxia present in at least 12 individuals from a very large kindred with this disorder, and examining possible linkage to chromosome 6.

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 are 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 dissemination 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 of Project:

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 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 train physicians to study genetic and environmental factors predisposing to increased cancer susceptibility.

Publications

None

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05146-04 CEB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Morbidity in Childhood Cancer Survivors and Their Offspring

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

John J. Mulvihill, Senior Investigator, CEB, NCI

COOPERATING UNITS *(if any)*

F. Rosner, Queens Hospital, New York, New York; H. Zarrabi, VA Medical Center, Newport, New York

LAB/BRANCH

Clinical Epidemiology Branch

SECTION

Clinical Genetics Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 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 *(Use standard unreduced type. Do not exceed the space provided.)*

Fertility and reproductive histories of cancer patients, especially of long term survivors of childhood cancer and of men and women who reproduced during cancer therapy are studied for information on the possible mutagenicity and teratogenicity of cancer treatment and to discover hereditary patterns of cancer. Current phases include intensive analysis of data from interviews and medical records of 2,200 survivors of childhood cancer and their sibling controls, to gather information on subsequent morbidity and mortality (especially, additional neoplasms), quality of life, fertility and health of offspring. A second phase is the completion of analysis of a voluntary registry of pregnancies in women with cancer that, to date, shows little, if any teratologic effect, but some excess wastage of pregnancies conceived within 12 months of completing chemotherapy. Finally the principles of mutation epidemiology, in particular, the use of so-called sentinel phenotypes, have been reviewed.

PROJECT DESCRIPTION

Name, Title, Laboratory and Institute Affiliations of Professional Personnel (other than the Principal Investigator) engaged on this Project:

M. H. Myers	Statistician	BB, NCI
S. Abbott	Statistician	BB, NCI
R. R. Connelly	Statistician	BB, NCI

Objectives:

To document fertility and reproductive outcome in patients who become pregnant before, during, and after cancer 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 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) Intensive interviewing and record abstracting in five collaborative centers are complete on 2,200 individuals who had cancer under age 19 years, survived at least five years, and achieved at least age 18 years. Over 3,000 controls, siblings of cases, were also studied for subsequent morbidity, mortality, quality of life, fertility and health of offspring. Intense analysis is underway, but preliminary tabulations showed that, in comparison to controls, the cases less often perceived their health as good to excellent and more often were unable to work or marry due to ill health, but had similar use of cigarettes and similar frequency of high school graduation, infertility, and birth defects in offspring. (See also Project Report Z01CP05158-03 CEB).

2) Continued analysis is underway of a registry of young women with cancer, assembled from physicians participating in Cancer and Acute Leukemia Group B. Preliminary analysis showed little, if any, teratologic effect, but some excess wastage of pregnancies conceived within 12 months of completing chemotherapy.

3) Book 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 in intensive analysis, with a series of planned publications. Additional populations suitable for investigation are being identified, as well as the feasibility of laboratory measures of mutation to increase the statistical power of subsequent studies.

A case report is in preparation illustrating the teratogenic potential of anti-folate agents, like methotrexate. Although the patient's mother did not reveal use of such agents, the patient's pattern of defects best matches the fetal aminopterin syndrome.

Publications

Mulvihill, J. J. and Czeizel, A.: Perspectives of mutation epidemiology: A 1983 view of sentinel phenotypes. Mutat. Res. (In Press)

Mulvihill, J. J. and Miller, J. R.: Mutation epidemiology: Prospects for detecting human germinal mutagens. In Kilbey, B. J. and Nichols, W. W. (Eds.): Handbook of Mutagenicity Test Procedures. Amsterdam, Elsevier Scientific Publishing Co., 1983. (In Press)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05194-03 CEB

PERIOD COVERED
October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
National Cancer Mortality Studies by Computer

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)
Robert W. Miller, Chief, CEB, NCI

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

LAB/BRANCH
Clinical Epidemiology Branch

SECTION

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 *(Use standard unreduced type. Do not exceed the space provided.)*

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, 1950-1979, for cancer mortality, and 1965-78, for deaths from other causes. 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

Name, Title, Laboratory and Institute Affiliations of Professional Personnel (other than the Principal Investigator) engaged on this Project:

F. W. McKay	Computer Systems Analyst	CEB, NCI
T. E. Goffman	Medical Staff Fellow	CEB, NCI

Objectives:

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

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

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, 1950-1979, 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. Graphs of childhood cancer mortality by type showed steep declines beginning usually in the early 1960s. This improvement applies especially to children under 15 years of age, and is much less pronounced from 15-19 years of age. Birth-cohorts showed a progressively improved picture, but may be reaching the limits possible through the use of present therapy. A bottoming-out is appearing for the types of cancer which respond best to therapy: Hodgkin's disease and kidney tumors.

2. At the suggestion of Dr. Goffman, economic subgroups of counties are being used to map type-specific cancers to determine high-risk areas. High rates of nasopharyngeal carcinoma have been observed along the Gulf Coast where

economic subgroups were studied. A case-control study may reveal the reason for this geographic (socioeconomic?) peculiarity.

3. Variability in the high-risk areas obtained when different years are used as the standard for age-adjustment are under study by Mr. McKay, assisted by Dr. Robert E. Tarone of the Biometry Branch.

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:

Continue to use and improve this resource.

Publications

Honeyman, M. S., Connelly, R., McKay, F. W. and Flannery, J. T.: Cancer Mortality in the White Population of the United States, 1950-1975. Connecticut Health Bulletin, 1983, Vol. 97, 237 pp.

Percy, C., Horm, J. W. and Goffman, T. E.: Trends in histological types of lung cancer. SEER 1973-1981. In O'Brien, S. J. (Ed.): Proceedings of the International Lung Cancer Update Conference. New York, Verlag Chemie, Inc. (In Press)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05279-02 CEB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Development of Epidemiologic Data Resources

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Gilbert W. Beebe, Expert Scientist, CEB, NCI

COOPERATING UNITS *(if any)*

Occupational Studies Section, EEB, NCI; Radiation Studies Section, EEB, NCI;

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 *(Use standard unreduced type. Do not exceed the space provided.)*

To develop a national system for occupational mortality several methods are being tested or explored in collaboration with other agencies: 1) updating the Continuous Work History Sample (CWHS) of the Social Security Administration with Internal Revenue Service information on occupation and with cause of death; 2) development of a program for state vital statistics offices to code occupation and industry on the death certificate, information now generally neglected; and 3) development of a large file of subjects of past Current Population Survey samples for periodic collation with the National Death Index to produce mortality tables by occupation, industry, and other demographic variables. A mesothelioma study is underway in which the SSA is experimenting with the construction of employment histories from SSA records. This does not look promising from a cost standpoint. Efforts are continuing to obtain access to the IRS address file for medical research and to enable the SSA to create industry-of-employment cohorts for mortality studies.

PROJECT DESCRIPTION

Names, Titles, Laboratory and Institute Affiliations of Professional Personnel (other than the Principal Investigator) engaged on this Project:

None

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 United States. Legislative changes are sought in the interests of epidemiologic research.

Major Findings:

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

2. The usefulness of SSA information on employment histories is being tested. 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. An interagency agreement has been signed with the SSA for obtaining death certificates and cause of death on 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 United States 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. This work will also create public use tapes enabling epidemiologists to use working population controls in lieu of general population controls in studies where the "healthy worker effects" hinders mortality comparisons.

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. Present efforts focus on the Current Population Survey (CPS) as a substitute for the 1980 Census sample. Census is putting together a sample of about 900,000 CPS subjects of past surveys in which the SS# was obtained.

5. Various legislative proposals were reviewed and commented upon, and a legislative initiative is being developed with 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 has been delayed by the OMB effort to prepare for the Congress the statistical "enclave" bill under which the major statistical agencies would have both greater freedom to link their files and greater protection of their privacy.

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, 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.: Long-term follow-up is a problem. Am. J. Public Health 73: 245-246, 1983.

Beebe, G. W.: Prospects for national data on occupational mortality. In Chiazze, L., Lundin, F. E. and Watkins, D. (Eds.): Methods and Issues in Occupational and Environmental Epidemiology. Ann Arbor Science Publishers, Ann Arbor, Michigan, 1983, pp. 75-82.

Norman, J. E., Kurtzke, J. F. and Beebe, G. W.: Epidemiology of multiple sclerosis in U.S. veterans: 2. Latitude, climate, and the risk of multiple sclerosis. J. Chron. Dis. (In Press)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05280-02 CEB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Carcinogenic Effects of Ionizing Radiation

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Gilbert W. Beebe, Expert Scientist, CEB, NCI

COOPERATING UNITS *(if any)*

Occupational Studies Section, EEB, NCI; Radiation Studies Section, EEB, NCI; Low Dose Radiation Branch, DCT, NCI

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 *(Use standard unreduced type. Do not exceed the space provided.)*

A-bomb survivors, Atomic Energy Commission - Department of Energy 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.

PROJECT DESCRIPTION

Names, Titles, Laboratory and Institute Affiliations of Professional Personnel (other than the Principal Investigator) engaged on this Project:

None

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 Findings:

1. A variety of exposures to ionizing radiation were studied for their potential contribution to the estimation of the carcinogenic effects of low doses. As a member of a Department of Energy epidemiology review panel, DOE sponsored epidemiologic studies at the Argonne National Laboratory, Battelle-Pacific Northwest Laboratories (Hanford), and Oak Ridge Associated Universities were reviewed. As a member of the committee advisory to the Hopkins study, the progress of the study of nuclear shipyard workers was monitored. From none of these studies did it seem likely that low-dose estimates of any considerable scientific or practical value would be forthcoming.

2. As a member of DCCP and NIH committees, recommendations were made as to methods of estimating the likelihood that a cancer, arising in an individual with known exposure to ionizing radiation, might have been caused by that exposure. The NIH Committee is an ongoing effort.

3. As a member of a National Council on Radiation Protection and Measurements task force comparing radiation and chemical carcinogenesis, a summary of the human data on radiation carcinogenesis has been drafted. Together with Dr. Robert W. Miller, a chapter on leukemia, lymphoma, and multiple myeloma was prepared for Dr. Arthur Upton's forthcoming volume on radiation carcinogenesis. For the volume, now in press, on the DCCP conference on radiation carcinogenesis held in Bethesda in May 1982, a summary chapter, "Developments in Assessing Carcinogenic Risks," was prepared. At the request of the organizing committee for the Seventh International Radiation Research Congress, a paper was prepared on the current status of human radioepidemiology, emphasizing uncertainities and

gaps in our knowledge. A methodologic review of health risks from exposure to ionizing radiation was published by the Society for Industrial and Applied Mathematics . A critical review of radiation epidemiology studies from the standpoint of their scope and methodology was prepared at the request of the Health Physics Society.

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.

Publications

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, 1982, pp. 3-21.

Beebe, G. W.: Current status of human radioepidemiology. In Broerse, J. J., Barendsen, G. W., Kal, H. B. and Van der Kogel, A. J. (Eds.): Proceedings of the International Radiation Research Congress. (In Press)

Beebe, G. W.: Developments in assessing carcinogenic risks from radiation. In Boice, J. D., Jr. and Fraumeni, J. F., Jr. (Eds.): Radiation Carcinogenesis: Epidemiology and Biological Significance. (In Press)

Beebe, G. W.: A methodologic assessment of radiation epidemiology studies. Health Physics (In Press)

Miller, R. W.: Radiation effects: Highlights of a meeting. J. Pediatr. 101: 887-888, 1982.

Miller, R. W.: Radiation injury. In Vaughan, V. C., McKay, R. J., Jr. and Behrman, R. E. (Eds.): Nelson Textbook of Pediatrics, ed. 12, Philadelphia, W. B. Saunders Co. (In Press)

Miller, R. W. and Beebe, G. W.: Radiation leukemia and lymphoma in man. In Upton, A. C. and Shore, R. E. (Eds.): Radiation Carcinogenesis. New York, Elsevier North-Holland. (In Press)

Miller, R. W. and Boice, J. D. Jr.: Radiogenic cancer after prenatal or childhood exposure. In Upton, A. C. and Shore, R. E. (Eds.): Radiation Carcinogenesis. New York, Elsevier-North Holland. (In Press)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05319-01 CEB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Epidemiologic Studies on Viruses and Genetics in the Etiology of Cancer

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Paul H. Levine, Senior Investigator, CEB, NCI

COOPERATING UNITS *(if any)* Institut Salah Azaiz, Tunis, Tunisia; University of Malaya, Kuala Lumpur, Malaysia; University of Ghana, Accra, Ghana; BB, EEB, FCRF, LCMB, & LTV, NCI; LVD, NIAID; Columbia University, New York, NY; Mayo Clinic, Rochester, MN; Roswell Park Memorial Institute, Buffalo, NY

LAB/BRANCH

Clinical Epidemiology Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.7	0.7	0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project uses a variety of epidemiologic techniques in conjunction with collaborating laboratories to investigate the relative role of viruses and genetics in human virus-associated tumors. Emphasis is placed on the intensive study of tumors occurring in unusual situations suggestive of an environmental etiologic factor. Evaluation of the immune response to specific candidate oncogenic agents and a search for chromosomal or other genetic markers are performed in an attempt to determine whether genetics affects the response to ubiquitous viruses in the appearance of malignancy. Among the findings in these studies are the following: Evidence for an oncogene was found in the leukemic cells of a woman with familial cancer in the course of studies of the entire family; evidence for pre-existing inability to produce IgG antibody following virus infection was apparent prior to the development of Hodgkin's disease in another patient; an antigen cross-reacting with gp52 of the mouse mammary tumor virus was found to be more prevalent in tumors from breast cancer patients in Tunisia than in those from patients in the United States; nasopharyngeal carcinoma (NPC) was found to have a previously unrecognized coastal pattern in the United States; analysis of NPC patients identified by the Surveillance, Epidemiology and End Results Program indicated that environmental factors in Hawaii contribute to a higher incidence of NPC in that state than in any other. HTLV was found to have a higher frequency in Ghana than in other parts of Africa, the United States, South Africa, Egypt and Singapore.

PROJECT DESCRIPTION

Name, Title, Laboratory and Institute Affiliations of Professional Personnel (other than the Principal Investigator) engaged on this Project:

None

Objectives:

To identify specific individuals, families, or populations which provide evidence for an etiologic role of viruses in the appearance of cancer. Laboratory tests are applied to search for specific candidate oncogenic viruses and, in addition, a battery of assays searching for genetic markers of susceptibility to cancer are applied.

Methods Employed:

Individuals in multiple case cancer families or individuals with cancer occurring under unusual circumstances or after unusual exposures are identified by attendance on ward rounds, at clinical meetings, or on referral. Populations of individuals at high risk of developing cancer or with unusual forms of cancer are identified by collaborating investigators in Tunisia, Malaysia, Denmark, Ghana, and other parts of the world. Serum samples, tumors, and other relevant biologic specimens are stored at the Tumor Virus Epidemiology Repository in Frederick, Maryland and are distributed to collaborating laboratories with appropriate assays to detect specific viral or genetic markers.

Major Findings:

1. Evidence was found for the presence of an oncogene in the bone marrow of a woman with acute myelocytic leukemia. The patient has a niece with Hodgkin's disease, two other relatives with cancer and two brothers with an undefined leukopenia.

2. Historical and in vitro evidence for a pre-existing inability to respond appropriately to viral infections was found in a young woman with Hodgkin's disease; a similar case was identified in the literature.

3. Nasopharyngeal carcinoma in the United States was found to have a predominantly coastal pattern in white males and females. The influence of place of birth on the subsequent appearance of NPC was noted in an analysis of data from the Surveillance, Epidemiology and End Results (SEER) Program; Japanese and white Americans born and living in Hawaii had a higher incidence of NPC than Japanese and white Americans living in the continental United States. Mortality studies using death certificates from more than 10,000 NPC patients also showed that there is a significantly greater excess of deaths in young black Americans from NPC than in any other United States racial or ethnic group.

4. Pathologic and hormonal studies in Tunisian breast cancer patients indicated a similarity between rapidly progressing breast cancer (RPBC) in Tunisia and

inflammatory breast cancer (IBC) in the United States. An evaluation of the SEER data on IBC in the United States indicated that pathologic identification of IBC has a greater prognostic impact than the clinical description of IBC. Biopsies from Tunisian breast cancer patients were noted to have an antigen cross-reacting with gp52 of the mouse mammary tumor virus (MMTV) more often than biopsies from breast cancer patients in the United States.

5. Seroepidemiologic studies showed that Danish children and adults in Greenland, an area with an increased incidence of NPC, have higher antibody titers to EBV than Danes remaining in Denmark. Studies on HTLV indicate a higher percentage of Ghanaians infected with HTLV than other Africans, Southeast Asians or North Americans.

Significance to Biomedical Research and the Program of the Institute:

Genetic susceptibility to oncogenic viruses in individuals, families, and/or populations and the interaction of environmental factors and genetics can be studied in depth by new laboratory techniques. This project has provided information and clinical specimens useful in studying the pathogenesis of cancer. Breast cancer is one of the most common tumors of women worldwide and is a significant public health problem in the United States. The development of a strong collaborative effort between the National Cancer Institute and the Institut Salah Azaiz in Tunisia is of great value in that it provides access to a group of patients with rapidly progressing breast cancer (RPBC). The high frequency of RPBC in Tunisia allows information on this entity to be accumulated more rapidly than in the United States. Clinical and pathological data thus far indicate that RPBC in Tunisia is no different than fulminating breast cancer in the United States and, therefore, information obtained from the Tunisian patients would be directly applicable to breast cancer patients in the United States. The finding in breast cancer of the high content of antigen cross-reacting with MMTV indicates that human material will be available that will accelerate studies on the involvement of viruses in the cause of breast cancer. The chemotherapy studies have already been of value to breast cancer patients in the United States and the results of these studies are encouraging American chemotherapists to treat patients with RPBC who in the past had not been treated with chemotherapy. The immunologic studies demonstrate the integrity of the immune system in patients with RPBC, providing guidelines to management of such patients.

Continued development of the Tumor Virus Epidemiology Repository (TVER) is assisting in the application of newer laboratory techniques to studies of the genetic susceptibility to viruses in individuals, families, and/or populations at risk of developing cancer. Initially developed for the study of the role of Epstein-Barr virus in the etiology of Burkitt's lymphoma in Ghana, the TVER is also being applied to studies on the role of HTLV in the etiology of T-cell leukemia, papovaviruses in the etiology of acute lymphocytic leukemia, and EBV in the etiology of NPC. Should other candidate oncogenic viruses be identified, the TVER will be of great value in epidemiologic studies of such viruses in different parts of the world.

The observation that a pre-existing inability to produce IgG antibody to viral antigens may be a pre-condition in Hodgkin's disease may lead to a diagnostic test for Hodgkin's disease and/or the opportunity to study people with inability to produce IgG antibody to viral antigens for their increased susceptibility to Hodgkin's disease. Identification of an oncogene in familial cancer may provide a means of determining which individuals in cancer prone families will be at highest risk of developing cancer.

Proposed Course:

Biological material will be collected from all members of the family of the patient with acute myelocytic leukemia to determine the relationship of this oncogene to the susceptibility to cancer in relatives. The studies in breast cancer will be continued with a more in depth epidemiologic study of the risk factors for aggressiveness associated with the disease in Tunisia. Findings from this study will be applied to a comparable study of inflammatory breast cancer in the United States. An immunoperoxidase study will be applied to a series of breast cancer patients in the United States and other countries in order to further determine the etiologic and prognostic significance of this antigen in breast cancer. More sensitive antibodies will be applied to the study of familial breast cancer to possibly identify the individuals in breast cancer families who might be at high risk of developing breast cancer. Studies of nasopharyngeal carcinoma will be extended in Malaysia and the United States with a particular emphasis on NPC occurring in the young.

Publications

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Costa, J., Webber, B. L., Levine, P. H., Muenz, L., O'Conor, G. T., Tabbane, F., Belhassen, S., Kamaraju, K. S. and Mourali, N.: Histopathological features of rapidly progressing breast cancer in Tunisia. Int. J. Cancer 30: 35-37, 1982.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05329-01 CEB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Hepatitis B Virus and Liver Cancer in Army Veterans of WWII

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Gilbert W. Beebe, Expert Scientist, CEB, NCI

COOPERATING UNITS *(if any)*

Medical Follow-up Agency, National Research Council; Veterans Administration (Five Hospitals); Liver Diseases Section, DIR, NIADDK

LAB/BRANCH

Clinical Epidemiology Branch

SECTION

Office of the Chief

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.4	0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The study is based on the epidemic of 50,000 cases of viral hepatitis in the United States Army in 1942, traced to yellow fever vaccine prepared by the Rockefeller Foundation and contaminated with a virus of hepatitis, now thought to have been the hepatitis B virus (HBV). A serologic survey to identify the virus with certainty will be performed by the VA and the NIADDK Liver Diseases Section on about 600 men, 200 who suffered from acute hepatitis during the 1942 epidemic, 200 who received vaccine from one of the seven contaminated lots but were not clinically ill, and 200 who did not receive the Rockefeller vaccine. Two epidemiologic studies will be performed with the Medical Follow-up Agency of the National Research Council: 1) a mortality study of three cohorts of 20,000 men each defined as in the serologic survey, with primary liver cancer the chief end-point; and 2) a case-control study of an estimated 1,400 WWII Army Veterans discharged from VA hospitals for primary liver cancer and 2,800 matched controls, the comparison to be based on immunization history with attention to the lot number of the yellow fever vaccine.

PROJECT DESCRIPTION

Names, Titles, Laboratory and Institute Affiliations of Professional Personnel (other than the Principal Investigator) engaged on this Project:

None

Objectives:

To confirm epidemiologic opinion that the virus responsible for the 1942 epidemic was HBV; to test the HBV-PHC hypothesis in an area of low natural incidence with a point-source infection of healthy young males; to determine the long-term (40 years) persistence of the type B antigen and antibodies; to contrast, as to later PHC, men with acute icteric hepatitis following yellow fever vaccination with men vaccinated with the same contaminated lots but showing no evidence of clinical disease; to estimate the likelihood of chronic hepatitis in 40 year survivors of infection with HBV; to test the hypothesis that the pathogenesis of HBV-associated PHC requires a prior cirrhotic stage; to explore other aspects of the natural history of viral hepatitis, e.g., its relation to cirrhosis; and to explore host and environmental factors for their possible influence on the association between HBV and PHC.

Methods Employed:

Assays for hepatitis viruses have become available that will positively identify persons with previous hepatitis A or B virus infection and those chronically infected with HBV. Blood will be obtained from about 200 men in each of the three groups described above and tested for serum aminotransferases, HBsAg, anti-HBs, anti-HBc, anti-HAV, HBeAg, HBsAg subtype, DNA-polymerase activity, HBsAg titer, and serum levels of HBV-DNA.

The three cohorts for the mortality study (and the serologic survey) will be defined on the basis of existing records of the Medical Follow-up Agency and the National Personnel Records Center in St. Louis. Establishment of the cohorts is straightforward except for Group II, men who received contaminated vaccine without becoming clinically ill. Because the 1973 fire caused extensive damage to the Army WWII records stored in St. Louis, most immunization records are no longer available and, for most men, vaccine lot number must be inferred from their presence in units known (from the records of clinical cases) to have received contaminated vaccine at particular times.

The cohorts will then be traced forward for mortality through the records of the VA system. Until October 1981 the VA extended a cash burial benefit as well as a flag and a burial plot to all honorably discharged war veterans, and this mortality ascertainment system has been shown to be 95 percent complete. Death certificate diagnoses of liver cancer and other liver diseases will be

investigated through hospital records and any available pathology material to refine the comparisons as to risk of death from primary hepatocellular carcinoma.

Because the definition of Group II is somewhat indirect, a case-control study is also to be performed on the basis of VA hospital discharges for primary liver cancer. The case-control study will yield more certain evidence of vaccine lot number than the cohort study, and there will be many more evidential immunization registers in the case-control study than there will be deaths from liver cancer in the cohort study. The case-control study alone would be inadequate, however, because the selection of men for VA hospitalization is completely unknown with respect to the variables under study here.

The estimated 1,400 VA hospital cases of liver cancer in WWII Army veterans and the 2,800 matched controls will be traced through military records in VA claims folders and the remaining records on file in St. Louis, for evidence of yellow fever vaccine lot number so that a comparison might be made as to the frequency of contaminated lots in each group. This comparison will then be refined by review of the liver cancer diagnoses in the evidential cases.

Major Findings:

None thus far. Contracts are to be awarded this fall.

Significance to Biomedical Research and the Program of the Institute:

Although many lines of evidence associate HBV with PHC, especially in Asia and Africa, there is great uncertainty as to 1) the probability that a single adult exposure will induce the chronic carrier state thought to lead to liver cancer, 2) the persistence of the carrier state, and 3) the role of cirrhosis in the pathogenesis of liver cancer. In addition, the long-term natural history of viral hepatitis remains ill-defined, especially with reference to the likelihood of chronic hepatitis and cirrhosis.

This study should make a positive contribution to the viral etiology of cancer as illustrated by the relationship between HBV and PHC. It should also further our knowledge of the natural history of HBV generally.

Proposed Course:

As outlined above.

Publications

None

ANNUAL REPORT OF

THE ENVIRONMENTAL EPIDEMIOLOGY BRANCH

NATIONAL CANCER INSTITUTE

October 1, 1982 Through September 30, 1983

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 were Drs. Jeffrey Clark and Stanley Weiss, both internists and medical oncologists. Both are participants in the medical/epidemiology fellowship program in which our Branch participates with the Medicine Branch, DCT, NCI. Katherine Chen was brought on as a computer programer in the Radiation Studies Section.

Dr. Sherri Bale, a statistical geneticist, was appointed as a Staff Fellow with the Family Studies Section, and Ms. Patricia Stewart was hired as a cancer expert to assist in our program of occupational studies. Dr. Margaret Tucker returned to the Branch after a two year leave of absence to complete her medical oncology training at Stanford University Hospital. Also joining the Branch for varying periods were visiting scientists from several countries, including China, England, Sweden, and Denmark.

RESEARCH PROGRAM:

Descriptive Studies: To identify systematically geographic variation and clustering, the Branch has analyzed U.S. cancer mortality on a county level. In the past, cancer death rates were developed and published along with maps illustrating the variation. By maintaining a file of demographic and potential exposure information at the county level, which was linked to the mortality data, a number of correlational or hypothesis-generating studies were also conducted. More recently, emphasis has been placed on the description of time trends. Mapping of cancer mortality for 1970 through 1975 has also been conducted for some of the more common tumors. Perhaps most striking was the updated map of lung cancer among white males, which revealed a shifting geographic pattern towards high rates in broad stretches of the south, and a decline of previously elevated mortality in northern metropolitan areas. Increases in mortality over time have also been documented for non-Hodgkin's lymphoma, particularly the histiocytic type, for multiple myeloma, especially among blacks, and for malignant melanoma. A survey of thyroid cancer in Connecticut revealed increases in the papillary and follicular cell types, consistent with the effects of radiation therapy given previously to children with benign conditions of the head and neck.

Special surveys have been conducted to clarify the mortality and incidence of malignancy in Alaskan natives. Increased risks were noted for cancers arising from the nasopharynx, salivary gland, kidney, gall bladder, liver,

and connective tissue. Time trends in this population revealed an increasing incidence of "western" tumors (e.g., lung, breast, colon) which were formerly uncommon.

Using the programs and methods developed for the cancer atlases, maps for non-neoplastic diseases were published, emphasizing conditions that predispose to cancer or share common etiologic factors. Most recently, with visiting scientists from the People's Republic of China, analyses of the geographic patterns of malignancy within China have been conducted, including analyses showing a similarity in the mortality patterns of cancers of the uterine cervix and penis, and a clustering of colorectal cancer and schistosomiasis in Chinese provinces bordering the Yangtze River.

Currently, studies are under way to evaluate differences in time trends by geographic areas for those tumor sites showing marked temporal variation (e.g., stomach, cervix, and lung), along with attempts to relate these differences to various static and dynamic measures of demographic characteristics in these same populations.

Field Studies in Special Risk Areas: The clues derived from the cancer maps and correlation studies performed by the Branch and others have been pursued actively, the final step being the testing of specific hypotheses by case-control investigations in high- or low-risk areas. Investigations of lung cancer in a high-risk area of Florida revealed significantly increased risks associated with employment in the shipbuilding industry, particularly during World War II.

A case-control study of cancer of the oral cavity among women in a high-risk area of North Carolina revealed that snuff dipping accounts for the excess risk of these tumors in the rural south. The use of mouthwash and employment in electronics manufacture were also both suggested as potential risk factors, findings based on small numbers, but for which there is support from other investigations. In a case-control study to evaluate an unusual cluster of high rates of colorectal cancer in areas of rural Nebraska, the excess risk was limited to persons of Czechoslovakian descent, with some evidence that both dietary and familial factors were involved. A case-control investigation to evaluate the clustering of renal cancer in the north central area of the U.S. indicated that cigarette smoking was the dominant risk factor and confirmed the suspicions of an excess risk among persons of German descent.

Recently, a case-control study of lung cancer was conducted in an industrialized area of eastern Pennsylvania. A significantly increased risk was found among men who worked in the steel industry, primarily long-term employees who began work before 1935. Some excess risk was also seen for zinc smelter workers employed for long durations. Earlier correlational studies conducted by the Branch had suggested the possibility of a general environmental reason for lung cancer excesses in counties with non-ferrous metal smelters emitting large amounts of inorganic arsenic. In this investigation, there was an excess risk of lung cancer related to the proximity of residence to the zinc smelter and to measures of arsenic in the soil around the homes.

Analyses are currently underway of case-control investigations of lung, stomach, and pancreas cancer in southern Louisiana. Preliminary results confirm the suggestion from a prior study that persons of Acadian ancestry were at excess risk of lung cancer, and indicate that the excess is apparently due to the heavy use of hand-rolled cigarettes. Analyses are also under way of a case-control investigation of bladder cancer in rural Vermont and New Hampshire, areas showing suprisingly high mortality rates for bladder cancer in both sexes. Preliminary results indicate no excess for the practice of eating "fiddlehead" (bracken) ferns, but do imply that prior employment in the textile industry is related to excess risk for both sexes.

Case-control investigations in areas with excess respiratory cancer among residents of New Jersey and the Gulf Coast areas of Texas have recently emerged from the field and are being prepared for analysis; of special interest will be an evaluation of occupational exposures, particularly in the chemical and petrochemical industries in these areas.

To pursue relationships between nasal cancer and woodworking seen in a death certificate survey, a case-control interview study was conducted in a high-exposure area in North Carolina and Virginia. Preliminary analyses suggest excess risks associated with furniture working, textile work, and smoking.

Recently, this program area has been expanded to include studies of special risk areas outside the United States. In particular, under the aegis of an Institute-to-Institute agreement with the Chinese equivalent of NCI, feasibility studies have been initiated to assess whether studies of the unusual geographic patterns of cancer in China could provide insights into cancer etiology.

Occupational Studies: Epidemiologic studies of occupational groups are valuable, since workers often have heavy and prolonged exposures to suspect carcinogens. Studies of these groups can therefore lead to measures to reduce the risk to workers, and can identify the potential hazard of agents which are also found in the general environment. In addition, detailed studies of groups occupationally exposed to known carcinogens can provide insights into the basic mechanisms of human carcinogenesis. The Branch initiates studies in the occupational area to (a) explain unusual geographic distributions of cancer incidence or mortality; (b) identify high-risk subgroups within broad industrial categories; (c) pursue clues provided by animal bioassays or clinical observations; and (d) assist outside agencies or institutions in evaluating the health experience of their workers. Typically, cancer patterns are determined through long-term follow-up of persons employed in specific plants and industries, and comparisons are made with the general population or other industrial groups when available. Proportionate mortality studies are conducted when population data are unavailable, or to provide leads for more analytic studies. Case-control studies of particular cancers are carried out in areas or industries where occupations of interest are concentrated, utilizing personal interviews and/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. In a study of servicemen in chemical processing companies during World War II who had been exposed to tetrachloroethylene and other chlorinated compounds while impregnating clothing against mustard gas, the cancer mortality experience was elevated due to excesses of leukemia, lymphoma, and cancers of the genital organs. The excess brain tumor mortality among petrochemical workers noted by scientists in this Branch and others is being pursued further in a multi-center collaborative, case-control investigation in areas where this industry is concentrated.

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 detailed analysis of risk in relation to temporal aspects of exposure suggests that arsenic acts in the manner of a promoting agent.

Mortality patterns among Florida pesticide applicators were similar to the general population, except for leukemia and cancer of the lung. The risk of lung cancer rose with the number of years licensed to apply pesticides to nearly a three-fold excess among those licensed seven or more years. Additional studies of pesticide-exposed populations are under way to pursue this lead.

A series of descriptive surveys and death certificate analyses have suggested a relationship of farming to certain tumors. In particular, leukemia and non-Hodgkin's lymphoma appeared excessive among farmers, with leukemia being higher in areas of corn production and the lymphomas higher in locales high in acreage treated with insecticides, planted with wheat, or planted with small grains. To evaluate these leads, case-control interview studies of leukemia and lymphoma have been initiated in Iowa and Minnesota. In addition, a case-control study of soft-tissue sarcomas has been started in Kansas to evaluate possible risks associated with the use of herbicides in crop management in this state, particularly in light of the concerns about contamination of these herbicides with dioxin residues.

A preliminary investigation of mortality among professional artists suggested that people in contact with commonly used art materials have an unusual cancer risk. In particular, male artists had more deaths from leukemia and bladder cancer, with the excess limited to painters. Follow-up on this lead with data from the National Bladder Cancer Study has revealed a three-fold excess risk of bladder cancer among artists similar to the risk from the mortality study (PMR=3.6).

Concerns from the laboratory investigation of formaldehyde and the widespread exposure to this substance prompted the Branch to study several groups occupationally exposed to this substance. A proportionate mortality study of embalmers revealed increased frequencies of deaths from cancers of the skin, kidney, and brain, particularly among decedents licensed only as embalmers (i.e., those likely to have greater exposure to formaldehyde). A large-scale cohort investigation including multiple industries where workers are exposed

to formaldehyde was launched in the past year and will cover approximately 28,000 exposed workers.

Exploratory studies 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. A similar exploratory study of corn wet-millers revealed an unexpectedly high risk of pancreas cancer in both whites and nonwhites employed in one process.

Recent efforts have been made to systematically evaluate the occupational and industrial risk of cancer among over 400,000 participants in the Dorn Veteran's Follow-up Study, after adjustment for smoking habits.

Radiation Studies: Studies of populations exposed to ionizing radiation are being conducted to investigate further the relationship between cancer risk and exposure to high and intermediate 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. We also view these studies of radiation-induced cancer as a promising approach to understanding mechanisms of human carcinogenesis. A multidisciplinary conference was held within the past year to review insights provided from these studies, and the proceedings will soon be published.

A new breast cancer incidence study among atomic bomb survivors in Japan has confirmed earlier data. In addition, an apparent anomaly in earlier data in which women exposed at age 40-49 were at significantly decreased risk disappeared. However, women older than age 40 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.

An international radiation study of cervical cancer patients, including over 200,000 women treated by radiation or surgery, is currently under way. 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 activity, such 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 as effective in inducing leukemia as are other radiation exposures that have been studied, but a slight risk may be associated with low-dose radiation received by marrow outside the pelvis; (6) the young and the very old are at the greatest risk for cancer induction; and (7) ovarian radiation may lower breast cancer risk at all ages.

A study of treated non-Hodgkin's lymphoma patients revealed positive correlation between radiation dose to the bone marrow and subsequent risk of acute non-lymphocytic 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. Preliminary findings indicate a two-fold risk of thyroid nodules in exposed persons, and a significant elevation of chromosome aberrations in circulating lymphocytes occurring more than 30 years after this partial-body irradiation.

Three case-control studies have been completed recently. An interview study of 200 thyroid cancer cases and 400 population controls has revealed a high risk associated with radiotherapy for benign head and neck diseases when exposure occurred under age 10. A study of childhood cancer in twins to investigate the risk associated with prenatal x-ray has revealed a two-fold excess risk which seems likely to be due to the radiation itself rather than the indications for pelvimetry. A study of 225 children who developed second primary cancers following treatment for childhood cancer in six countries has revealed preliminary evidence for dose-response relationships between the magnitude of radiation received and the risk of malignancy in radiosensitive tissues. Among 9,000 two-year survivors of childhood cancer, the cumulative probability of developing a second cancer after 25 years was 12%.

A variety of ongoing projects are under way, including a collaborative investigation of 10,000 children irradiated for ringworm of the scalp in Israel (preliminary data indicate elevated risks of cancer of the thyroid and brain, and leukemia); a follow-up study of 170,000 x-ray technologists; a case-control study of approximately 2,000 cases of leukemia and lymphoma in a pre-paid health plan to evaluate the risk of lifetime diagnostic x-ray exposures; and several studies involving cancer registries or clinical trials to evaluate the carcinogenic effects of radiation therapy and chemotherapeutic agents. In addition, the Branch's long-term collaborative relationship with the Radiation Effects Research Foundation (RERF) in Japan is continuing. Recently, case-control interview investigations of breast cancer and lung cancer at RERF have completed the field phase. Emphasis in these analyses will be placed on evaluation of relationships between ionizing radiation and other risk factors for these malignancies.

Medicinal Agents: The Branch conducts a variety of studies to assess drug-induced cancer. The rationale for this is two-fold. Such studies have been valuable in the discovery of previously unrecognized carcinogenic hazards, and they have allowed insights into mechanisms of carcinogenesis. This has been so, not necessarily because of the presence of a large burden of drug-induced cancer in our society, but rather because drug exposure usually involves high doses which can be assessed by standard epidemiologic approaches. In conducting this program, epidemiologic, clinical, and laboratory observations are monitored for candidate drugs that can be evaluated for carcinogenic effects utilizing special resources developed by the Branch. These special resources include the monitoring of 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. In recent years, the focus of this program has been primarily on hormonal medications and cytotoxic drugs, although a variety of other agents have also been evaluated.

A case-control study using the Breast Cancer Detection Demonstration Project (BCDDP) has allowed the evaluation of several suspect drugs in breast cancer

etiology. No excess risk was observed following thyroid hormone exposures, but a reduced risk of breast cancer was found among women with untreated hypothyroidism and goiter. Examination of tranquilizer use has shown no excess risk associated with diazepam, as suggested by some animal investigations; in fact, a reduced risk of disseminated disease was suggested among users of this medication. Although based on small numbers, the use of phenothiazines seemed to be related to a slight excess of breast cancer risk, with some evidence of dose-response. Overall in this population, there was no relationship between breast cancer risk and the use of oral contraceptives. However, non-significant excess risks were associated with contraceptive use among women exposed in the perimenopausal period and among several other groups, including women with a family history of breast cancer or a previous biopsy for benign breast disease. The latter association was restricted to those women who developed benign disease while taking the contraceptive, implying that the excess risk was only an apparent one due to the nature of the benign disease that develops among contraceptive users. High-dose estrogen therapy for breast cancer was found to be associated with an increased risk of endometrial cancer in a cancer registry, case-control study.

The Branch has continued its program to evaluate potential carcinogenicity of various cytotoxic agents used in the treatment of cancer and some non-neoplastic conditions. Follow-up studies of patients with ovarian cancer, Hodgkin's disease, non-Hodgkin's lymphoma, and a variety of gastrointestinal tumors, have established the leukemogenicity of a variety of alkylating agents, including the first quantitative study of melphalan and the nitrosourea, methyl-CCNU. These studies have indicated differences in leukemogenic potential both related to dose and to particular type of drug. Several investigations have thus far failed to reveal any excess risk of malignancy associated with the use of the anti-metabolites. A study of cancer risk in patients with various rheumatologic diseases treated with relatively small doses of alkylating agents has also shown an excess risk of leukemia. A case-control study of children who developed multiple primary cancers is also under analysis for the role of therapy in the development of second tumors.

A recent evaluation of dapsone, under suspicion because of positive animal tests and used by patients with Hansen's disease, has failed to reveal any evidence of a causal relationship between this medication and cancer risk up to 25 years after first use. A case-control interview study of cancers of the renal pelvis has indicated an association with phenacetin-containing analgesic drugs, and has also suggested that heavy users of compounds with acetaminophen may also be at excess risk.

Nutritional Studies: Indirect evidence that diet and nutrition are related to cancer risk is substantial. Recently, the Branch has expanded its activities in diet and nutrition to test some of the current hypotheses and to generate additional testable hypotheses. Initially, these efforts involved the addition of a nutritional component to a study being conducted mainly for other reasons. In this manner, dietary factors were sought in case-control evaluations of oral cancer among women in North Carolina, and of lung cancer in several high-risk areas. A dietary component was also incorporated into investigations of the unusually high colorectal mortality in rural Nebraska,

and the excesses of pancreatic and stomach cancer in southern Louisiana. The case-control study of oral cancer suggested that consumption of fruit and vegetables was consistently low in the diet of the cases. The excess risk of colorectal cancer in rural Nebraska was primarily among people of Czechoslovakian ancestry, and was associated with a high intake of meat and dairy products in this ethnic group. The nutritional data from the lung cancer studies are currently under analysis.

Recently, a case-control study of invasive and in situ cervical cancer has been developed in five cancer centers, with a nutritional aspect that measures the intake of various micronutrients, both by interview about usual adult dietary patterns and by a laboratory assay of blood samples.

With the experience gained through incorporating nutritional elements in other studies, we have recently initiated studies whose major rationale is dietary. A collaborative study with three population-based registries in areas covering substantial Oriental-American populations is focusing on diet, particularly at a young age, and its relation to breast cancer risk among Oriental Americans. The study also involves measures of hormones and macro- and micro-nutrients. In addition, a study is under way to evaluate a suggestion from geographic studies of colorectal cancer that the risk among migrants from high-risk northern areas to low-risk retirement areas in the south declines rapidly to the low southern rate. Initially, a telephone interview study of the next-of-kin of persons dying of colorectal cancer and persons dying of other reasons is being conducted to establish whether the observed pattern is worth further analytic pursuit or is simply an artifact relating to migration.

With HANES I, the first Health and Nutrition Examination Survey of the United States, 1971-1974, the National Center for Health Statistics (NCHS) assessed the nutritional status of 23,000 representative American adults. The Branch is assessing correlations between dietary and biochemical data from this study and the county mortality rates for various cancers. The data are also being utilized for a variety of methodological studies of nutritional epidemiology. In collaboration with other Institutes and the NCHS, the Branch is tracing and reinterviewing the adults examined in HANES I, in an effort to relate dietary habits with the subsequent risk of cancer.

Case-Control Studies: The Branch conducts a variety of case-control studies of selected cancer sites that are not necessarily limited to high-risk areas or targeted to test one particular hypothesis. These studies are initiated for tumors where there is a wide variety of etiologic leads that need to be tested, or for tumors for which little is known but which seem right for a "fishing expedition" to generate new etiologic leads for more analytical testing.

One of the larger examples of this type of investigation is the National Bladder Cancer Study. Recent findings from this study include no association with the use of hair dyes, a diminished risk with having never drunk coffee (but no dose-response relationship among coffee drinkers), a two-fold risk associated with multiple urinary tract infections, a two-fold risk with bladder stones, and a three-fold risk with inhalation of pipe smoke. A number

of investigations are currently under way using this data set to focus on various occupational hazards and the risk of bladder cancer. Suspicions raised in geographic analyses concerning work in the automobile industry were tested in this study, and no relationship was found. However, the high mortality rates in areas where automobile manufacture is prominent seem to be related to the presence of elevated risks for a variety of machinist occupations and with truck driving, with some evidence suggesting a role for diesel emissions. Detailed analyses of other occupational groups (e.g., chemical, petrochemical, leather) are currently under way, along with comprehensive analyses of the relationship of tobacco use and usual source of drinking water to the risk of bladder cancer.

A number of risk factors other than medicinal agents have been examined in the breast cancer study within the BCDDP. Familial susceptibility to breast cancer appeared to be mediated through hormonal factors that operate early in a women's life, and showed some evidence of synergistic interaction with the presence 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 which are unlike those of benign breast disease. The differences that did occur implied that obesity and oophorectomy may be involved in the promotion of already-initiated carcinogenic processes.

As previously noted, a multi-center study of cervical cancer is in the field to evaluate several risk factors, including sexual variables, tobacco use, oral contraceptives, nutritional status, and frequency of prior pap smears. A study of 300 cases of testicular cancer in young men is currently under analysis. This study focuses on childhood and young adult exposures, as well as in utero exposures identified via interviews of mothers. Preliminary findings indicate a four-fold increase associated with maldescent, but this risk is limited to the side of maldescent and is reduced and eliminated with earlier ages at surgical correction. No independent risk was identified for inguinal hernia. Further, no evidence was found to support the suggestions that external means of elevating testicular temperature increase the risk of this tumor. Analyses of occupational and prenatal exposures are nearing completion.

Three other case-control investigations have recently completed data collection and are under analysis: a study of 350 patients with ovarian cancer in Washington, D.C., to help clarify the influence of reproductive factors, exogenous hormones, contraceptive methods, and other possible risk factors; a study of 400 patients with intraocular melanoma in collaboration with the Wills Eye Institute in Philadelphia; and a study of 300 patients with cutaneous T-cell lymphomas (mycosis fungoides), to test various etiologic hypotheses developed in an earlier descriptive study.

Infectious Agents: For some time, there has been little epidemiologic evidence to support an infectious etiology for any but a few human tumors. One Branch member has focused on African Burkitt's lymphoma. In collaboration with the University of Ghana Medical School, studies have been done to characterize the Epstein-Barr virus antibody titers and their relationship to incidence and progression of the disease.

Recently two events triggered considerable concern and activity in the area of possible infectious agents and cancer. The explosive outbreak of the Acquired Immunodeficiency Syndrome (AIDS), associated with Kaposi's sarcoma in many cases, is one of these events. While initially confined to promiscuous homosexuals, the syndrome has been identified in drug addicts, Haitian refugees, and hemophiliac patients. In an effort to clarify mechanisms, the Branch conducted a study of apparently normal Danish homosexual males, and found a high frequency of T-lymphocyte aberrations that strongly suggested that a transmissible agent is involved in etiology, and that the source of this agent for the cases in Denmark has been sexual contact with U.S. citizens. Preliminary data from surveys in New York City (high risk) and Washington, D.C. (low risk) suggest that: (a) the risk factors for altered helper/suppressor ratios (H/S) are the same as those previously identified for AIDS itself; and (b) important differences in risk factors exist between high- and low-risk communities. Thus, in New York, the key determinant of low H/S ratios was being the recipient of anally directed sexual acts, while in Washington the major risk factor was having had sex with a partner from a high-risk area. To clarify the risk among hemophiliacs, a collaborative investigation has been initiated to assess the dose of factor replacement and the possibility of common batches of serum being responsible for the observed cases. Early analysis has suggested a correlation between the dose of clotting factor transfused and the risk of depressed H/S ratios.

The second major finding has been the recent isolation and identification of a human retrovirus from patients with certain T-cell leukemias and lymphomas. The type of disease associated with positive antibody or virus isolates is quite characteristic clinically, shows an unusual geographic distribution, and seems to vary concominantly with the prevalence of antibody to the virus in various populations, but is always in great excess in the cases compared to any population series of controls. In collaboration with Dr. Robert Gallo's laboratory at NCI and other investigators, the Branch has initiated an ambitious program of studies to evaluate the epidemiologic aspects of this new virus-cancer relationship. Jamaica appears to be an endemic area for both the disease and the virus, and a series of studies have been initiated to describe the epidemiology of the viral infection and its temporal relationship to the diseases of interest. In addition, a variety of serum banks world-wide have been tapped in order to survey for prevalence of virus antibodies, and these have been augmented with surveys for the characteristic clinical entity that appears to be associated with the infection.

The Branch is involved in one follow-up survey utilizing a serum bank from the 1960s, one large-scale case-control study of invasive cervical cancer in the U.S., and the analysis of a similar case-control investigation in Panama, all of which include an evaluation of the possible relationships between candidate viruses, particularly herpes simplex virus type II, and the risk of cervical cancer.

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 now includes over 2,000 families. Utilizing this capability, statistical genetic approaches were applied in: (a) the analysis of patterns of cancer occurrence in families prone to sarcomas and diverse neoplasms; (b) segregation and linkage analyses of melanoma-prone families; and (c) linkage analysis of HLA in families prone to Hodgkin's disease.

Perhaps the best example of how the interdisciplinary approach provides insights into the mechanisms of host susceptibility to cancer is illustrated by the evaluation of familial melanoma and its relation to the dysplastic nevus syndrome (DNS). Based on a detailed study of over 400 members of 14 melanoma-prone families, the clinical and pathologic features of DNS have been precisely defined. It was demonstrated that careful surveillance of such high-risk individuals permits the detection of substantial numbers of surgically curable melanomas. An autosomal dominant mode of inheritance for hereditary melanoma/dysplastic nevi has been established along with the suggestion that this trait is linked to the Rh locus. A significant cellular abnormality has been identified in affected persons, manifested by sensitivity to the cytotoxic and mutation effects of both UV radiation and certain chemical carcinogens. These studies led to the recognition that dysplastic nevi play an important role in the etiology of sporadic melanoma as well; at least half of all melanomas are now thought to arise from dysplastic precursors.

Finally, our work with DNS and melanomas has bridged the gap between etiology and prevention by establishing and coordinating a free-loan program for the distribution of three educational video tapes which were developed by the Branch to disseminate information about this syndrome to concerned health professionals and the general public. It is estimated that 45,000 persons viewed these materials in this past year.

Immunogenetic studies in families prone to various lymphoproliferative disorders have identified an association between Hodgkin's disease and a new HLA specificity (MB-1), and revealed an HLA haplotype sharing in sibs with hairy cell leukemia.

Follow-up on clinical observations indicate that polymastia is a risk factor for testicular cancer. In a clinical follow-up of families prone to ovarian cancer reported earlier, 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.

Finally, a repository of biologic specimens from high-risk family members has been maintained as a valuable source of materials for experimentalists investigating susceptibility mechanisms in carcinogenesis. After years of minimal interest by our laboratory colleagues, the development of a variety of new molecular probes and the identification of a number of different putative human oncogenes has led to considerable enthusiasm for access to the material from particular families.

Immunoepidemiology: The Branch is engaged in a number of studies to evaluate populations with altered immune function to clarify the relationship between immune function and malignancy. The risks of cancer of different sites are quantified for these 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, including end-stage renal disease, sicca syndrome, gluten-sensitive enteropathy, sarcoidosis, scleroderma, and Hansen's disease.

Detailed analysis of renal transplant recipients has indicated a 25-fold increase in risk of lymphoma which appears within a year of transplantation, is greater among recipients of cadaver versus sibling grafts, and is more prominent among recipients in the earlier years of transplantation compared to more recent periods. At the University of Minnesota, a registry of cancers occurring in patients with genetically determined immune deficiency diseases has revealed a pattern of cancer risk resembling that noted among transplant recipients. In addition, patients with sicca syndrome have a lymphoma risk similar to transplant recipients, and the magnitude of the risk seems to be related to clinical indicators of the severity of the antigenic stimulation in this disease.

There is little evidence of a marked elevation of lymphoma risk among Hansen's disease patients. In addition, the study of 3,000 "hyperimmunized" former employees of a biologic warfare research center has also failed to reveal any marked excess of hematopoietic tumors.

Particular tumors other than the lymphomas have also shown excesses in some of these populations, and some differences and similarities in these risks between populations similar to the lymphoma risks have been noted. The implication from this wide variety of studies is that in a general sense, impaired immunologic surveillance is probably not etiologically important for most human malignancy. Rather, particular immune disorders are related to specific patterns of malignancy; and linkage of the clinical immunologic aberrations present in these diseases with the cancer patterns may provide exciting insights into the mechanisms of immune regulation of human carcinogenesis.

Veterinary Studies: One Branch member, a veterinarian, conducts surveys and more analytic investigations of cancer and other diseases in domestic animals, particularly dogs. By epidemiologic comparisons with human cancer, these studies are designed to clarify risk factors in human cancer and related diseases, characterize animal models that may be useful in further research, and identify sentinels that may act as early predictors of environmental hazards. The main resource used in these studies is data collected by 16 veterinary medical teaching hospitals and clinics in the U.S. and Canada.

Cholangiocarcinoma in dogs appears associated with recent infestations of blood-letting intestinal nematodes. The human counterparts of these parasites infest geographic areas of the world where presently there are unexplained high incidence rates 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.

Recent acquisition of the service records and a complete computerized file of standard post-mortem data on the military working dog population has permitted the initiation of a systematic linkage of disease to prior medical history, "occupational" exposures, diet, and other factors among 7,000 military working dogs.

General Environment: The impact on cancer of general environmental exposures (air and water pollution) are perhaps the most difficult of all to assess. Difficulties include: (a) a lack of specificity of hypotheses; (b) exposures that are difficult to measure, particularly for the time period of relevance of cancer induction; and (c) average doses which could be expected to produce low-level elevations in relative risk. Historically, we have done little as a Branch in this general area. Recently, we have ventured into some of the assessments by attempting to take advantage of some improvements in methodological approaches. A variety of laboratory studies have raised suspicions about a number of different chemical carcinogens in drinking water. Correlational studies of cancer mortality rates and direct or surrogate measures of these chemicals gave varying results, with a few relatively consistent findings, among them a positive association with urinary bladder cancer. Because of this, an aggressive attempt was made to test this hypothesis in the National Bladder Cancer Study. Specifically, persons were interviewed for their lifetime residence histories, which included questions about the source of their drinking water at these various locations. In parallel with this, we collaborated with the Environmental Protection Agency in surveying the areas for past practices as to the source of the water and its chemical treatment. In addition, chemical measurements in current water supplies were taken and related to characteristics of the source of the water and its chemical treatment. Analysis has failed to yield consistent evidence of increased risk with exposure to water likely to be relatively high in trihalomethanes and chemicals related to them. On the other hand, regional differences in the analysis have raised the question of increased risk related to other contamination of water supplies, such as chemicals used in agriculture. These same general methods of exposure assessment have been applied to a number of field studies of other cancer sites that were initiated for other reasons.

Our attempts at evaluating air pollution have been fewer. Because of the known relationship between arsenic exposure and lung cancer risk in the occupational setting, the substantial amount of arsenic placed in the atmosphere by non-ferrous metal smelters, and the uniformly elevated rates of lung cancer in both sexes in counties containing such smelters, we recently completed a study which attempted to assess the influence of general environmental pollution. In this investigation, the risk of lung cancer was directly related to two measures of potential general environmental exposures among non-occupationally exposed persons (proximity to the smelter and arsenic in the soil). This study has given us some enthusiasm for the ability to test at least some concerns about general environmental air pollution. The

proposed study of lung cancer in Shanghai, China, is also being undertaken because of a suspicion about general environmental exposures being responsible for the elevated rates among Chinese women among whom cigarette smoking is not prominent.

Methodologic Studies: Both by design and by the necessities of the types of studies conducted, a variety of methodologic investigations are performed by the Branch. This ranges from the development and testing of large data collection systems for their applicability to epidemiologic needs, through tests of alternate methods of conducting field investigations, to the adaptation and development of statistical methods for epidemiologic studies.

Potential epidemiologic resources within the National Center for Health Statistics, the Social Security Administration, the Health Care Financing Administration and the Veterans Administration have all been evaluated and have undergone extensive testing for utility as epidemiologic resources.

Research in methods of survey research have included: (a) the first applications of random digit dialing to control selection; (b) assessments of the comparability of information from surrogate respondents to that from the subjects themselves; (c) comparisons of hospital and population-based control series on histories of coffee drinking, artificial sweetener use, etc., with particular attention to reasons for hospitalization; and (d) the exploration of alternate methods for control selection for cases coming from referral cancer treatment facilities. Innovative approaches to exposure ascertainment have been notably successful. A comprehensive radiation dosimetry program has contributed accurate estimates of dose to specific organs from a variety of medical exposures and the value of cytogenetic aberration data as a biological dosimeter in persons with partial-body irradiation is being explored in three separate populatons.

In the area of statistical methods, a library of programs for epidemiologic analysis using a programmable calculater was updated and expanded into a second edition. An alternate means of analysis of failure time data by the Cox proportional hazards model was developed, and the extent to which unconditional logistic analyses overestimate odds ratios from matched data sets was examined in a simulation study. Peculiarities of relative risk estimation from case-control studies within cohort studies, depending on the criteria for control selection, were also assessed. Several reports expanded methodology for use in occupational and other cohort studies. SMR and PMR techniques were compared. New tests for equality of and trends in SMRs were reported on, as well as a detailed method of regression analysis for SMRs. A substantial amount of work has been done in the area of estimating cancer risk from low doses of ionizing radiation, including the effect on statistical power of using general models reflecting the current state of knowledge about mechanisms of radiation carcinogenesis. Methodologic issues were also explored with respect to descriptive and correlational studies, the evaluation of familial aggregations of cancer, and the calculation of HLA phenotype frequencies. Further development of occupation and exposure linkage systems for the study of occupational carcinogenesis has been accomplished and applied to several data sets.

Reviews: A major role of the Branch is to continue to provide comprehensive and critical reviews of etiologic factors in cancer. The co-editing of a reference volume entitled Cancer Epidemiology and Prevention included Branch reviews of several risk factors and cancers, including lung, biliary tract, multiple myeloma, non-Hodgkin's lymphoma, bone cancer, soft-tissue sarcoma, and non-melanoma skin cancer. The epidemiologic evidence for environmental carcinogenesis and genetic susceptibility was also reviewed in detail. Particular environmental hazards have also been reviewed, including ultraviolet radiation, medicinal agents, occupational exposures, parasites, water pollution, and food additives. The edited proceedings of a Branch-sponsored conference entitled Radiation Carcinogenesis: Epidemiology and Biological Significance focused on insights provided by radiation studies on the mechanisms of cancer induction. Additional reviews of the health effects of ionizing radiation, including a general overview as well as a review of cancers following medical irradiation, the effect of radiation on the immune system, the statistical and epidemiologic issues concerning estimation of cancer risks from low doses of ionizing radiation, the implications of studies on radiogenic breast cancer for models of human carcinogenesis, and methodologic approaches to studying cancer-prone families have all been performed by Branch staff. The Branch views these critical reviews as not only an opportunity to stay current in a variety of fields, but to develop new directions for the Branch's own research programs.

OTHER ACTIVITIES:

The Branch continued to provide a liaison for epidemiologic reasearch 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 binational agreements with the People's Republic of China, Italy, France, and Japan.

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.

SUMMARY REPORT

ANALYTICAL STUDIES SECTION

PROGRESS ON RESEARCH CONTRACTS

The studies supported by our progress on research contracts are concerned with unique or rare opportunities to study populations with unusual risk patterns and exposures in order to better understand the etiology of certain cancers.

One contract was initiated to update cancer incidence and mortality among Alaskan natives and examine trends in incidence from 1969 onwards and mortality from 1960 to the present; to investigate several striking familial aggregations of cancer among natives, particularly nasopharyngeal and hepatocellular carcinoma; to confirm the initial report of a human T-cell lymphoma virus in a patient with lymphoma; and to test for mutagenic activity in samples of dried and salted fish in coastal villages where cancer rates are high.

Results from the cancer incidence for the years 1969-82 and cancer mortality surveys for the years 1959-78 show striking excesses among Alaskan natives (15-fold or more compared to rates in U.S. whites and blacks) for nasopharyngeal carcinoma (NPC), with increased incidence and mortality also evident for salivary gland, kidney, liver, and gallbladder cancers. In contrast, the total cancer rates are at or slightly below U.S. norms. The patterns in natives are changing, however, as lung, colorectal, and breast cancers have become the leading cancers in recent years. Clustering of NPC and primary liver cancer has been observed in 9 families, prompting biochemical and immunogenetic workups. Samples of fish in Eskimo villages where NPC is common have been collected for Ames testing, since in preliminary analyses a high percentage of dried fish showed mutagenic activity, a finding consistent with the association of salted fish intake with the high rates of NPC in Hong Kong and elsewhere in Southeast Asia.

To examine the feasibility of conducting collaborative epidemiologic research in China, a contract was begun to conduct pilot investigations for a case-control study and a nutrition intervention trial in an area of North Central China where esophageal cancer rates are the highest in the world; for a case-control study of lung cancer in Shanghai, where rates are exceptionally high in women even though few smoke; and for a case-control study of choriocarcinoma in Beijing. Research protocols that detailed methods for case ascertainment, control selection, blood and urine collection, and field survey procedures were developed, providing explicit guidelines for the conduct of the pilot studies now underway. Initial results show that the societal organization structures do indeed facilitate epidemiologic study, since population-based cases and controls have been efficiently identified and selected both in rural and urban areas, and since registration systems permit an essentially complete tracing of individuals in follow-up studies.

ENVIRONMENTAL EPIDEMIOLOGY BRANCH

RESEARCH CONTRACTS ACTIVE DURING FY 83

ANALYTICAL STUDIES SECTION

Institution/Principal Investigator/ Contract Number	Title
Center for Disease Control Ann Lanier YO1 CP 00500	Epidemiologic Studies of Cancer in Alaskan Natives
Chinese Academy of Medical Sciences Li Bing NO1 CP 21012	Feasibility Studies for Collaborative Epidemiologic Cancer Research in China

SUMMARY REPORT

ENVIRONMENTAL STUDIES SECTION

PROGRESS ON RESEARCH CONTRACTS

The studies of the Environmental Studies Section that are supported by the contract mechanism were initiated to clarify the role of various environmental and host determinants of the etiology of malignant neoplasms. Specifically examined are associations of cancer and nutritional factors, drugs, other lifestyle factors, and prior disease. The areas covered by these contracts include 1) studies examining breast cancer in Oriental Americans, 2) studies on environmental cancer using pre-paid health plans, and 3) a follow-up study of women evaluated for infertility.

Studies Examining Breast Cancer in Oriental American Women (3 Contracts):

There is some reason to believe that, if a substantial amount of the international variation in breast cancer is due to dietary differences, the relevant age is likely to be the pre-pubertal period and perhaps adolescence. These ages have also been considered a critical time for breast cancer induction from entirely different lines of evidence (e.g., the interactions of other breast cancer risk factors with age, and from experimental studies). A recent Breast Cancer Task Force workshop on diet and breast cancer concluded that dietary investigations of breast cancer needed to be shifted towards evaluation of dietary differences at younger ages, particularly in view of the lack of any major positive findings from recent studies relating adult diet to risk of breast cancer.

Oriental Americans constitute a group in which diet in the home at young ages can be assessed by the case-control method. Cases and controls and their mothers will be interviewed in order to assess lifetime dietary patterns. The most important analytic variable will be one which can be assessed reasonably well in a case-control interview context, that is, "traditional" diet versus more westernized diet. It is hoped to evaluate differences in risk between a primarily traditional diet in the home throughout life, a primarily westernized diet throughout life, and a relatively traditional diet within the home at young ages but a westernized diet later. It is also planned to quantify the difference in rates between Oriental Americans and Caucasians in relation to recognized breast cancer risk factors (including age at menarche), and the differences explained more directly by diet or other factors.

The study design involves identification of newly incident cases of breast cancer among young Oriental Americans (less than age 50, or perhaps less than age 55), and interview of these women and a comparable matched control series of Oriental Americans to obtain standard breast cancer risk factors, and some information about diet (particularly major dietary changes over life). At the time of interview, the name, location and phone number of the subjects' mothers will be obtained. Telephone interviews with mothers will be carried out to assess general dietary practices in the home when the study subject was growing up.

Since the interview study will involve a substantial number of premenopausal Oriental Americans, and since most hypotheses concerning the mechanism of action of diet concern steroid hormone levels and metabolism, we intend to assess variation in hormone levels by case-control status and by degree of westernization. An advisory committee of endocrinologists will assist in determining which hormonal assays should be done.

The Cancer Center of Hawaii, Northern California Cancer Program, and University of Southern California possess population-based registries which have been in existence for at least two years and which cover an Oriental-American population large enough to yield at least 100 breast cancers among Oriental Americans less than 55 over a 5-year period. Epidemiologists from these areas will collaborate with the NCI Project Officer in developing a protocol and questionnaire for the study. Following this phase, each area will collect the information designated in the protocol. The analyses and interpretation of the results will be a collaborative effort between NCI investigators and these epidemiologists.

Studies on Environmental Cancer Using Pre-Paid Health Plans (3 Contracts):

The objectives of these studies are: (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 with 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.

In the Southern California contract, the surgical record books from the Southern California Permanente Medical Group from 1952 through 1970 have been 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 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.

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.

In Northern California data collection was completed for a case-control study of ovarian cancers similar to that in Portland. Using 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 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.

In Portland, time trend analyses of cancer incidence in this plan are currently underway. 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. Case-control studies of cholesterol level and risks of colon and lung cancer in men, and diagnostic irradiation and the risk of leukemia and lymphoma, are in the data-collection phase.

New studies in Portland and in Northern California which are planned include evaluations of the interrelationships between oral contraceptive use and benign and malignant breast disease, drug use and reproductive factors for endometrial cancer, and follow-up studies based on findings from the cholesterol, occupational, and radiation studies currently being conducted or analyzed.

A Follow-up Study of Women Evaluated for Infertility (1 Contract):

Nulliparous women and women with a late first pregnancy are at an increased risk of developing certain neoplasms, including those of the breast, endometrium, and ovary. The reasons for this excess risk are not known. Among the possible reasons suggested have been either a change in the hormonal milieu consequent to first pregnancy or abnormal profiles associated with absolute or relative infertility. Studying a cohort of women treated for infertility will allow comparison of disease incidence among subgroups with differing abnormalities, and may aid in understanding the mechanisms of carcinogenesis. If an abnormal hormonal milieu is the important factor in

contributing to an excess cancer risk among infertile women, those women with specific identifiable hormonal problems will be of particular interest. The follow-up study of women at the Mayo Clinic with recognized problems of infertility will allow evaluation of an hypothesis regarding breast cancer etiology that has received recent widespread attention. This hypothesis proposes that women who exhibit luteal phase defects may be at increased risk due to their relatively unopposed state of endogenous estrogens. The present study will not only allow evaluation of effects of varying hormonal states, but will allow examination of various infertility treatment effects. Of particular interest will be radiation exposures to the pituitary and/or ovaries and use of progestational agents.

The proposed methodology is a retrospective cohort study. Approximately 2,500 women evaluated for infertility during a thirty year period (1935-1955) will be followed to the present time for subsequent disease risk. Medical records will be abstracted to obtain the following information: demographic characteristics, menstrual and reproductive history, the diagnostic work-up for infertility, and infertility treatment details. Follow-up information on subsequent reproductive events, development of malignancies and vital status (including cause of death) will be sought through personal contact and through a population-based tumor registry. Data will be analyzed using standard methods for prospective studies, using internal comparisons (e.g., comparing events rates for those with and without a progesterone deficiency) and external comparisons.

ENVIRONMENTAL EPIDEMIOLOGY BRANCH

RESEARCH CONTRACTS ACTIVE DURING FY 83

ENVIRONMENTAL STUDIES SECTION

Institution/Principal Investigator/ Contract Number	Title
Cancer Center of Hawaii Abraham Nomura NO1 CP 21036	Breast Cancer in Oriental Americans
Kaiser Foundation Research Institute Los Angeles, California Harry Ziel NO1 CP 11038	Studies on Environmental Cancer Utilizing Pre-Paid Health Plans
Kaiser Foundation Research Institute Oakland, California Gary Friedman NO1 CP 11037	Studies on Environmental Cancer Utilizing Pre-Paid Health Plans
Kaiser Foundation Research Institute Portland, Oregon Andrew Glass NO1 CP 11009	Studies on Environmental Cancer Utilizing Pre-Paid Health Plans
Mayo Foundation George D. Malkasian NO1 CP 11023	Follow-up Study of Women Evaluated for Infertility
Northern California Cancer Program Donald Austin NO1 CP 21010	Breast Cancer in Oriental Americans
Southern California, University of Malcolm Pike NO1 CP 21038	Breast Cancer in Oriental Americans

SUMMARY REPORT

FAMILY STUDIES SECTION

PROGRESS ON RESEARCH CONTRACTS

During FY 1983 this Section had 3 research contracts. Two of them, Contracts No. NO1-CP-31006 and No. NO1-CP-31015 were recently initiated, therefore there is no progress to report.

During FY 1983 the immunology/immunogenetics laboratory supported by Contract No. YO1-CP-50500 contributed substantially to studies of high risk families, AIDS, and HTLV. The specific results are discussed in the progress of the Section. Highlights are (1) the demonstration of shared cell surface markers in familial CLL; (2) common immunogenetic markers in sporadic and familial Hodgkin's disease; (3) subclinical immunologic perturbations in AIDS and persons at high risk of AIDS; and (4) immunogenetic markers associated with HTLV infection. Future studies will focus on extending these observations to other study populations.

ENVIRONMENTAL EPIDEMIOLOGY BRANCH

RESEARCH CONTRACTS ACTIVE DURING FY 83

FAMILY STUDIES SECTION

Institution/Principal Investigator/ Contract Number	Title
Gorgas Memorial Institute Department of Epidemiology William Reeves NO1 CP 31015	Epidemiology of Human T-Cell Leukemia/Lymphoma Virus in Republic of Panama
Uniformed Services University of the Health Sciences Michael Strong YO1 CP 50500	Immunologic Studies of Disease Susceptible Families

Institution/Principal Investigator/ Contract Number	Title
West Indies, University of W. Nigel Gibbs NO1 CP 31006	Epidemiology of Human T-Cell Leukemia/Lymphoma Virus in Jamaica, West Indies

SUMMARY REPORT

OCCUPATIONAL STUDIES SECTION

PROGRESS ON RESEARCH CONTRACTS

Occupational studies supported by research contracts are concerned with the role of environmental exposures, particularly those associated with agriculture, in the origin of leukemia and non-Hodgkin's lymphoma (NHL). Geographic and death certificate studies have noted a high mortality from these tumors among farmers. To follow up these leads, 600 cases of histologically confirmed leukemia and 600 NHL (300 of each from Iowa and Minnesota) among adult white men will be interviewed. Twelve hundred population based controls (600 from each state) are selected for comparison.

Study subjects, or their next-of-kin, are interviewed to obtain information on agricultural exposures including insecticides, herbicides, and fungicides, non-agricultural occupations, medical history, residential history and sources of drinking water, use of unpasteurized dairy products, and contact with sick pets. To date approximately 1600 subjects have been interviewed. Interviewing is complete in Minnesota and will be finished in Iowa by January 1984. Since field work is not yet complete, findings are not available at the present time.

ENVIRONMENTAL EPIDEMIOLOGY BRANCH

RESEARCH CONTRACTS ACTIVE DURING FY 83

OCCUPATIONAL STUDIES SECTION

Institution/Principal Investigator/ Contract Number	Title
Iowa, University of Peter Isacson NO1 CP 11020	A Study of Environmental Factors in the Origin of Leukemia and Non-Hodgkin's Lymphoma among Adult Males from Rural Areas
Minnesota, University of Leonard Schuman NO1 CP 01033	A Study of Environmental Factors in the Origin of Leukemia and Non-Hodgkin's Lymphoma among Adult Males from Rural Areas

SUMMARY REPORT

POPULATION STUDIES SECTION

PROGRESS ON RESEARCH CONTRACTS

The Population Studies Section has responsibility for the acquisition and utilization of resources to facilitate epidemiologic studies. These studies range from descriptive to analytic, including case-control and cohort studies. Liaison is maintained with government and non-government sources to realize these objectives. That which follows are summaries of several activities within the Section which are supported by contracts.

Population Estimates: The Bureau of the Census is developing estimates of the resident population of the U.S. at the county level by age race and sex for the 1980s. Models have been developed which utilize special censuses, decennial censuses and medicare registration to provide these estimates. Prior estimates for the 1970s are being revised using the 1980 census.

Assessment of Screening:

Data have been abstracted on all persons ever screened for bladder cancer as well as all persons known to have developed bladder cancer among the Chambers Works employees regardless of whether they had been screened. Complete work histories were abstracted which included dates of employment by job title for the duration of employment by DuPont. Medical histories have been abstracted for both cases and non-cases, which include: the dates and results of every urinary cytologic reading, the dates and results of every urinary blood test, and the dates and type of every physical exam which the persons had been given, as well as vital status information. For each bladder cancer case detailed clinical histories were abstracted to provide information concerning signs or symptoms of bladder cancer, procedures performed, findings of these procedures, and any recommendations made. Detailed pathologic information has also been abstracted to include type, grade, stage, evidence of multicentricity, metastatic sites, and also any second primary sites of malignancy.

Lung, Stomach, and Pancreas Cancer in Louisiana: As a follow-up to a death certificate study in Louisiana, a case-control interview study of the above cancer sites was conducted in 26 southern Louisiana parishes. Analysis of the lung cancer data is nearly complete and interviewing for stomach and pancreatic cancer is continuing. An extensive questionnaire was administered to gather information on lifetime occupational and residential histories and tobacco use, as well as dietary practices and alcohol consumption. A significant 30 percent reduction in lung cancer risk was seen for frequent consumers of fruits and vegetables. An increased risk seen for Cajun/Acadians was consistent with the death certificate study, and seems to be due to differential patterns of tobacco use, in particular the use of hand-rolled cigarettes. Analysis of occupational factors and a more refined analysis of tobacco use, including exposure to passive smoking, is now in progress.

Preliminary results show a significant excessive risk for lung cancer among white males employed in lumber manufacturing and forestry, even when adjusted for smoking habits. Preliminary data suggest that persons residing near a saw mill or lumber manufacturing plant also have an increased rate of lung cancer.

Bladder and Lung Cancer in New Jersey: We have worked collaboratively with the New Jersey State Department of Health on studies of bladder cancer and lung cancer. New Jersey was a participant in the National Survey of Environment and Health (NSEH) which attempted to evaluate health effects due to the ingestion of artificial sweeteners. Analyses were made of the role of occupation and industry in the development of bladder cancer among males in New Jersey. Significant relative risks were detected for employment as rubber workers, gas station attendants, and miscellaneous laborers, and also for exposure to leather, rubber, paint, printing ink and other organic compounds. Interactions between various occupational exposures and cigarette smoking are also being evaluated.

The data collection phase has been completed for a case-control study of incident lung cancer among white male residents of high-risk areas of New Jersey. The questionnaire which was used in this study was designed to collect detailed data on cigarette smoking, including a lifetime brand use and information on the usual dietary intake of vitamin A and beta-carotene. The detailed longitudinal cigarette smoking history is being utilized to investigate the effects of duration, intensity, total dose, time since having stopped smoking, age first started smoking, and the effect of changing from high to low-tar cigarettes on the development of lung cancer. Attempts will be made wherever possible to analyze the data by cell type. The role of occupational hazards and air pollution will also be evaluated.

A companion study of lung cancer among non-white males will remain in the field until the end of calendar year 1983. A statewide study of incident lung cancer among females in New Jersey is also in progress. The case ascertainment period for this study will end in the summer of 1983. The subsequent editing and preliminary analyses should be completed by Spring of 1984. Special emphasis will be placed on an evaluation of nutritional status, passive smoking, and indoor pollution, as well as hormonal and other factors.

Biomedical Computing: A number of major systems are currently in various stages of development. These systems range from the development of cost efficient systems which provide longitudinal hospitalization histories for patients, to the calculation of chemical-specific exposure indices for cohort studies.

ENVIRONMENTAL EPIDEMIOLOGY BRANCH

RESEARCH CONTRACTS ACTIVE DURING FY83

POPULATION STUDIES SECTION

Institution/Principal Investigator/ Contract Number	Title
Bureau of the Census Richard Irwin Y01 CP 20517	Population Estimates by Age, Race, and Sex for the 1980s
E.I. DuPont de Nemours & Co., Inc. William Vogler N01 CP0 1038	Study of DuPont Chambers Workers Bladder Cancer Screening Program
Louisiana State University Pelayo Correa N01 CP 91023	Cancer in Southern Louisiana: A Case Control Study of Lung, Pancreas Stomach Cancers
New Jersey State Department of Health Ronald Altman N01 CP 61031	Etiologic Studies of Cancer in New Jersey
ORI, Inc. Norman Shusterman N01 CP 01054	Biomedical Computing Design and Implementation

SUMMARY REPORT

RADIATION STUDIES SECTION

PROGRESS ON RESEARCH CONTRACTS

The studies of radiation-induced cancers supported by the research contract mechanism (9 contracts, $2,298,506) are to strengthen the quantitative basis for risk estimation, especially at low doses, to improve the understanding of the role of host and environmental factors on radiogenic cancer risk, and to provide insights into carcinogenic mechanisms. Specific studies are discussed below.

Radiation Risk Estimation in Israeli Children Irradiated for Tinea Capitis. The objectives of this study are to determine the incidence of cancer in 10,000 Israeli children irradiated for ringworm of the scalp, 10,000 nonexposed persons selected from the general population, and 5,000 nonexposed siblings. The methods employed are as follows: 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 obtained. Death certificates will be obtained for those who have died, and the vital status as of 1981 will be determined for all enrolled persons. Malignancies of particular interest include: thyroid, brain, parotid gland, breast, bone, lung, esophagus, larynx, skin, leukemia, and lymphoma. A preliminary report has indicated a risk of malignant and benign tumors of the thyroid, cancer of the brain, and leukemia as associated with scalp irradiation during childhood.

Risk of Cancer Following Multiple Chest Fluoroscopies for Tuberculosis in Connecticut and Massachusetts. The objectives of this study are 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. All eligible patients discharged alive from major Massachusetts and 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 also be used to ascertain the incidence of cancer. Mail questionnaires will be sent to living persons. Preliminary mortality analyses of the Massachusetts population do not suggest an increase of cancer mortality among tuberculosis patients. Among the exposed population, no statistically significant excess of deaths was found for malignancies that might have been expected to be elevated, i.e., the breast, lung, and leukemia. Preliminary results for cancer incidence indicate a slight, but not statistically significant, increased risk of breast cancer when the exposed are compared to the nonexposed patients. The distributions of risk by age and time since exposure apparently differ from previous studies.

International Radiation Study to Evaluate the Risk of Radiation Exposure in Cervical Cancer -- European Segment. The objectives of this study are to: (1) quantify the risk of radiogenic cancer in cervical cancer patients for sites that are not well-studied, such as the stomach and pancreas, (2) evaluate the risk of low-level exposures to the breast and thyroid, and (3) evaluate the influence of host factor (such as age) on subsequent radiogenic risk. Over 200,000 women with cervical neoplasia who have been reported to one of 15 cancer registries are being evaluated for the risk of second malignancies. Case-control studies for specific cancer sites are being conducted to provide detailed information on radiation dose for risk assessment. More than 22,000 patients treated for cervical cancer in 22 European clinics are being evaluated for the occurrence of second cancers subsequent to radiotherapy. Radiation doses to body organs outside the pelvis are relatively low, under 100 rad, and can be accurately characterized. Detailed dosimetry information is being abstracted from hospital records. Morbidity and mortality are being determined through active follow-up. The cancer registry cohort analyses are almost complete and will be published as a monograph. Findings indicate excess risks, related to radiation, for cancers of the rectum, kidney, ovary, and corpus uteri, as well as acute nonlymphocytic leukemia and multiple myeloma. A deficit of breast cancer, possibly related to ovarian ablation, was also observed. Ongoing case-control studies will determine the extent to which cancer risk is related to radiation dose.

Risk of Cancer in X-Ray Technologists. The objective of this study is to evaluate the long-term effects of chronic exposures to radiation due to occupation among 170,000 registered American radiologic technologists. 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 (140,000) 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. 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. This project has been under way for an insufficient period of time for a more significant report.

Epidemiologic Studies of Cancer Among A-bomb Survivors. 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. 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. 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. 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. Interviews of next-of-kin of lung cancer cases and age-sex matched controls were completed. 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 those exposed under age 10 years. 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 before menopause, were strengthened by the new data.

Irradiation for Benign Menstrual Disease. The objectives of this study are to determine cancer incidence and mortality and estimate the risks of radiation-induced cancer in women treated for benign gynecological disorders (BGD). A study size of at least 6,000 exposed women should be sufficient to provide adequate statistical power to detect and evaluate dose-response relationships for radiogenic leukemia and solid tumors. We have issued a competitive RFP, and will consider multiple awards. Medical, therapeutic, and follow-up information will be abstracted from medical records. Death certificates would be obtained for those who died, and questionnaires will be sent to those located alive. Mortality analyses will be made, and we are also planning comparisons with Connecticut population rates for cancer incidence, and with women treated without radiation for BGD if a large enough cohort of unexposed women can be assembled. Organ-specific radiation doses will be determined for individual BGD patients.

Leukemia and Lymphoma Associated with Diagnostic X Ray. The objectives of this study are to quantify the risk of leukemia and lymphoma possibly associated with lifetime histories of diagnostic x-rays. Using the resources of pre-paid health plans in California and Oregon, 2,000 cases of leukemia and lymphoma and 2,000 controls are being identified, and all histories of diagnostic x-rays are being abstracted from medical records. Diagnostic x-ray exposures are being converted to radiation dose to the active bone marrow. Initial record abstraction has been completed in Oregon for all study subjects, and in California for approximately one-third of the cases and controls. Preliminary bone marrow dosimetry determinations, using the California Nomenclature Coding System, have been made. Analyses of results are ongoing.

ENVIRONMENTAL EPIDEMIOLOGY BRANCH

RESEARCH CONTRACTS ACTIVE DURING FY 83

RADIATION STUDIES SECTION

Institution/Principal Investigator/ Contract Number	Title
Chaim-Sheba Medical Center Baruch Modan NO1 CP 01042	Radiation Risk Estimation in Israeli Children Irradiated for Tinea Capitis
Harvard University Richard R. Monson NO1 CP 81058	Follow-up of Fluoroscopically Examined Tuberculosis Patients In Relation to Incidence of Cancer
International Agency for Research on Cancer Rudolfo Saracci NO1 CP 11017	International Radiation Study to Evaluate the Risk of Radiation Exposure in Cervical Cancer--European Segment
Minnesota, University of Jack S. Mandel NO1 CP 21015	Risk of Cancer in X-Ray Technologists
National Academy of Sciences Seymour Jablon NO1 CP 01012	Epidemiologic Studies of Cancer Among A-bomb Survivors
Yale University Jennifer Kelsey NO1 CP 01029	Risk of Cancer Following Multiple Chest Fluoroscopies for Tuberculosis
Kaiser Foundation Research Institute Andrew Glass NO1 CP 11009	Studies of Environmental Cancer Utilizing Pre-Paid Heatlh Plans
Kaiser Foundation Research Institute Gary Friedman NO1 CP 11037	Studies of Environmental Cancer Utilizing Pre-Paid Health Plans

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP04378-08 EEB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

U.S. Cancer Mortality Survey

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)

Thomas J. Mason, Chief, Population Studies Section, EEB, NCI

COOPERATING UNITS *(if any)*

National Center for Health Statistics Bureau of the Census, National Oceanic and Atmospheric Administration, Environmental Protection Agency

LAB/BRANCH

Environmental Epidemiology Branch

SECTION

Population Studies Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 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 *(Use standard unreduced type. Do not exceed the space provided.)*

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 hypothesis. An update to our earlier work in this area has been completed in the form of a three volume publication containing cancer mortality rates and changes in rates for counties of the United States for 1950-1959, 1960-1969, and 1970-1979. Age-adjusted mortality rates are ranked nationally by percentiles for 1970-1979, and for percentage change in the rates from 1950-1959 to 1970-1979. These volumes should facilitate the identification of places where additional study should be considered.

PROJECT DESCRIPTION

Names, Titles, Laboratory and Institute Affiliations of Professional Personnel (other than the Principal Investigator) engaged on this Project:

J.F. Fraumeni, Jr.	Associate Director	FS&S	NCI
R.N. Hoover	Acting Chief	EEB	NCI
W.J. Blot	Chief, Analytical Studies Section	EEB	NCI
B.L. Stephensen	Computer Specialist	EEB	NCI
R.I. Ramsbottom	Computer Specialist	EEB	NCI
A. Blair	Chief, Occupational Studies Section	EEB	NCI
L.W. Pickle	Mathematical Statistician	EEB	NCI
H.M. Hayes	Veterinarian	EEB	NCI

Objectives:

To examine the cancer mortality experience in the United States relative to cancer etiology. Special emphasis is placed upon the selection 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:

A three-volume publication was completed containing cancer mortality rates and changes in rates for counties of the United States for 1950-1959, 1960-1969, and 1970-1979. This report includes the number of deaths and cancer mortality rates age-adjusted to the 1970 U.S. population for each of 35 site-specific cancers for the four race-sex groups. Further, counties are ranked nationally by percentiles for 1970-1979 age-adjusted death rates and for percent change from 1950-1959 to 1970-1979.

Over the three periods covered by this report, there have been marked fluctuations in mortality for several site-specific cancers. Total cancer mortality rates increased 16% for white males and 43% for nonwhite males. Among the ten major cancer sites, lung cancer mortality increased most noticeably. White male lung cancer mortality increased 112% from 1950-59 to 1970-79 and white female lung cancer mortality increased 185%. Nonwhite lung cancer rates rose even more sharply. Both nonwhite male and female lung cancer mortality increased approximately 200% Interestingly, the largest part of this increase occurred between the first two periods for white and nonwhite males, while this increase occurred between the second and third periods for female lung cancer mortality. Consistent increments in pancreatic cancer mortality were observed for all race/sex groups. Increments were 18% and 15% for white males and females, while nonwhites experienced greater increases of

41% and 55%. Mortality from cancer of the large intestine increased among white males (17%), nonwhite males (53%), and nonwhite females (31%). Only white females experienced a decline (7%) for this site. Stomach cancer mortality continued to decline. All four race/sex groups experienced a decrement of greater than 40% from 1950-59 to 1970-79. Rectal cancer also declined for all race/sex groups. For this site, the decrements for white males, white females, nonwhite males, and nonwhite females were 34%, 42%, 16% and 34%, respectively.

Marked changes over time also occurred for several less common cancer sites. Most striking among these changes was the large increment in multiple myeloma. Mortality from this cause of death increased sharply for white males (78%) and females (84%), and even more sharply for nonwhite males (132%) and females (145%). Melanoma mortality continued to rise in the four race-sex categories, white males (84%), white females (50%), nonwhite males (60%), and nonwhite females (21%). Laryngeal cancer mortality rose only slightly for white males (6%), but white females (47%) and especially nonwhite males (77%) and females (70%) exhibited larger increases. Esophageal cancer showed little change for whites, but nonwhites experienced increments of 60% and 78% for males and females, respectively. Less common cancer sites characterized by decreasing mortality included cancer of the bone and, for females, cancer of the uterine corpus. Bone cancer mortality declined 39%, 41% 24%, and 38% among the four race/sex groups. Mortality from cancer of the chorion and uterus declined 44% for whites and 53% for nonwhites.

One of the results of the publication of our recent Atlas of Mortality from Selected Diseases was a study of the geographic patterns of motor neuron mortality rates when indicated that rates west of the Mississippi River were generally higher than rates to the east. These rates were positively correlated with rural farming and socio-economic status, but not with urbanization, physician-population ratios, lead and mercury exposure, or rates for a number of cancers.

The geographic distributions of 11 major ethnic groups within the United States were illustrated by computer-generated State Economic Area maps. The Scandinavian, German, and Russian ethnic groups were similarly concentrated primarily in the North Central region, while the Irish, Polish, other east European and south European groups were clustered predominantly in the Northeast. Other ethnic groups had patterns which were different from either of the above. These maps correspond to the atlases of mortality from cancer and other diseases. Although ecologic analyses are subject to a number of potential biases, and especially to the ecological fallacy, visual comparison of disease mortality and ethnic concentrations may be useful for formulating hypotheses regarding genetic, cultural, and/or environmental determinants of disease.

Significance to Biomedical Research and the Program of the Institute:

This survey provides a continually expanding data set which has generated specific data set into specific racial and geographic subsets (e.g., county

level analyses). It 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:

Bharucha, N. E., Schoenberg, B. S., Ravens, R. H., Pickle, L. W., Byar, D. P., and Mason, T. J.: Geographic distribution of motor neuron disease and correlation with possible etiologic factors. Neurology, 1983. (In Press).

Morin, M. M., Pickle, L. W., and Mason, T. J.: Geographic patterns of ethnic groups in the United States. Am. J. Public Health, 1983. (In Press).

Riggan, W. B., VanBruggen, J., Acquavella, J. F., Beaubier, J., and Mason, T. J.: U.S. Cancer Mortality Rates and Trends: 1950-1978, Vols. I, II, III. Washington, D.C., U.S. Government Printing Office, 1983. (In Press).

CONTRACT IN SUPPORT OF THIS PROJECT

NATIONAL OCEANIC AND ATMOSPHERIC ADMINISTRATION (Y01-CP-80506)

Title: Automated Graphics Support for Environmental Epidemiology

Current Annual Level: $2500.00

Man Years: 0.3

Objectives: To provide 35mm film images from magnetic tape input from the NCI computer graphics system.

Major Contributions: This support contract has been used extensively to provide film copy of the geographic distributions of diseases under investigation by branch professionals. These investigations have resulted in the publication of several major atlases which identify regions of the U.S. where additional study would seem warranted.

Proposed Course: This interagency agreement will be continued until this resource is no longer needed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP04401-07 EEB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Immunologic Factors in Cancer Etiology

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)

Robert N. Hoover, Acting Chief, Environmental Epidemiology Branch, NCI

COOPERATING UNITS *(if any)*

Veterans Follow-up Agency of the National Academy of Sciences, End Stage Renal Disease Program, Oxford University, University of Minnesota Immunodeficiency Cancer Registry

LAB/BRANCH

Environmental Epidemiology Branch

SECTION

Environmental Studies Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to characterize the cancer experience of populations with altered immune status. Risks of cancer of different sites are quantified for various groups of such patients, compared with each other and with national rates, and characteristics and possible determinants of unusual risks are sought. The populations studied include renal transplant recipients, kidney dialysis patients, patients with inherited and acquired immunologic disorders, and groups of immunostimulated persons. Evidence from these studies suggests that some immune factors, while apparently not of major relevance to some tumors, are important in the development of certain others, particularly non-Hodgkin's lymphoma.

PROJECT DESCRIPTION

Names, Titles, Laboratory and Institute Affiliations of Professional Personnel (other than the Principal Investigator) engaged on this Project:

J.F. Fraumeni, Jr.	Associate Director	FSS	NCI
T.J. Mason	Chief, Population Studies Section	EEB	NCI
A.F. Kantor	Senior Staff Fellow	EEB	NCI
L.A. Brinton	Senior Staff Fellow	EEB	NCI
E.J. Martin	Expert	EEB	NCI

Objectives:

(1) To identify and study populations with altered immunologic states experiencing unusual rates of malignancy. (2) To determine whether populations with similar risk patterns share immunologic abnormalities which could then delineate causal mechanisms.

Methods Employed:

1. Final analysis was completed on 16,739 patients who received renal transplants. The number of cancers observed among these patients was compared to that expected based on cancer incidence rates in the general U.S. population. Analyses included a search for the determinants of the unusual lymphoma risk in this group.

2. A collaborative arrangement has been established with the End-Stage Renal Disease Program of the Health Care Financing Administration (HCFA) to evaluate cancer risks among patients on dialysis. The study population includes 28,049 dialysis patients. Detailed analyses of cancer risk in these patients are now being concluded. The number of cancers observed in these patients was compared with that expected based on cancer incidence rates prevailing in the general population.

3. A number of analyses in the past year have been carried out to characterize the risk of cancer among patients with genetically determined immunodeficiency diseases. This has been done in collaboration with the University of Minnesota.

4. Nearing completion is a follow-up study to evaluate cancer mortality of 3000 former employees of Fort Detrick, Maryland, who were "hyperimmunized" by repeated vaccinations. Complete immunization histories have been obtained for these skilled craftsmen who were repeatedly immunized against biological agents under development at the Fort Detrick facility from 1945 to 1970. Follow-up is now complete, and these data are being analyzed.

5. A study of cancer risk in patients with Hansen's disease (leprosy) has recently been completed. This disease is associated with impaired cellular immunity, prolonged antigenic stimulation, and lymphoproliferation. A cohort of 1678 patients admitted to the National

Hansen's Disease Center in Louisiana from 1939 to 1977 were followed to determine excess cancer mortality. (See Project Z01CP04412-07 EEB).

6. Planning has been completed to utilize data from the Veterans Administration for a series of projects relating several immunologic disorders to cancer risk. Longitudinal medical histories have been constructed for all persons admitted to Veterans Administration Hospitals since 1970, and work is continuing to include all persons admitted since 1963. Studies are under way to obtain more precise estimates of the risk of developing various forms of cancer. Preliminary analyses in populations with acquired immune disorders include patients with sicca syndrome (Sjogren's disease), scleroderma, celiac disease, ulcerative colitis, and Crohn's disease.

7. Cancer risk is being evaluated in servicemen who were diagnosed during World War II with sarcoidosis, a disease also associated with impaired cell-mediated response and lymphoproliferation. Analysis of the 800 veterans under study is nearing completion.

Major Findings:

1. There was a 15-fold excess risk of lymphoma among recipients of renal transplants, which was due primarily to non-Hodgkin's lymphoma. The risk of other cancers was increased two-fold due to elevated relative risks for cancers of the hepatobiliary system, lung, bladder, thyroid, and soft-tissue sarcoma. Nonsignificant excesses were seen for malignant melanoma, brain tumors, and leukemia. The risk for lymphoma showed a substantial reduction over calendar time, an elevation with repeated transplantation, and an inverse association with the "closeness" of the donor-recipient relationship. These results suggest that the risk of lymphoma in immunosuppressed individuals is influenced by the degree of antigenic stimulation or a highly correlated factor, although the same mechanism is less likely to explain the risk of other cancers.

2. In comparison with national incidence rates, the overall relative risk of cancer in dialysis patients was not significantly different from unity. Slight excesses in non-Hodgkin's lymphoma, leukemia, and cancer of the gallbladder were found, but were not statistically significant. The risk for non-Hodgkin's lymphoma was significantly elevated in patients with glomerulonephritis suggesting a role for nonuremic factors, such as immunosuppressive drug therapy.

3. Preliminary analyses of data from the Veterans Administration hospital system suggest that patients with sicca syndrome, a disorder associated with abnormal immunoregulatory function, has revealed elevated risks of lymphoma, leukemia, and cancers of the buccal cavity. Similar patterns are being seen in patients with scleroderma, a disorder involving autoimmunity and sharing certain immunologic features with sicca syndrome. In addition, scleroderma patients show excess risks of esophagus and lung cancer. Celiac disease, which involves repeated antigenic stimulation and

abnormal immunologic reaction in intestinal mucosa initiated by gluten, has also shown a strong association with these same tumors. Preliminary analyses of patients with ulcerative colitis and Crohn's disease reveal risk patterns similar to previous studies, indicating the reliability of this large data resource for estimating cancer incidence in cohort studies.

4. Among patients with Hansen's disease, a slight increase in lymphoma risk was observed, but was not statistically significant and did not occur in those patients with the most severe immune alteration. This cohort is also being evaluated for possible cancer risk associated with the antibacterial dapsone (sulfone) which was used in the treatment of leprosy. Slight excesses of cancers of the mouth, esophagus, liver, kidney, and bladder were observed in patients exposed to dapsone; however, a dose-reponse relationship was not apparent for most sites. Of some concern are excesses of oral and hepatobiliary cancers among patients with extended intervals since first exposure to sulfone therapy, but findings must be considered tentative until further data on drug exposure and other possible risk factors are obtained.

Significance to Biomedical Research and the Program of the Institute:

We believe epidemiologic evidence regarding the role of immune factors is particularly important in delineating causal mechanisms. Animal experiments indicate that immune mechanisms are central to tumor development in a number systems, but the relevance of these studies to humans has been debated. Insights into immunological determinants may come from observations on human populations whose immune systems have been altered in major ways. Epidemiologic studies suggest that some immune factors, while apparently not important to many tumors, influence the development of other neoplasms, particularly the lymphomas. Studies in immunoepidemiology initiated by the Branch are providing more precise estimates of the risk of developing various forms of cancer in populations of patients who are immunosuppressed on the basis of therapy, genetic traits, or acquired disorders, or who have received prolonged antigenic immunostimulation. These studies are remarkable both for the similarities and the differences in the patterns of cancer risk from group to group. It appears that a simple generalized principle of immunologic surveillance is not involved in human carcinogenesis, but the patterns of risk may provide clues to the nature of the immune factors involved in the development of lymphomas and certain other neoplasms.

Proposed Course:

1. Analyses of the studies of a variety of patient groups will be completed. These include patients with sarcoidosis, celiac disease, scleroderma, sicca syndrome, and end stage renal disease, as well as the hyperimmunized employees of Fort Detrick. The kidney transplant patients covered by the HCFA and the VA will be followed, and any excess cancer risks will be related to more specific measures of immune function and antigenic stimulation. Other patient groups with diseases that alter immune status

will be identified within the VA data base, and their risk of malignancy assessed. The populations showing excess risks of particular malignancies will be studied more intensively in a case-control manner. In these studies, clinical and laboratory measures of immune function will be related to the risk of malignancy.

2. 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.

3. 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.

Publications:

Brinton, L. A., Hoover, R., Jacobson, R. R. and Fraumeni, J. F., Jr.: Cancer mortality among patients with Hansen's disease. JNCI (In Press)

Hoover, R. and Fraumeni, J. F., Jr.: Cancer risks in renal transplant recipients. N. Engl. J. Med. (In Press)

Kantor, A. F., Hoover, R. N., Kinlen, L. J. and Fraumeni, J. F., Jr.: Cancer in patients receiving long-term dialysis treatment. JAMA (In Press)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP04410-07 EEB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies of Persons at High Risk of Cancer

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

William A. Blattner, M.D., Chief, Family Studies Section, EEB, NCI

COOPERATING UNITS *(if any)* Laboratory of Tumor Cell Biology, NCI; Medicine Branch, NCI; Clinical Epidemiology Branch, NCI; ORI, Inc.; Department of Surgery, Uniformed Services Univ. of the Health Sciences; Flow Laboratories, Inc.; Westat, Inc.; Biotech Laboratories, Inc., Laboratory of Human Carcinogenesis

LAB/BRANCH

Environmental Epidemiology Branch

SECTION

Family Studies Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
13.5	10.5	3.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☒ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

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. Mechanisms of host susceptibility are seen in DNA repair defects in familial melanoma, and in families prone to sarcomas and other rare cancers. Investigative laboratory collaborations have clarified the relationship between lifestyle, subclinical immunologic perturbations and the epidemic of Acquired Immunodeficiency Syndrome in male homosexuals and other high risk groups. Seroepidemiologic studies of the newly discovered candidate human leukemia retrovirus, HTLV, have defined patterns of virus infection and disease relationships. Studies of leukemias and other cancers as second primary malignancies due to exposures to therapeutic agents have quantified the risk of a variety of chemotherapy and radiation exposures.

PROJECT DESCRIPTION

Names, Titles, Laboratory and Institute Affiliations of Professional Personnel (other than the Principal Investigator) engaged on this Project:

M.H. Greene	Senior Clinical Investigator	EEB	NCI
M.A. Tucker	Clinical Investigator	EEB	NCI
R.J. Biggar	Senior Clinical Investigator	EEB	NCI
J.J. Goedert	Cancer Expert	EEB	NCI
D.W. Blayney	Research Associate	EEB	NCI
D.J. Tollerud	Research Associate	EEB	NCI
S. Weiss	Research Associate	EEB	NCI
J.W. Clark	Research Associate	EEB	NCI
M.C. Fraser	Nurse Epidemiologist	EEB	NCI

Objectives:

To document the occurrence of cancer in high-risk groups, and to study such groups by clinical, epidemiologic, and laboratory investigations, in an effort to elucidate genetic mechanisms and host-environmental interactions contributing to carcinogenesis. To develop educational materials and provide counseling to high-risk groups. To coordinate the distribution of tissue and blood specimens from such high risk groups to interested investigators for etiologic studies by cytogenetic, immunologic, viral, endocrine, biochemical, tissue culture, and other methods. To apply innovative analytic approaches to these studies, including statistical genetic approaches.

Methods Employed:

Protocols for study of high risk groups are developed, outlining study aims and methods, and are reviewed by section professionals to maximize efficient use of personnel and laboratory resources. Study subjects 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 questionnnaire 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 study subjects, stored in biospecimen repositories, and transmitted to collaborating laboratories. Analysis includes application of computerized genetic, as well as traditional approaches.

PROJECT 1: CLINICAL, BIOLOGICAL AND GENETIC STUDIES OF CANCER-PRONE FAMILIES

Family Studies Resources:

An integrated computerized and manual data base has been developed to maintain the registry of cancer-prone families (over 2400 in number) which forms the core resource for this project. These families comprise a non-population-based series of kindreds ascertained from NIH and extramural physicians and by self-referral of concerned family members. Designed to facilitate accurate record-keeping and easy retrieval of data, this system includes a computerized clinical information file which can be linked to biospecimen inventory and laboratory-generated data files, thus simplifying our record-keeping and permitting computer-based data analysis. Three contracts provide critical laboratory support to these studies: (a) a laboratory for the processing and storage of biological specimens (Biotech Research Laboratories); (b) a laboratory for the establishment and maintenance of fibroblast cell lines (Flow Laboratories); and (c) an immunogenetics laboratory for HLA-typing and in vitro immune function assessment (Uniformed Services University of the Health Sciences). Contract-based resources shared with the Clinical Epidemiology Branch provide laboratory support for studies of DNA repair, cytogenetics and genetic linkage. Through the NIH Cancer Nursing Service, we have obtained the services of an Epidemiologic Research Nurse. She has added an invaluable component to our clinically oriented studies, and has begun to assume responsibility for research projects of her own design.

Malignant Melanoma: Since April of 1976, we have been studying a series of melanoma-prone families, using the approach outlined above. Key findings to date include: (a) the identification and characterization of a precursor to familial melanoma, the dysplastic nevus syndrome (DNS); (b) the recognition that similar lesions underlie a significant fraction of non-familial melanomas as well; (c) demonstration that the melanoma/DNS trait in high-risk families fits a Mendelian autosomal dominant model of inheritance; (d) the suggestion that this disease susceptibility locus may be linked to the Rh gene on chromosome 1; (e) quantification of the risk of melanoma in these families; (f) demonstration that careful evaluation of high-risk family members leads to the diagnosis of a substantial number of surgically curable melanomas; (g) development and nationwide dissemination of a series of educational videotapes to make information on this subject available to physicians and high-risk family members; and (h) a nursing assessment of the impact of high-risk status and study participation on the lives of family members.

The major finding in laboratory studies to date is the identification of a significant cellular abnormality in cultured fibroblasts from melanoma/DNS patients, manifest by enhanced in vitro cytotoxicity following exposure to ultra-violet radiation and certain chemical carcinogens. These same cells have now been shown to be hypermutable following both UV and chemical carcinogen exposure, making hereditary melanoma/DNS only the second hypermutability disorder identified in man (xeroderma pigmentosum is the first).

A report of two kindreds with both melanoma of the skin and eye, plus the DNS, has been prepared; the data suggest that choroidal melanoma is not related to the DNS, as some authors have hypothesized. Section staff have collaborated with members of the Environmental Studies Section in a case-control study of intra-ocular melanoma. Analysis of these data has just begun. Families prone to melanoma plus breast cancer and to melanoma plus lymphoma are also under evaluation. To permit quantitative assessment of the risk of melanoma and other cancers in the relatives of patients with melanoma, a survey has been conducted utilizing 432 consecutive, newly diagnosed melanoma patients from the MD Anderson Hospital in which careful family histories were taken and all cancer diagnoses verified. These data, just entering analysis, should permit us to estimate the proportion of all melanoma that occurs in a familial context, as well as applying various sophisticated genetic analysis techniques to further clarify the heritability of this cancer. The recognition of a sporadic variant of the DNS has led to the need for a clearer understanding of the prevalence of these lesions in the general population. In collaboration with epidemiologists in New Zealand, a population-based survey of nevi has been undertaken. Preliminary data suggest that 8% of the 380 adults in the survey had at least one dysplastic nevus; 4% of the cohort had multiple lesions of this type.

Lymphoproliferative and Hematopoietic Cancers: A detailed study of HLA types in 8 families prone to Hodgkin's disease, 58 non-familial Hodgkin's disease patients, and 214 controls, has identified an association with the newly recognized HLA specificity designated MB-1. The association suggests a relation to prognosis rather than etiology; segregation analysis in these families failed to confirm a Mendelian mode of inheritance, and no linkage with either HLA or a limited panel of polymorphic markers was found.

An HLA association was also reported in an unusual kindred in which 3 sibs developed hairy cell leukemia. Of special interest was the presence of an undefinable DR specificity on the disease-related HLA haplotype, suggesting that a defective DR gene product may be of importance in the pathogenesis of this family's illness. A large study of HLA phenotype frequency in patients with cutaneous T-cell lymphomas is now in the specimen collection phase. Data from patients with Burkitt's lymphoma have confirmed the association between this cancer and HLA-DR7.

Clinical/laboratory studies of families prone to chronic lymphocytic leukemia (CLL) have revealed that all cases in one family share the same cell surface phenotype, including one patient who has undergone a spontaneous "remission" but still harbors this particular clone of cells. Chromosome 12 abnormalities have been documented in two of these patients. Various immunologic abnormalities have occurred in both affected and unaffected members of families prone to CLL and to non-Hodgkin's lymphoma; further studies are under way to clarify these observations and their relation to disease susceptibility. A family with two cutaneous T-cell lymphoma patients has been studied in detail, but no specific disease-related abnormalities could be identified. A survey of a series of cutaneous T-cell lymphoma patients

reporting lymphoma or leukemia in close relatives revealed an excess of Hodgkin's disease in these families compared with the general population.

Comprehensive reviews of the epidemiology of multiple myeloma, Waldenstrom's macroglobulinemia, non-Hodgkin's lymphoma, and mycosis fungoides were published. A recent study conducted with the Occupational Studies Section documented an excess of acute leukemia and some B-cell malignancies in workers exposed to benzene. An analysis of multiple myeloma mortality in the U.S. (with the Occupational and Population Studies Sections) documented ethnic and regional variations and provided clues to host and occupational factors. A death certificate study of multiple myeloma in Nebraska revealed an excess risk in various agricultural occupations. Preliminary review of data from death certificates in North Carolina suggests an increased risk of myeloma in wood-related occupations. This project was a joint undertaking with the Environmental and Analytic Studies Sections. Section staff have also played central roles in the design and implementation of EEB case-control studies of non-Hodgkin's lymphoma in Iowa and Minnesota, and cutaneous T-cell lymphomas in Philadelphia. No results are available from these two surveys.

Sarcomas: Section staff have been evaluating 25 families prone to sarcomas and diverse neoplasms including brain and neural tumors, leukemia, and breast cancer identified from the Family Studies Registry. The goal is to further characterize the spectrum of cancer types in the "Li-Fraumeni syndrome" and to assess whether a single gene model of inheritance fits the distribution of disease. Preliminary analysis revealed significant excesses of sarcomas, nervous system and lymphoreticular malignancies, and cancers of the breast, pancreas, lung and endocrine organs, compared with the general population. Prospective follow-up of the original four families confirmed a 30-fold excess of cancer, underscored that specific cancers accounted for this excess, and documented the propensity of family members to develop multiple primary cancers.

One very large kindred has been evaluated intensively; laboratory studies have led to the recognition of a new ionizing radiation repair phenotype, i.e., radioresistance. Cells cultured from affected family members were unusually resistant to the killing effects of radiation, suggesting a novel mechanism by which abnormalities in cell repair systems might be related to cancer susceptibility. This phenotype has now been observed in members of three additional families.

A young man with radiation-related breast cancer and a family history of diverse malignancies (including sarcomas) was found to have in vitro sensitivity to both ionizing radiation and bleomycin (a radiomimetic chemical), as did his sister and mother. The data suggested that the cancer susceptibility in this family might be related to unusual cellular sensitivity to environmental carcinogens.

Genitourinary Cancer: Sixteen families prone to ovarian cancer are being studied. The occurrence of disseminated intra-abdominal carcinomatosis in 3 high-risk family members who had previously undergone prophylactic oophorectomy suggested that the target organ at risk in these families

includes other derivatives of the coelomic epithelium, such as the peritoneum. A descriptive review of the cancers which have occurred in these families is under way, as is segregation analysis to test for a single gene trait.

A previously reported family prone to bladder cancer was re-studied; three new cases of bladder cancer were documented, second primary lung cancers were found in two of the earlier cases, and cigarette smoking was implicated as a risk factor for both tumor types. All 3 cases studied had a rapid acetylator phenotype, suggesting an interaction between host susceptibility and environmental carcinogens in this family.

Section staff participated in the design and conduct of a case-control survey of testicular cancer, with the Environmental and Analytic Studies Sections. This study demonstrated that first-degree relatives of men with testicular cancer were at 6 times greater risk of this cancer compared with relatives of controls. From the cases in this survey, and the Family Studies Registry, eight families prone to testicular cancer have been identified and studied. Urogenital anomalies occurred frequently in unaffected family members, particularly inguinal hernia and hydrocele, which were 3-7 times more common than in the general population. Clinical evaluation of 73 men with testicular cancer and 299 controls revealed that polymastia was associated with a relative risk of 4.5.

In an earlier case-control survey of renal adenocarcinoma, polymastia was found to be a significant risk factor. Polymastia is known to be associated with congenital anomalies of the kidney, and now appears to be a marker of increased risk to renal and testicular cancer. A population-based survey of 35 renal cancer patients with features suggesting genetic susceptibility failed to identify a consistent cytogenetic abnormality, as had been previously suggested by the familial occurrence of renal adenocarcinoma and 3:8 translocation.

Gastrointestinal Cancer: Studies of families prone to colorectal cancer, both with and without polyposis, have revealed a variety of laboratory abnormalities, including abnormal intestinal mucosa crypt cell kinetics and abnormal cytoskeletal matrix (actin cable) formation in cultured skin fibroblasts. These studies offer the prospect of identifying specific family members at high risk of colorectal cancer. Early results from a new survey indicate significant differences in colonic crypt cell labeling between patients at low risk of colon cancer (Seventh Day Adventists) and non-vegetarian controls. In another project, the prevalence of cytoskeletal abnormalities was found to increase with age in normal persons. A case-control study of colorectal cancer in rural Nebraska was designed and analyzed with members of the Environmental and Population Studies Sections. We observed increased risk among persons of Czechoslovakian ancestry and in association with high dietary fat and beer consumption. A formal genetic analysis of these data is currently under way. This represents our first effort to elucidate the extent to which familial risk factors have contributed to a geographic cluster of malignancy ascertained from the cancer maps. Twenty-six members of three Alaskan native families prone to primary

hepatocellular carcinoma have also been studied. HLA typing and peripheral blood mononuclear cell subset studies are just beginning. This study presents an opportunity to assess immunogenetic determinants of liver cancer risk in a population where hepatitis-B infection is endemic. A survey of second malignancies in patients with salivary gland cancers revealed no significant excess risk of cancer, including breast cancer as previously reported.

PROJECT 2: HUMAN T-CELL LEUKEMIA VIRUS (HTLV)

The discovery of the first human retrovirus, HTLV, has given new impetus to the hypothesis that viruses cause some human cancers. The objectives of this project are to undertake a series of epidemiologic, clinical and experimental studies aimed at defining the distribution and determinants of HTLV infection and the role of HTLV as a cause of human cancer.

HTLV is the first true human, type-C virus. Unrelated to any known animal virus, HTLV is an exogenous human retrovirus demonstrable in malignant T-cells of certain patients with T-lymphoproliferative malignancy, as well as from virally infected, clinically normal individuals. HTLV was first isolated in the Laboratory of Tumor Cell Biology (LTCB) from two patients thought to have typical cutaneous T-cell lymphoma (CTCL). Disease-oriented serologic studies by the LTCB in United States patients with mycosis fungoides (MF) failed to confirm this association. However, the results suggested a serologic association with a new disease described by the Japanese: adult T-cell leukemia/lymphoma (ATL). ATL exhibited a curious clustering in the southwestern islands of Japan, as well as a novel constellation of clinical and laboratory features. It was a leukemia of mature T-cells, with skin involvement, visceromegaly, and hypercalcemia. The Section became involved in collaborative studies with the LTCB in the spring of 1981. By applying epidemiologic principles, a broad range of clinical, etiologic, genetic and experimental studies have been implemented whose results have played a key role in focusing laboratory investigations on populations suitable for serological and molecular analysis. In addition, Section staff have provided insights into the development of assay techniques suited for seroepidemiologic surveys by quantifying quality control, assay standardization, and measures of sensitivity and specificity.

Relationship of HTLV to Human Disease:

When the Section joined this project, the relationship of HTLV to human malignancy was poorly characterized. A large sero-survey of various T-cell malignancies had proven negative except for the finding of HTLV antibodies in group of Japanese ATL patients. We recognized that the original virus-positive cases were atypical for MF, and postulated a relationship between cases with lymphosarcoma cell leukemia (LCL) of T-cells from the West Indies and Japanese ATL. These observations led to the recognition of HTLV as a marker for a distinct subset of lymphoreticular neoplasms.

A group of LCL patients was shown to be serologically and virologically associated with HTLV. Through preliminary serologic studies of a population-based sample, HTLV infection was shown to be endemic in the Caribbean area. HTLV antibody prevalence in normal individuals was 3 percent, and apparently increased with age. Women predominated in the original series of leukemia patients.

Based on these early insights, it was hypothesized that a single clinicopathologic entity, which closely resembled or was identical to ATL, was associated with HTLV. Because of the similarity of clinical features between patients from the Caribbean area with LCL from Japan with ATL, and from the first patient from whom HTLV was isolated, it seemed likely that HTLV and these similar diseases were related. Further, because the original descriptions were disease-based, a study was undertaken to assemble a series of patients, selected because of the presence of HTLV serum antibodies, and then describe common features of the disease.

A detailed evaluation of three patients with HTLV antibodies and shared clinical features formed the basis for our subsequent description of the "sentinel disease." A careful single institution review of clinical records, pathologic material, and field interviews and examinations of surviving patients, showed that 10 of 13 members of a group of patients with lymphoreticular malignancies and HTLV serum antibodies had the same clinical syndrome. This "sentinel" HTLV-associated disease was characterized by hypercalcemia, cutaneous involvement, leukemic transformation, Ann Arbor Stage IV presentation, and short survival. U.S.-born cases were blacks from the southeastern U.S., while the remainder were born in South America, Japan, Israel, and Alaska.

Three members of this serologically defined case series did not fit into the clinicopathologically defined entity of ATL. Investigation of one man, a Caucasian U.S. resident with hairy cell leukemia (leukemic reticuloendotheliosis), provided epidemiologic support for the laboratory finding that his virus isolate, dubbed "HTLV-II," was a separate strain from the original HTLV isolate. Investigation of the other two atypical cases suggested that one man acquired his infection via repeated visits to the Caribbean endemic area, while another may have been coincidentally infected because of birth in a suspect U.S. endemic region. Studies of clinical staging, natural history and treatment (conducted with physicians from the Division of Cancer Treatment), have shown the utility of HTLV antibodies as a marker identifying a subset of non-Hodgkin's lymphoma patients in whom metabolic abnormalities and unexpected sites of involvement can be anticipated. An aggressive treatment regimen is being developed to determine the utility of antibody status as a prognostic measure.

A serologic survey of 89 patients from the University Hospital of the West Indies identified 20 HTLV antibody-positive patients, of whom 19 had lymphoproliferative malignancies. Thirteen of the 19 had non-Hodgkin's lymphoma (NHL), 4 had chronic lymphocytic leukemia (CLL) and 2 had acute lymphocytic leukemia (ALL). Of an incident series of 16 NHL patients

prospectively ascertained, 11 (69%) were HTLV antibody-positive. All had stage IV diffuse lymphoma with the clinical features previously found in HTLV disease. The predominance of females (70%) in this series contrasted with the overall male predominance for lymphoreticular malignancies in Kingston, and suggested an as yet unexplained susceptibility to HTLV infection or disease in females. This survey documented the utility of HTLV antibodies as a marker for a particular subset of lymphoreticular malignancies, and indicated that HTLV may contribute substantially to risk for lymphoma and lymphocytic leukemia in some geographic areas.

Sero-surveys to evaluate the prevalence of HTLV in lymphoreticular malignancies have documented cases in South Africa, Nigeria, Israel, India, Taiwan, Columbia, and numerous centers in Japan. Most of these virus antibody-positive cases have shared features of the sentinel disease that we have helped to characterize. In addition, the prevalence of serum HTLV antibodies in several other diseases with clinical features similar to the sentinel disease have been investigated. Two patients with the newly described acquired immunodeficiency syndrome (AIDS) also had HTLV proviral sequences in their DNA. Patients with T-cell CLL, and also patients with the non-malignant T-cell disease, sarcoidosis, do not have serum antibodies to HTLV. Thus, our studies have: (a) demonstrated that the "sentinel disease" associated with HTLV antibodies in a population is ATL; (b) shown that clusters of HTLV-related ATL occur in the Caribbean, the United States, South America and elsewhere, in addition to southwestern Japan; and (c) characterized the clinical features and natural history of the disease, to alert physicians to the staging and prognostic significance of HTLV antibodies in their patients.

Distribution of HTLV Infection:

Convenience sero-surveys of specific geographic areas in Japan, the Caribbean, and the southeastern United States, have documented an elevated prevalence of HTLV antibody positivity in normal persons from the same populations from which ATL cases originate. In Japan there is a correlation between the prevalence of antibody positivity and prevalence of clinical ATL; the highest prevalence rate in our survey is 16% for the island of Kyushu. In the ATL non-endemic areas of Kanagawa, Hokkaido, and Shimame the rate is less than 1%. A survey on the island of St. Vincent, West Indies, documented a prevalence rate of 2 - 5.4% in 3 villages; 4.6% of persons over 50 years of age were HTLV antibody-positive, compared with 1.5% of younger persons. Similarly, in Japan, the prevalence of HTLV antibodies increases with age. A survey of sera from the Georgia State Department of Health revealed that 2 of 95 Georgia blacks were HTLV antibody-positive while all 32 whites were sero-negative. This is consistent with the observation that most virus-positive ATL cases detected in U.S.-born patients have been blacks from the southeastern U.S. Our data suggest that ATL clusters in geographic regions where HTLV infection is prevalent in the general population.

Preliminary results of surveys of sera from various geographic areas in Venezuela indicate a low prevalence rate (3 to 5%). These data are being used

to determine the influence of altitude, race, geographic area, and presence of arthropod-borne disease on HTLV antibody prevalence. Sera from 3 population-based surveys in Panama have shown a prevalence of over 10% in some groups. Detailed demographic data are available on the patients studied. The data suggest that socioeconomic and lifestyle factors strongly influence HTLV antibody prevalence, with highest rates in persons with subsistence income and farming occupations.

HTLV antibodies have been detected in normal persons from Singapore, Egypt, Alaska, and in non-lymphoid cancer patients from Tunisia. Burkitt lymphoma patients from Ghana have a prevalence rate of approximately 4%. Negative results have been observed in 1000 serum samples from normal persons in Germany, England, and the U.S. A survey of over 150 laboratory workers, who are a group at high risk of exposure to HTLV, has documented only one positive: a Jamaican-borne glass washer whose antibodies to HTLV antedated the discovery of HTLV and T-cell tissue culture work by over 10 years.

These studies document for the first time the prevalence of HTLV virus antibodies in populations at risk for ATL and the non-uniform distribution of virus infection in various geographic areas.

Determinants of HTLV Infection:

Systematic studies to evaluate the mode(s) of virus transmission and host-susceptibility factors are under way or are planned. Approaches involve epidemiologic studies of informative populations and interdisciplinary laboratory protocols patterned after the approach pioneered by the Section. One study focuses on the distribution and determinants of HTLV infection in families, especially in close relatives of HTLV-positive ATL cases. To date, 25 families have been ascertained. The prevalence of HTLV antibodies in close family members is 3 to 4 times greater than that seen in the corresponding normal population in each region. In several cases, cell culture of antibody-positive, clinically normal individuals has resulted in HTLV isolations. In one Japanese family, a sibling of an ATL case was shown to harbor HTLV virus in cultured lymphocytes, while detectable virus antibodies were absent. The mother of these siblings has an unexplained lymphocytosis and HTLV antibodies; HTLV antigens are expressed when her T-cells are cultured. The presence of circulating cells with morphologic features of ATL suggest that this woman has an early or pre-leukemic state. Antibody positivity has been documented in the mothers of all ATL cases tested to date. Testing for immunogenetic markers and cytogenetic abnormalities is currently under way.

The pattern of HTLV distribution is similar to that of hepatitis B. We are investigating the risk of blood-borne HTLV transmission by: (a) defining the prevalence of HTLV serum antibodies and antigen in blood bank donors; (b) establishing the rate of HTLV seroconversion in recipients of blood products from HTLV seropositive donors; and (c) attempting to establish the attack rate of lymphoreticular disease in recipients who seroconvert. Serum collection, maintained by the American Red Cross, representing blood donor populations of Birmingham, Alabama and Burlington, Vermont, is being tested. We hypothesize

that the Birmingham, Alabama sample, which contains more lower socioeconomic class and black donors, will have a higher prevalence of HTLV antibodies. To determine the rate of seroconversion after transfusion with HTLV positive blood, the resources of the Houston Center of the Transfusion Transmitted Virus (TTV) study (a prospective seroepidemiologic study initially conceived to study the transmission of hepatitis B by blood transfusion) is being utilized; 455 samples representing Houston blood donors who did not have evidence of hepatitis B have been tested, and nine had serum antibodies to HTLV, in titers ranging from 1:60 to 1:1620. This prevalence rate (2%) is within the range seen in other population samples. Testing of serial follow-up samples from recipients of blood from these seropositive donors is under way.

HLA typing of virus positive ATL cases revealed the presence of more than the expected number of HLA-A and B antigens, particularly antigens of the HLA B-5 cross-reactive group. A monoclonal antibody 4D12 (which detects an epitope of this cross-reactive antigen series) was also expressed on these malignant T-cells. The 4D12 antibody was developed against a T-cell line HUT-78, recently shown to harbor genomic DNA fragments of HTLV. Expression of 4D12 correlates with the presence of HTLV in that it is detectable in virus-positive but not negative cell lines. Studies of cord blood co-culture-infected cells document the appearance of HLA-DR, extra HLA-A and B antigens and induction or intensification of the 4D12 surface marker in association with successful viral infection. Molecular studies are now under way to evaluate whether these alterations reflect a chromosome 6 integration site or HLA molecular mimicry in the viral envelope gene. Such studies have documented homology between cloned HTLV env genes and cloned HLA genes. These findings also raise the possibility that persons expressing antigens of the B5 group may have increased susceptibility to virus infection and/or development of disease. Studies of families should help to clarify this question. These and related immunologic studies provide a model for evaluating possible etiologic mechanisms of lymphoid malignancy or diseases of altered immunity.

HTLV Computer Data Base:

Section personnel have implemented comprehensive computer-based systems in support of this project. The existing Family Studies computer system was readily adapted to HTLV studies even though the HTLV project is considerably more complex. Thus, we are now able to link existing clinical information to the biologic specimens. The inventory management system retains the ability to track individual specimens, and now incorporates features designed to facilitate receipt and handling of larger batches of serum. As of 1 March 1983, the inventory system had tracked 23,281 samples as they were received, disbursed, and tested. Data from several assays have been keyed, and statistical evaluation of their sensitivity, specificity, and reproducibility are being performed in conjunction with Biometry Branch statisticians.

Epstein-Barr Virus Studies:

A series of clinical and laboratory investigations of Burkitt's lymphoma were undertaken in 1979 and 1980 by Dr. Robert J. Biggar at the University of Ghana in Accra. Clinical review of changes in presenting features in 430 patients documented a shift away from facial presentation toward a higher proportion with abdominal disease, a pattern similar to that seen in low incidence areas. This may reflect a decrease in incidence of the classical African form of disease. The prognosis of patients between the ages of 6 and 9 was poorer than that for other ages; males and females did equally well in response to therapy. The introduction of high-dose cyclophosphamide as a consolidation therapy did not improve survival. Patients who achieve 12 months of remission, but who retain persistence of high EBV-EA antibodies, may still be at risk of relapse. Antibodies to EBV-VCA, myelin and cerebroside immune complexes, and oligoclonal antibodies, were examined in the cerebrospinal fluid of patients with Burkitt's lymphoma. Ninety-two percent of patients with CNS lymphoma had at least one of these markers compared with 10% of patients without CNS disease. This suggests the utility of those markers as an early predictor of CNS involvement. Surveys of EBV antibodies in urban and rural populations documented that EBV seroconversion occurred earlier in the rural area, and that females developed significantly higher antibody titers. HLA typing in a series of Burkitt's cases documented excesses of Dr7, A-1 and Bw44 in cases compared with controls. Cytogenetic analysis documented the occurrence of the 14 q+ marker in 67% of direct aspirate biopsy material from Burkitt's cases, and by evaluating multiple tumor sites the clonal origin of multiple tumors in the same patient was documented. This series of interdisciplinary studies provided new information on the role of host factors in the etiology of Burkitt's lymphoma, and suggested shifts in the patterns of Burkitt's lymphoma in the high incidence area toward patterns more typical of low incidence areas. A fall in the incidence of Burkitt's lymphoma is one explanation for this pattern.

PROJECT 3: ACQUIRED IMMUNODEFICIENCY SYNDROME (AIDS)

AIDS is an outbreak of highly lethal opportunistic infections and Kaposi's sarcoma that first appeared in homosexual men in New York City and California in 1979. Since that time more than 1300 AIDS cases have been diagnosed, and the number of cases has doubled in each subsequent six-month period. In addition, AIDS has been identified in other groups, including drug addicts, Haitians, and hemophiliacs.

The Section approach of interdisciplinary clinical and laboratory studies in high-risk groups was applied in order to assess risk factors affecting both the overt outcome and the subclinical immunologic changes that antedate clinical disease. The Section had in place both the epidemiologic resources and the sophisticated immunologic laboratory required to study AIDS at the national and international level, when this urgent medical problem became manifest.

Our overall strategy has been to identify the risk factors related to the occurrence of AIDS; to develop a repository of information and biological specimens for testing hypotheses regarding the etiology, early detection and prevention of AIDS; and to examine these hypotheses with relevant clinical, epidemiologic and laboratory tools by coordinating diverse resources available at NIH and elsewhere.

Rather than attempt to duplicate efforts already under way regionally in a number of university and medical centers, or to undertake the case-finding role that is the province of the Centers for Disease Control, the Section has applied strategies traditional for EEB studies. We selected study locations to permit comparisons between high, intermediate, and low risk areas, and to keep survey design and laboratory test procedures as comparable as possible. In addition, provisions for follow-up have been implemented to study the natural history and outcome of the study cohorts, as defined by various exposure measures. The analysis to date has provided substantial support for the hypothesis that AIDS is caused by a transmissible agent. This approach has also produced a unique resource of banked biological specimens on persons in different risk groups, in different localities, and at different times in the course of the outbreak.

Studies of Apparently Healthy Homosexual Men:

Our Section has pioneered the approach of identifying and evaluating cohorts of apparently healthy members of high risk groups for evidence of altered immunity. A pilot study in New York City examined the immunologic status of 15 homosexual men and related their helper-to-suppressor ratios to drug use, sexual practices, and cytomegalovirus antibody levels. These data suggested that abnormal helper/suppressor ratios were surprisingly common in these patients, and were associated with nitrite inhalant use. As a consequence, experiments with mice and monkeys were developed to test the effects of nitrites on the immune system, but we failed to confirm any change in the phenotype distribution of helper and suppressor cells in these animals.

In November 1981, 259 homosexual men in Denmark were interviewed. A cohort of 80 men classified as cases (based on nitrite use or travel to the U.S.) or matched controls who lacked these exposures, were recontacted and phlebotomized. Laboratory studies on this cohort showed that some healthy homosexual men in low risk areas also have low ratios. The strongest correlate with depressed helper/supressor ratios was having visited the United States for homosexual sex (relative risk = 7.7). Study participants having sexual contact with a known AIDS case in Denmark also had a high relative risk (relative risk = 6.9). A longitudinal follow-up involving this subgroup of 80 patients is currently in the laboratory testing phase and will focus on the question of the stability of the previously recognized laboratory abnormality.

The next study included 245 homosexual men in New York and Washington, D.C., all of whom have been characterized clinically, demographically, and immunologically. Preliminary analysis suggests that (a) low helper-to-suppressor ratios are more common in New York; (b) important differences

exist in the determinants of low ratios in high-risk (New York) and low-risk (Washington) areas; and (c) being the recipient of anal homosexual intercourse is an important risk factor for low ratios in both areas. The number of different sexual partners was thus the key risk factor in New York, but sexual contact with a man from a high-risk area was most important among residents of the low-risk area. The observed risk factors for having a low ratio are essentially the same risk factors that have been found for homosexual men with Kaposi's sarcoma. Thus, this is the first study to demonstrate that the known risk factors for AIDS are the same as for those having a low helper-to-suppressor ratio. This result helps to verify the usefulness of the ratio as a marker for AIDS.

A study of alpha-interferon levels in homosexual men revealed the presence of an unusual acid-labile variant in 63% of those with AIDS, 29% of those with lymphadenopathy, and 8% of apparently healthy male homosexual New Yorkers (see section on Hemophilia for more details). Subsequently, elevated interferon was found in 10% of the Danish homosexual men, although this did not correlate with helper-to-suppressor ratios. Assessment of other possible laboratory markers of AIDS indicated that thymosin was not useful, while levels of $beta_2$-microglobulin may hold some promise. Currently the usefulness of other biological markers is being ascertained, including HLA type and antibody against components of syphilis, hepatitis, CMV, and other biological agents.

Studies on Hemophiliacs:

In late 1982, a cohort of 50 hemophilic patients, two of whom had AIDS, were evaluated immunologically with compilation of their lifetime exposure to concentrated plasma clotting factors. Depressed helper/suppressor ratios were observed in 20% percent, and preliminary analysis demonstrated a correlation between the number of clotting factor transfusions and low ratios. This suggests a dose-response relationship to a blood-borne pathogen in the etiology of the immune dysfunction in hemophiliacs. Acid-labile alpha interferon was also evaluated as a possible marker for AIDS in hemophiliacs. Interferon was not detected in 45 healthy hemophiliacs. The assay was positive, however, in three hemophiliacs with AIDS; and in two of them this laboratory abnormality antedated the onset of clinical illness by 3-10 months. This suggests that assay of serum interferon may be of value in screening members of groups at high risk of AIDS, and that an unusual biologic agent may be inducing this unusual type of interferon in AIDS.

PROJECT 4: THE CARCINOGENICITY OF CYTOTOXIC DRUGS

Employing various strategies, this project is designed to: (1) assess and quantify the cancer risk associated with specific cytotoxic drugs; (2) seek clinically relevant differences in risk among the various agents studied; (3) determine whether cancer risk increases as a function of drug dose; (4) learn whether there is an interaction between cytotoxic drugs and therapeutic radiation in cancer risk; (5) elucidate host characteristics which might permit identifying sub-groups of patients which are unusually susceptible to

treatment-related cancers; and (6) gain insights into mechanisms of human carcinogenesis.

The use of cytotoxic drugs in the management of various diseases represents a very special circumstance in which humans are deliberately exposed to potentially toxic chemicals, many of which are known to be carcinogenic in laboratory animals. It is reasonable to use such therapy in patients with advanced malignant disease, most of whom would die without treatment. However, these drugs are now being used with increasing frequency in cancer patients with a much more favorable prognosis (in whom long-term survival can be anticipated), and in the management of various non-neoplastic conditions. Therefore, the need has arisen to clarify the late carcinogenic risks associated with the use of these compounds. Further, such studies provide a unique opportunity to explore mechanisms of carcinogenesis in human subjects. Accordingly, EEB has designed a series of studies to address these issues. Among the strategies employed are: (1) cohort studies - follow-up of patients with a particular index disease, with ascertainment of subsequent cancers and correlation of treatment for the index disorder with the risk of specific malignancies; (2) randomized cohort studies - similar to (1) except that patients studied are participants in randomized therapeutic trials; and (3) case-control studies - patients with a specific index disease and a specific subsequent cancer are compared to persons with the same index disease who have not developed a subsequent cancer, to assess the role of therapy as a cancer risk factor.

Ovarian Cancer:

A study of 1400 women with ovarian cancer who were treated in 5 randomized clinical trials was completed and reported. A substantial (RR = 109) risk of acute nonlymphocytic leukemia (ANL) was linked to the alkylating agents melphalan and chlorambucil. The addition of radiation therapy to these drugs did not increase the risk of ANL. A positive correlation between initial drug dose and risk of ANL was observed. This study provided the first quantitative data on melphalan as a human leukemogen, the first formal documentation of ANL risk in patients receiving adjuvant chemotherapy, and important support for the hypothesis that the risk of ANL is related to drug dose. A follow-up study of 2600 one-year survivors of ovarian cancer is now in analysis. Two special features of the new survey are: (a) the availability of complete chemotherapy dose data on every drug received by each patient; and (b) an opportunity to directly compare the leukemogenicity of melphalan and cyclophosphamide. Very preliminary results suggest that melphalan is significantly more leukemogenic than cyclophosphamide.

Non-Hodgkin's Lymphoma:

A study of 517 NCI patients with non-Hodgkin's lymphoma (NHL) was completed and reported. The excess cancer incidence observed in this cohort was completely attributable to ANL. A case-control study within the cohort revealed that the greatest ANL risk occurred in patients treated with combined

modality therapy (i.e., radiation plus chemotherapy), especially if total body or hemi-body radiation was employed. A positive correlation between cumulative radiation dose to the bone marrow and risk of ANL was demonstrated, the first such evidence in cancer-treatment-related radiation exposure. A chemotherapy dose-response was suggested as well. The data also indicated that NHL patients may be at increased risk of primary bronchogenic carcinoma, an association that was unrelated to therapy.

Brain Cancer:

A recent EEB study has demonstrated that the nitrosourea methyl-CCNU is a human leukemogen when used in the adjuvant treatment of patients with gastrointestinal cancer. It is now important to determine whether other nitrosourea compounds have similar toxicity. A study has just been initiated to evaluate 2200 brain cancer patients treated in 6 clinical trials conducted by the Brain Tumor Study Group. Fifteen hundred patients received a nitrosourea drug, of which 85% was BCNU. The data collection has just begun.

Gestational Trophoblastic Neoplasms:

This study was undertaken to evaluate the carcinogenicity of methotrexate and actinomycin-D, two agents for which such data in humans are sparse. A cohort of 1800 women with gestational trophoblastic neoplasms treated at 5 regional referral centers is now under active study. Data abstraction is complete on 1200 patients, and follow-up has just begun; no results are yet available. The results of this survey are of great potential importance since they will address the hypothesis (advanced on the basis of laboratory studies) that methotrexate is not carcinogenic in humans.

Childhood Cancer:

In collaboration with the Late Effects Study Group, a survey of second malignancies in 9200 persons surviving cancer in childhood has been conducted and is now in analysis. Preliminary results suggest significant excesses of: (a) acute leukemia, thyroid cancer, and osteogenic sarcoma in survivors of Hodgkin's disease; (b) bone and soft tissue tumors in survivors of retinoblastoma; (c) acute leukemia, bone, soft tissue, and thyroid cancers in survivors of Wilms' tumor; and (d) bone, soft tissue and thyroid cancers in survivors of neuroblastoma. The cumulative risk of all second cancers combined in this survey was 12% at 25 years. Detailed treatment data collected from 200 patients with second tumors and 400 controls are now being analyzed. These data will be particularly valuable in distinguishing second cancers that are treatment-related from those that are not, a special problem in childhood cancers. There is a paucity of data on late treatment effects in children which will be addressed by these data.

Laboratory Studies of Long-Term Survivors of Hodgkin's Disease:

A multi-disciplined laboratory evaluation of long-term survivors of Hodgkin's disease treated at NCI with combination chemotherapy with or without radiation

therapy has been undertaken. To date, 77 of 97 potential participants have been evaluated. In analyses conducted thus far, bone marrow histology, in vitro function, and cytogenetics have all been normal. No cases of ANL or preleukemia have been observed during the 2 years of study. Additional laboratory studies are planned, as is a case-control analysis of ANL following treatment of Hodgkin's disease at the NCI. The latter will focus on a detailed assessment of drug and radiation dose as they relate to ANL risk. This project represents our first effort to apply a laboratory approach to our epidemiologic studies of treatment-related carcinogenesis.

Miscellaneous:

A survey of subsequent malignancy among 840 patients receiving alkylating agents (primarily thio-TEPA and nitrogen mustard) for various rheumatoid diseases has documented a 45-fold excess of acute nonlymphocytic leukemia. The risk of ANL increased as the number of courses of drug therapy increased, suggesting a dose-response relationship. Unexpected excesses of pancreatic, lung, and kidney cancers were also observed. Data analysis is now under way on a cohort of patients with primary bronchogenic carcinoma treated by the Veterans Administration Surgical Oncology Group. A case-control study within the cohort of gastrointestinal cancer patients treated with adjuvant methyl-CCNU has been designed to evaluate the relationship between dose of nitrosourea therapy and risk of ANL.

Significance to Biomedical Research and the Program of the Institute:

Studies of persons and groups at high risk for cancer lead to insights concerning risk factors and mechanisms for cancer. Results are significant because high risk groups identified through these studies can be monitored for early diagnosis, educated to their risk, and where possible cancer prevented by modification of life style. These well-characterized high risk groups are suitable for participation in interdisciplinary studies where laboratory approaches can be applied to provide fundamental insights at the molecular level, concern the mechanisms of their cancer risk.

Proposed Course: Over the past 18 months the scope of Section research activities has greatly expanded, particularly in studies of known and suspected biologic agents in cancer. However, the underlying philosophy of applying in-depth multidisciplinary approaches to the investigation of these study areas continues. In each of the major program areas this research philosophy will be applied as new advances warrant.

In the Section, future emphasis will be in 2 areas: 1) the design and implementation of protocols modeled after our approach to the dysplastic nevus syndrome, which will include studies of families prone to sarcomas and ovarian cancer; and 2) the application of newer techniques in molecular biology and molecular genetics to selected families. The application of gene transfection and cloning technique to search for novel onc genes in sarcoma families, and restriction fragment genetic polymorphism analysis in melanoma- and lymphoma-prone kindreds are currently under way.

Studies of the carcinogenic risks of biologic agents are at an early stage in development. In HTLV, comprehensive interdisciplinary studies are currently being established in high-risk areas of the world. The focus of these studies will be on defining the distribution and determinants of HTLV infection and the relationship of HTLV to human malignancy.

Investigations of the AIDS syndrome will focus on in-depth epidemiologic analysis of risk factors with altered T-cell subsets as a marker of exposure. The validity of this approach will be investigated by longitudinal follow-up of previously identified cohorts, and extension to other populations at both high and low risk of AIDS.

Studies of cytotoxic agents as risk factors for second primary cancer will focus on continued collaborative studies with the Division of Cancer Treatment to evaluate various classes of agents in cohorts that are emerging, with sufficient person-years of follow-up to allow for valid risk estimates. The emphasis in these studies will be on previously unstudied classes of agents, with an attempt to quantify the interaction of various agents. The results of the prospective laboratory investigation of the Hodgkin's disease cohort will be evaluated as a model for future studies to identify host susceptibility factors and subclinical precursor lesions.

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CONTRACTS IN SUPPORT OF THIS PROJECT

BIOTECH RESEARCH LABORATORIES, INC. (NO1-CP-21007)

Title: Laboratory Support for Processing and Storage of Biological Specimens from Persons at High Risk of Cancer.

Current Annual Level: $279,509

Man Years: 3.0

Objectives: To provide specimen processing and storage for a wide variety of biological materials obtained from persons at high risk of cancer.

Major Contributions: A total of 30,365 vials of various specimen types are currently in the repository.

Proposed Course: Samples will continue to be collected and processed according to quality control and processing guidelines.

FLOW LABORATORIES, INC. (NO1-CP-21021)

Title: Biological Specimen Repository for Patients at High Risk for Cancer.

Current Annual Level: $80,000

Man Years: 3.0

Objectives: To maintain and develop a repository of skin fibroblasts and epithelial strains on persons at high risk of cancer.

Major Contributions: Over 3,000 cell strains have been collected to date.

Proposed Course: Additional samples will be collected and processed. Bulk cultures for DNA cloned analysis will be grown.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP04411-07 EEB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Cancer and Related Conditions in Domestic Animals: Epidemiologic Comparisons

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Howard M. Hayes, Staff Associate, EEB, NCI

COOPERATING UNITS *(if any)*

LAB/BRANCH

Environmental Epidemiology Branch

SECTION

Environmental Studies Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 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 *(Use standard unreduced type. Do not exceed the space provided.)*

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 investigation 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 polybrominated biphenyls; 3) morbidity among military working dogs who had considerable exposure to Agent Orange; 4) the epidemiologic features of colorectal cancer, prostatic cancer, and gastro-intestinal cancer in pet dogs; 5) a case-control study of the long-term effects of Promone and Ovaban in female dogs; and 6) a case-control study of feline hip dysplasia in purebred animals.

PROJECT DESCRIPTION

Names, Titles, Laboratory and Institute Affiliations of Professional Personnel (other than the Principal Investigator) engaged in this Project:

R.N. Hoover	Acting Chief	EEB	NCI
J.F. Fraumeni, Jr.	Associate Director	FSS	NCI
L.W. Pickle	Senior Staff Fellow	EEB	NCI
R.J. Biggar	Staff Associate	EEB	NCI
K.L. Milne	Staff Associate	EB	NIEHS

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 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. Relative risks for various factors (i.e., age, breed, sex, and various environmental variables) are calculated for the diseased animals. Other analytical techniques employed may include case-control comparisons for factors associated with disease in man. Other animals are studied whenever another resource is available (e.g., military working dog autopsy file of the Armed Forces Institute of Pathology).

Major Findings:

1. Investigation of canine biliary carcinoma showed no apparent genetic predisposition, although a sex differential was suggested. Evidence was available suggesting that past infestation with blood-letting intestinal parasites occurs more frequently in cases of cholangiocarcinoma than expected. The geographic distribution of analogous human parasites is consistent with the presently unexplained high incidence of human cholangiocarcinoma.

2. A retrospective study 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 the latter could serve as a model.

Significance to Biomedical Research and the Program of the Institute:

Canine cholangiocarcinoma appears associated with recent infestations of bloodletting intestinal nematodes (Ancylostoma caninum, Trichuris vulpis). The human counterparts of these parasites (Necator americanus, Trichuris

trichiura) infest geographic areas of the world where presently there are unexplained high incidence rates of cholangiocarcinoma. It seems possible that these parasites could migrate up the bile ducts for the same unexplained reason as does Ascaris lumbricoides in man, and cause some localized alteration eventually resulting in malignancy. 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, including that 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 epidemic 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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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP04412-07 EEB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Carcinogenic Effects of Therapeutic Drugs

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*
(Name, title, laboratory, and institute affiliation)

Robert N. Hoover, Acting Chief, 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, Boston, MA; Division of Cancer Therapy, NCI

LAB/BRANCH

Environmental Epidemiology Branch

SECTION

Environmental Studies Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	2.0	0.5

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☒ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

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, 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

Names, Titles, Laboratory and Institute Affiliations of Professional Personnel (other than the Principal Investigator) engaged on this Project:

J.F. Fraumeni, Jr.	Associate Director	FSS	NCI
J.D. Boice, Jr.	Chief, Radiation Studies Section	EEB	NCI
M.H. Greene	Clinical Investigator	EEB	NCI
L.A. Brinton	Senior Staff Fellow	EEB	NCI
M.A. Tucker	Clinical Investigator	EEB	NCI
R.A. Kleinerman	Epidemiologist	EEB	NCI
A.F. Kantor	Senior Staff Fellow	EEB	NCI
E.J. Trapido	Staff Fellow	EEB	NCI
P. Hartge	Epidemiologist	EEB	NCI
C. Schairer	Health Statistician	EEB	NCI
G. Gridley	Health Statistician	EEB	NCI

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 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 risk (see Project No. Z01CP04501-06 EEB). Recently completed analyses have evaluated the effects of thyroid medications on risk and diazepam use on progression of breast cancer. Ongoing analyses are considering the relationship of oral contraceptives and menopausal estrogens to risk of benign breast disease and of antihypertensives to breast cancer risk.

2. A retrospective cohort study of over 3,000 women treated for hyperthyroidism allowed evaluation of the effects of thyroid supplements among those who became hypothyroid or euthyroid.

3. A study of the long-term effects of the immunosuppressive drugs is described in detail in Project No. Z01CP04401-07 EEB, "Immunologic Factors in Cancer Etiology."

4. A population-based, record-abstract, case-control study of approximately 500 cases of ovarian cancer and 600 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 risk of this malignancy. The drugs being evaluated include estrogens, oral contraceptives, major tranquilizers, and other drugs affecting the pituitary-ovarian axis.

5. 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 using data from two pre-paid health plans in order to evaluate the risk of breast cancer associated with the use of replacement estrogens by women who had a bilateral oophorectomy.

6. A case-control study, recently completed in the Washington, D.C. area, obtained home interviews from 300 ovarian cancer cases diagnosed in a 36-month period in 33 hospitals and from 300 age- and race-stratified hospital controls. Extensive information collected on oral contraceptives and menopausal estrogens will allow effects to be evaluated in relation to overall risk of disease, as well as to specific histologic subtypes.

7. 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 Z01CP04501-06 EEB).

8. The feasibility of conducting a large case-control study of choriocarcinoma is currently being tested in the People's Republic of China. Of primary interest will be the assessment of oral contraceptives on development of choriocarcinoma and on promotion of hydatidiform mole to malignancy.

9. A case-control study of in situ and invasive cervical cancer has been conducted in Panama. Interviews as well as sera were obtained from 156 of 169 surviving patients and from 309 age-matched neighborhood controls. The relationship of oral contraceptives to risk is being evaluated in conjunction with information on sexual behavior of study subjects.

10. The relationship of oral contraceptive use to risk of a variety of female tumors was assessed through a population-based prospective cohort study conducted in Massachusetts. In 1969, 97,000 questionnaires were sent to currently married female residents of Boston and 14 contiguous towns, eliciting information on oral contraceptive use. Two subsequent surveys, one in 1973 and another in 1979, updated information on oral contraceptive use and medical events. Cancer incidence was determined through review of pathology logs and discharge lists of 34 area hospitals for the period 1970-1976.

11. A study of patients with Hansen's disease has recently been completed. The predominant mode of therapy of these individuals for a number of years has been the drug dapsone, which has 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. Z01CP04401-07 EEB).

12. A case-control study in four U.S. cancer registries and in Denmark has recently been completed. Approximately 500 women who developed

endometrial cancer as a second cancer following breast cancer therapy are being evaluated along with matched controls. Detailed information was collected on medical histories and estrogen exposures, allowing the risk of endometrial cancer to be evaluated in relation to cumulative estrogen use.

13. 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 analyzed. This program of studies is being done in collaboration with the Division of Cancer Treatment. 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 Gynecologic Oncology Group, the Veterans Administration Surgical Oncology Group, the Eastern Cooperative Oncology Group, the Gastrointestinal Tumor Studies Group, the Brain Tumor Study Group, and the Southwest Oncology Group. Several large individual institutions (e.g., MD Anderson Hospital, Mayo Clinic, Princess Margaret Hospital) are also collaborating in these studies. Drugs being evaluated include: thioTEPA, melphalan, methyl-CCNU, BCNU, cytoxan, chlorambucil, 5-fluorouracil, nitrogen mustard, and others.

14. A follow-up study of mortality and cancer incidence in 840 patients treated with alkylating agents (primarily thioTEPA and nitrogen mustard) for rheumatoid arthritis is currently being analyzed.

15. 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.

16. The risk of acute nonlymphocytic leukemia (ANL), acute myelodysplatic syndrome, and preleukemia was evaluated among 3600 patients with gastrointestinal cancer treated in nine randomized trials. The exposure of interest was methyl-CCNU, a nitrosourea alkylating agent.

17. A follow-up study of 1400 women treated with melphalan or chlorambucil for ovarian cancer in five randomized clinical trials has been conducted to evaluate the risk of subsequent acute nonlymphocytic leukemia and other cancers. A follow-up study of 2600 one-year survivors of ovarian cancer in two large hospitals is now in analysis. Two special features of this new survey are: (a) the availability of complete therapy dose data on every drug received by each patient and (b) an opportunity to directly compare the leukemogenicity of melphalan and cyclophosphamide (cytoxan).

18. A study of approximately 1800 women treated for gestational trophoblastic diseases at 5 regional referral centers is under way to evaluate the carcinogenicity of methotrexate and actinomycin-D, two agents for which such data in humans are sparse.

19. A study has been initiated to evaluate the toxicity of nitrosourea compounds among 2200 brain cancer patients treated in 6 clinical trials conducted by the Brain Tumor Study Group. Fifteen hundred patients received a nitrosourea drug, of which 85% was BCNU.

20. A multi-disciplinary laboratory evaluation of long-term survivors of Hodgkin's disease treated at NCI with combination chemotherapy with or without radiation therapy has been undertaken. In addition to follow-up for cancer incidence, data are being obtained to assess bone marrow histology, in vitro function, and cytogenetics.

21. A case-control study of 200 children with second malignant neoplasms, and 400 controls evaluating the relationship between the therapy they received for their first malignant neoplasm and the development of their second, is currently under analysis. These children were treated with a wide range of chemotherapy agents.

22. 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.

Major Findings:

1. 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. In addition, there was no indication of increasing risk with years of use or years since initial use, despite slight excess risks among users of high-dose preparations. However, nonsignificant excess risks associated with pill use were seen among premenopausal women who reported a family history of breast cancer in a sister or previous biopsies for benign breast disease.

2. Further analysis of data from the Breast Cancer Detection Demonstration Project showed no evidence that diazepam promotes or accelerates breast cancer growth. In addition, the study provided an opportunity to evaluate the relationship of thyroid supplements to risk of breast cancer. Neither women who used thyroid hormones for treatment of thyroid disease nor those who used them for other reasons (primarily weight loss or fertility problems) were at a significant risk of breast cancer. Women with untreated hypothyroidism or goiter, however, appeared to be at a significantly reduced risk.

3. Another study that evaluated the promoting effects of thyroid supplements on breast cancer risk found no increased risk among 1600 women treated for

hypothyroidism. No breast cancer excess was observed among women who remained euthyroid, and the risk was not influenced by type of drug, duration of use, or dose, nor was there any increased risk among nulliparous women who were long term users of thyroid preparations, a group previously reported at elevated risk.

4. 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. Z01CP04401-07 EEB for a more complete description.)

5. Analysis of data from the Massachusetts follow-up study of oral contraceptive users showed no overall increase in endometrial cancer, and the risk did not vary consistently by length of use, years since first use, or age at initial use. Rates of hospitalization for cervical and ovarian cancer were similar among oral contraceptive users and nonusers, with rate ratios being 0.9 and 1.2, respectively.

6. Evaluation of the effects of dapsone in patients with Hansen's disease provided no evidence for a generalized carcinogenic effect. Of some concern was an elevation of hepatobiliary cancers among patients with extended intervals since first exposure, but this finding must be considered tentative until further data on drug exposure and other possible risk factors are obtained.

7. Preliminary analysis of data from the case-control study of breast cancer patients who developed endometrial cancer indicated a significant risk associated with estrogen therapy. All known risk factors for endometrial cancer were also observed.

8. The survey of second malignancies among patients receiving alkylating agents for rheumatoid diseases has documented a 45-fold excess of acute nonlymphocytic leukemia. The risk of ANL increased as the number of courses of drug therapy increased, suggesting a dose response relationship. Unexpected excesses of pancreatic, lung, and kidney cancers were also observed.

9. Analysis of the follow-up of 517 patients treated for non-Hodgkin's lymphoma (NHL) at the NCI showed an excess of acute non-lymphocytic leukemia (ANL). A case-control study within the cohort revealed the greatest ANL risk in patients treated with combined modality therapy (i.e., radiation plus chemotherapy), especially if total body or hemi-body radiation was employed. A positive correlation between cumulative radiation to the bone marrow and risk of ANL was demonstrated, the first such evidence in cancer treatment-related radiation exposure. A chemotherapy dose-response was suggested as well. The data also indicated that NHL patients may be at an increased risk of bronchogenic carcinoma, an association that was unrelated to therapy.

10. Analysis of data from the follow-up study of patients with gastrointestinal cancer showed that those given methyl-CCNU as adjuvant therapy were at a 12-fold excess risk of developing leukemia. This study provided the first quantitative evidence that nitrosoureas are leukemogenic in man, and confirms previous observations that adjuvant chemotherapy with alkylating agents may increase the risk of leukemia. An intrinsic predisposition to leukemia was ruled out in an evaluation of over 44,000 patients treated in Connecticut for gastrointestinal cancer between 1935-1974, i.e. before the advent of nitrosourea chemotherapy.

11. The study of women treated with melphalan or chlorambucil for ovarian cancer in five randomized clinical trials found a very high risk of ANL that apparently followed a dose-response relationship. The risk of ANL among patients who were treated with chemotherapy alone was indistinguishable from that observed in patients receiving both radiation and chemotherapy. The seven-year cumulative risk of ANL was 9.6%. Preliminary analysis of the follow-up data on 2600 one-year survivors suggests that melphalan is significantly more leukemogenic than cyclophosphamide.

12. In the study of low dose adjuvant chemotherapy in colorectal cancer, patients treated with thio-TEPA and FUDR were not found to be at increased risk of cancer.

13. Preliminary analysis of 77 of 97 potential participants in the study of patients extensively evaluated following a diagnosis of Hodgkin's disease shows bone marrow, in vitro function, and cytogenetics to be normal. No cases of ANL or preleukemia have been observed during the two years of study.

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 humans 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 safety of treatment of various malignancies with these agents.

Proposed Course:

1. Another evaluation of the relationship between a number of drugs and the risk of breast cancer and benign breast disease will be undertaken using the data from the case-control interview study done in conjunction with the Breast Cancer Detection Demonstration Projects (see Project No. Z01CP04501-06 EEB for a more complete description), and through the study of breast cancers in oophorectomized women in pre-paid health plans.

2. The studies of ovarian cancers in pre-paid health plans and in Washington, D.C. will be analyzed, and potential for other drug evaluations in the health plans will be assessed.

3. 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. Cohort studies currently being considered include women with breast cancer treated with adjuvant chemotherapy and men with testicular cancer treated with combination chemotherapy. These efforts will be supplemented, where appropriate, by 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.

4. Analyses will be done among non-cancer patient groups who have received substantial amounts of immunosuppressive or cancer chemotherapeutic drugs, and their cancer risks evaluated where possible.

5. The analyses of the mentioned ovarian cancer trials and the extended patient series will be completed. 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:

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP04480-07 EEB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies of Occupational Cancer

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Aaron Blair, Ph.D., Chief, Occupational Studies Section, 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 Admin., New Jersey Dept. of Health, Univ. of Louisiana, Univ. of Pennsylvania

LAB/BRANCH

Environmental Epidemiology Branch

SECTION

Occupational Studies Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
13.7	10.7	3.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Epidemiologic studies of occupational groups are conducted to identify and clarify the role of environmental factors in the origin of cancer. Workers often experience heavy and prolonged exposure to environmental agents. Potentials for heavy exposure, coupled with the availability of work history records, make occupational groups invaluable for epidemiologic investigations of cancer, and studies of numerous worker groups are under investigation. Completed during the past year were studies of 1) pesticide applicators revealing an excess of lung cancer that rose with the number of years employed; 2) veterinarians with an excess mortality from leukemia, particularly among those practicing during the period when radiologic safety procedures were lax; 3) chemical workers exposed to benzene who had an excess of lymphatic and hematopoietic cancer; 4) professional artists exposed to paints and solvents, revealing high frequencies of deaths from cancers of the bladder, kidney, brain, colon, prostate, and breast; 5) pottery workers exposed to talc and other mineral dusts who experienced an unusual mortality from cancers of the skin, kidney, and brain; 6) professional photographers likely to have contact with developing chemicals who had an increased frequency of deaths from pancreatic cancer; 7) shoeworkers with increased frequencies of cancers of the colon, liver, and gallbladder; 8) workers in the rubber industry where a case-control study found no excess of brain cancer; and 9) iron hematite miners where there were excesses for cancer of the stomach and lung. Other investigations under way include proportionate mortality studies of plumbers, foresters, and tobacco workers; cohort mortality studies of formaldehyde workers, anatomists, dry cleaners, furniture workers, shipyard workers, aircraft mechanics, potters, and chemists; and case-control studies of leukemia, lymphoma, soft-tissue sarcoma, mesothelioma, colon polyps, and brain cancer.

PROJECT DESCRIPTION

Names, Titles, Laboratory and Institute Affiliations of Professional Personnel (other than the Principal Investigator) Engaged on the Project:

M. Alavanja	Health Scientist Administrator	SRB	DRG
W. Blattner	Clinical Investigator	EEB	NCI
K. Cantor	Epidemiologist	EEB	NCI
J. Fraumeni	Associate Director	FS&S	NCI
D. Grauman	Computer Systems Analyst	EEB	NCI
M. Heid	Technical Information Specialist	EEB	NCI
S. Hoar	Epidemiologist	EEB	NCI
R. Hoover	Acting Chief	EEB	NCI
J. Lubin	Health Statistician	EEB	NCI
B. Miller	Epidemiologist	EEB	NCI
J. Sontag	Staff Scientist	OD	NCI
R. Spirtas	Health Statistician	EEB	NCI
P. Stewart	Industrial Hygienist	EEB	NCI
T. Thomas	Epidemiologist	EEB	NCI
J. Walrath	Epidemiologist	EEB	NCI
D. Winn	Epidemiologist	EEB	NCI

Objectives:

To identify and evaluate groups at high risk of developing cancer because of contact with carcinogenic materials in the work environment. To develop methods and resources to further research opportunities in the area of occupational epidemiology.

Methods Employed:

Cancer patterns are determined through long-term follow-up of persons employed in specific plants, industries, and occupations. Records from companies, unions, professional organizations, state health departments, and tumor registries are used to identify exposed populations. 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 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:

1. 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.

2. An occupation and exposure linkage system has been developed to facilitate categorization of study subjects by exposure. Less sophisticated classification systems based on occupation and industry currently available may misclassify subjects and reduce or obscure associations between specific exposures and disease.

3. Analysis of cause of death among professional artists revealed an excess of leukemia and cancers of the bladder, kidney, brain, colon, rectum, and prostate among men. 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 women (particularly women painters), higher than expected proportions of cancers of the colon, 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.

4. 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.

5. 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 during the late 1950s and early 1960s.

6. 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.

7. Irritant effects of formaldehyde are well known and recent laboratory experiments raised serious concerns regarding its carcinogenicity. A proportionate mortality study of embalmers was undertaken to evaluate potential hazards among humans. Increased frequencies of death from cancer of the skin, kidney, and brain were observed, particularly among those licensed only as embalmers. 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.

8. 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.

9. A review of findings from epidemiologic studies of cancer among farmers completed at NCI and elsewhere indicated that, despite a generally low overall mortality, farmers appear to experience excessive mortality from leukemia and cancers of the brain, stomach, prostate, and skin.

10. A cohort mortality study of structural pest control workers uncovered excess deaths from leukemia and cancers of the brain and lung. The risk of lung cancer rose with number of years licensed and was greater among those first licensed at younger ages. Although information on tobacco use was not available, the absence of excess mortality from other tobacco-related causes of death and the rising risk with number of years licensed makes it unlikely that smoking would entirely account for the lung cancer excess.

11. In a study of mortality among members of two shoe worker unions, elevated frequencies of cancer of the liver and gallbladder occurred among men and women in one union and increased mortality from cancer of the rectum among men and women in both unions.

12. Professional photographers may come in contact with various photographic developing chemicals during their career. A mortality study uncovered increased relative frequencies of cancer of the pancreas among professional photographers.

13. The pattern of causes of death among tobacco workers revealed no striking elevations, although more deaths from colon cancer occurred than expected among men and women, whites and blacks.

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 are also extremely valuable in evaluating possible hazards to the general population because workplace exposures are often heavier and more well-defined than exposures in the non-occupational environment. Studies of occupational groups also provide information on the mechanism of chemical and physical carcinogens.

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 where further research is needed to identify more accurately carcinogenic agents associated with the workplace and to better quantify the cancer risks. Special attention will be focused on the use of biochemical measurements to quantify effective exposures and to assess biological damage.

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CONTRACTS IN SUPPORT OF THIS PROJECT

ABT ASSOCIATES (NO1-CP-11019)

Title: Support Services for a Case-Control Study of Brain Cancer and Occupation.

Current Annual level: $100,000

Man Years: 4.0

Objectives: To provide 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.

Major Contributions: This project has been under way for an insufficient period of time for a significant report of results. Approximately 250 interviews have been completed.

Proposed Course: Continue the project as planned.

GENERAL SERVICES ADMINISTRATION (Y01-CP-20514)

Title: Retrieval of Civilian Personnel Records.

Current Annual Level: $45,000

Man Years: 1.0

Objectives: To review civilian personnel records of individuals formerly employed as airplane maintenance workers at Hill Air Force Base, Ogden, Utah.

Major Contributions: This Interagency Agreement provides NCI with access to Official Personnel Folders which contain very detailed occupational histories of the study subjects.

Proposed Course: Expiration of the present agreement will occur on September 30, 1983. The Project Plan for this effort has been approved through 1984, and it is anticipated that the work will continue according to the methodology outlined above.

GENERAL SERVICES ADMINISTRATION (Y01-CP-20515)

Title: Retrieval of Military Personnel and Health Records and Civilian Personnel Records.

Current Annual Level: $45,000

Man Years: 0.67

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.

Major Contributions: This Interagency Agreement provides NCI with access to Official Personnel Folders which contain very detailed occupational histories of the study subjects.

Proposed Course: Expiration of the present agreement will occur September 30, 1983. The Project Plan for this effort has been approved through 1984, and it is anticipated that the work will continue according to the methodology outlined above for items (1) and (3). Data retrieval for item (2) was completed during the past fiscal year.

KANSAS, UNIVERSITY OF (N01-CP-01027)

Title: A Case-Control Study of Lymphoma and Soft-tissue Sarcoma: Association with Herbicide Exposure.

Current Annual Level: $223,000

Man Years: 5.0

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 carcinogenic risk of herbicide exposure among farmers in Kansas.

Major Contributions: Cases and controls have been selected, and interviewing is underway. Field work will be completed by September 1983.

Proposed Course: The contract is scheduled to expire September 29, 1983. Delay in obtaining some data may require a three-month, no-cost extension of this contract.

SOCIAL SECURITY ADMINISTRATION (Y01-CP-10502)

Title: Determination of Vital Status and Personal Data of Epidemiological Study Cohorts.

Current Annual Level: $46,199

Man Years: 1.0

Objectives: To determine the vital status and obtain such demographic information as sex, race, and date of birth for members of epidemiology study groups.

Major Contributions: This Interagency Agreement provides the critical information needed for each retrospective cohort mortality study, namely the vital status of members of the study groups. 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 will occur on September 30, 1983. 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.

WESTAT, INC. (N01-CP-01020)

Title: Mortality among Airplane Maintenance Workers.

Current Annual Level: $416,000. (Funds are provided by the Department of Defense through an Interagency Agreement.)

Man Years: 4.0

Objectives: To compare the mortality experience of workers exposed to solvents and other hazardous substances with that of internal and external control groups.

Major Contributions: Results are not yet available. Data collection is under way. Additional time is necessary to produce a significant report.

Proposed Course: The level of effort must be increased because of the difficulty in estimating historical exposures.

WESTAT, INC. (NO1-CP-11006)

Title: Support Services for a Mortality Study of Workers Exposed to Formaldehyde.

Current Annual Level: $284,898

Man Years: 4.0

Objectives: To provide technical and managerial support for data collection for a cohort mortality study of formaldehyde-exposed workers.

Major Contributions: Results are not yet available. The cohort has been assembled and work histories have been abstracted. 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. Because of unexpected field costs, an additional $150,000 has been requested for completion of this contract.

WESTAT, INC. (NO1-CP-91034)

Title: Support Services for Occupational Studies.

Current Annual Level: $71,537

Man Years: 2.0

Objectives: To provide technical, managerial, and clerical support for occupational studies conducted by the Environmental Epidemiology Branch.

Major Contributions: This contract has provided support for some 25 different projects. Contributions were made to items 1, 3, 5, 8, 10, 11, and 12 under Major Findings for this Intramural Research Project.

Proposed Course: Expiration date for this contract is September 29, 1983.

WESTAT, INC. (NO1-CP-21009)

Title: Support Services for Occupational Studies.

Current Annual Level: $861,000

Man Years: 20.0

Objectives: To provide technical, managerial, and clerical support for occupational studies by the Environmental Epidemiology Branch. This contract replaces NO1-CP-91034, which expires September 29, 1983.

Major Contributions: This contract provides support for some 25 different projects. Contributions were made to items 1, 3, 5, 8, 10, 11, and 12 under Major Findings for this Intramural Research Project.

Proposed Course: This contract is scheduled to run until September 30, 1985.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP04481-07 EEB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies of Radiation-Induced Cancer

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

John D. Boice, Jr., Chief, Radiation Studies Section, EEB, NCI

COOPERATING UNITS *(if any)*

Clinical Epidemiology Branch, NCI; Radiation Effects Research Foundation, Japan; National Center for Devices and Radiological Health, FDA; Medical Follow-up Agency, NAS; Department of Energy; IARC, France

LAB/BRANCH

Environmental Epidemiology Branch

SECTION

Radiation Studies Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 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 *(Use standard unreduced type. Do not exceed the space provided.)*

This project (1) examines cancer incidence and mortality among populations exposed to ionizing and non-ionizing radiation, especially at low dose levels; (2) characterizes 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 (3) examines, tests, and formulates models of radiation carcinogenesis to help define basic mechanisms. Group studies include the Japanese A-bomb survivors, and several large populations with documented therapeutic (e.g., cervical cancer patients), diagnostic (e.g., tuberculosis patients), and occupational (e.g., x-ray technologists) exposures to ionizing radiation. Program members serve on committees advising the Government as well as international agencies.

Results of studies suggest that: (1) the risk of radiogenic cancer remains throughout life; (2) large doses to limited volumes of some sites, such as the rectum, may induce cancer; (3) ovarian damage caused by radiation may lower breast cancer risk, even among post-menopausal women; (4) repeated relatively low radiation doses pose some future risk of breast cancer; (5) susceptibility to radiogenic breast cancer declines with increasing age at exposure, and children under age 10 are at high risk; (6) children irradiated for benign conditions of the head and neck are at high risk of developing thyroid neoplasia; (7) chromosome aberrations following partial-body irradiation persist in circulating lymphocytes for over 40 years; (8) prenatal x-ray in twins increases the risk of childhood cancer; (9) the risk of developing a second cancer following treatment for a childhood cancer reaches 12% after 25 years; (10) women treated with high-dose radioactive iodine may not be at increased risk of dying from cancer; (11) childhood cancer mortality in Utah may not be associated with radioactive fallout from nuclear weapons testing.

PROJECT DESCRIPTION

Names, Titles, Laboratory and Institute Affiliations of Professional Personnel (other than the Principal Investigator) engaged on this Project:

Charles E. Land	Health Statistician	EEB	NCI
Gilbert W. Beebe	Health Statistician	CEB	NCI
Daniel A. Hoffman	Epidemiologist	EEB	NCI
Ruth A. Kleinerman	Epidemiologist	EEB	NCI
Elizabeth B. Harvey	Epidemiologist	EEB	NCI
Stella G. Machado	Expert Statistician	EEB	NCI
Katherine W. Chen	Computer Programmer	EEB	NCI
William J. Blot	Chief, Analytical Studies Section	EEB	NCI
Linda M. Pottern	Epidemiologist	EEB	NCI
Mark H. Greene	Clinical Investigator	EEB	NCI
Margaret A. Tucker	Clinical Investigator	EEB	NCI
Rochelle E. Curtis	Statistician	BB	NCI
Joseph Scotto	Demographer	BB	NCI
T. Ishimaru	Visiting Scientist	EEB	NCI
Marianne Ewertz	Visiting Scientist	EEB	NCI

Objectives:

(1) To plan and conduct studies to identify and quantify the risk of cancer in populations exposed to ionizing radiation (e.g., x rays) and nonionizing radiation (e.g., ultraviolet light); (2) to evaluate the influence of physical factors, such as size or fractionation of dose, and interactions with biological modifiers of response (e.g., age) and environmental co-factors, upon the risk of radiogenic cancer; (3) to examine possible analogs of radiation carcinogenesis in man, such as the induction of cytogenetic abnormalities in circulating lymphocytes, and to test and formulate models of radiation carcinogenesis to help define basic mechanisms of cancer induction; and (4) to advise and collaborate with other Government agencies involved in radiation research and the health and safety of activities involving radiation exposure.

Methods Employed:

The relationship between cancer risk and radiation is an especially promising area for epidemiologic research because quantitative descriptions of exposure are usually straightforward. As Doll has put it, "studies of the quantitative relationships between dose and effect, of the conditions which modify the effect of a specific exposure and of the time relations between duration of exposure, intensity of exposure, length of induction period and the rate of progress of the clinical disease will enable the epidemiologist to take part in formulating and testing hypotheses about the mechanisms by which cancer is produced" (Acta Un. Int. Cancer 20: 747, 1964). The proceedings (in press) of a multi-disciplinary conference organized in 1982 focus on insights provided from such studies. The program of radiation studies is summarized in four

project areas: Medical Exposures, Atomic Bomb Survivors, Occupational and Environmental Exposures, and Methodologic Studies.

1. Medical Exposures. Studies of exposed patients are conducted to strengthen the quantitative basis for risk estimation, especially at low doses, to improve understanding of the role of host and environmental factors on radiogenic cancer risk, and to provide insights into carcinogenic mechanisms. Studies of populations exposed to medical irradiation have great potential for quantifying late radiation effects because (1) exposures can usually be accurately estimated, (2) nonexposed patients are often available for comparison, (3) useful information on other risk factors can frequently be obtained from existing records, and (4) medical facilities often follow patients for long periods of time after treatment. Radiation studies may be a particularly useful approach to understand the mechanism by which cancer is produced since quantitative descriptions of exposure are usually straightforward, an advantage not available for most other carcinogens. For certain relatively insensitive tissues, the only evidence that a cancer can be induced by ionizing radiation comes from patient populations given high-dose, partial-body, therapeutic irradiation. For other sites the best evidence on low-dose risk comes from populations given multiple, low-dose, diagnostic irradiation resulting in high cumulative exposures. The radiation studies program tries to assure that maximum benefit is derived from existing epidemiologic resources, and attempts to initiate studies of populations not previously evaluated, but which offer unusual potential for new information. Fifteen medically irradiated populations are currently under study: cervical cancer patients, children with lymphoid hyperplasia, children with cancer, children with tinea capitis, thyroid cancer patients, women with benign gynecologic disorders, women with breast cancer, men with testis cancer, tuberculosis patients, twins, patients with leukemia and lymphoma, scoliosis patients, thyrotoxicosis patients, and women with endometrial cancer.

2. Atomic Bomb Survivors. Studies are conducted to investigate cancer risk in the survivors of the Hiroshima and Nagasaki atomic bomb explosions in order (1) to clarify the relationships between risk and radiation dose in terms of dose response, temporal distribution of risk following exposure, dependence on sex, age at exposure, and other host factors, variation by organ site and histological classification, and interactions with risk factors other than radiation; (2) to strengthen the data base by supporting efforts to improve coverage of the tumor and tissue registries in the two cities, and, through formal pathology reviews, to improve accuracy of diagnosis; and (3) to extend the value of the data base by investigating cancer risk factors other than radiation. Investigations based on the Life-Span Study (LSS) sample of 82,000 A-bomb survivors plus 26,000 non-exposed residents are carried out at the Radiation Effects Research Foundation (RERF) in Hiroshima and Nagasaki. The general philosophy of the program is to take advantage of the unique RERF resources to investigate interesting research questions as they arise. A typical pattern is as follows: an incidence survey for a particular cancer site is conducted using all locally available sources of diagnostic information. Attempts may be made to ascertain non-fatal cases occurring outside the two cities or, at least, to adjust risk estimates for migration.

Diagnostic information is carefully reviewed, including examination of histological materials if available, by a local investigator, either from RERF or another organization, usually a university medical school. A formal pathology review, by a specially constituted panel, may be performed to resolve difficult questions of diagnosis, or to provide more detailed information on histology. Statistical analyses are performed to examine questions of ascertainment bias, and to estimate risk as a function of radiation dose, age at exposure, and time after exposure. A separate pathology study may be initiated to examine the possibility that precancerous lesions may be more prevalent in autopsy cases without clinical evidence of cancer. Cases and matched controls may be investigated for risk factors other than radiation exposure in a separate interview study. Because the population-based incidence study contains all the information with respect to dose response that could possibly be obtained from a case-control study based on the LSS sample, cases and controls can be matched with respect to radiation dose as well as age at exposure, city, and sex, in order to maximize statistical power for the investigation of possible interactions between radiation and other factors. Finally, once the incidence study has been completed, the A-bomb data may be combined with basic data from other irradiated populations in parallel analyses using identical strata for dose, age at exposure, and time after exposure. The LSS sample is large, has a wide dose distribution and a natural age distribution, and has been followed uniformly since 1950. Moreover, exposure occurred independently of any existing disease. Data from the LSS sample, therefore, can serve as a bridge by which inferences from other, less general, data sets can be extrapolated beyond their more restricted exposure ages, dose levels, lengths of follow-up, and circumstances of exposure.

3. Occupational and Environmental Exposures. The objectives of this project area are to evaluate the long-term effects of chronic exposure to radiation as a consequence of occupational or environmental exposures, and to collaborate with other Government agencies involved in radiation research. Although the possibility of increased cancer risk associated with chronic occupational exposure to low-LET radiation is of concern both for public health and radiation standard-setting, the only valuable quantitative information available to estimate this risk is derived from populations with acute and largely high-dose exposures. These estimates are subject to uncertainties associated with the assumed shape of the dose-response function used for downward extrapolation of risk. The existence since 1926 of a professional registry of about 170,000 medical x-ray technologists offers a unique possibility for studying a large and well-defined population occupationally exposed to highly fractionated low-LET radiation. The registry offers the possibility of studying the two most sensitive organ sites for radiation carcinogenesis in women, the breast and the thyroid, at the level of incidence in a population with at least some exposure at particularly vulnerable ages. Because other Government agencies are sponsoring large-scale investigations of occupationally exposed workers, this area has not received much attention from our program over the years. Section members, however, have been called upon to assist in the evaluation of controversial studies, such as the studies on the effects of fallout from nuclear weapons tests in the western United States.

4. Methodologic Studies. This project area focuses on methods for increasing the information from existing bodies of data, and for treating difficult analytic problems that arise during the course of other studies. A particular approach involves simultaneous reanalyses in parallel of basic data from published studies of cancer risk in different irradiated populations, in collaboration with the original authors of these studies.

5. Consultant activities and services on expert committees. The Section members 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 Three Mile Island Public Health Advisory Group, the International Commission on Radiation Protection, and the World Health Organization.

6. Review papers. Several review papers concerning health effects following exposure to ionizing radiation were written, including a review of cancer following medical irradiation, the epidemiology of radiogenic cancer of the digestive tract and other organs, the statistical aspects of estimating cancer risks from low doses of ionizing radiation, the importance of latent period, the effects of fetal irradiation, the long-term effects of radiation upon children, and the risk of cancer following treatment with radioactive iodine.

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 implications of radiation studies to models of carcinogenesis were stressed, and the proceedings are in press.

Major Findings:

1. Medical Exposures. An international study of 200,000 cervical cancer patients treated with radiation and/or surgery suggests that the risk of radiogenic cancer remains throughout life; exposures at young ages may carry the greatest relative risk, but exposures at both young and old ages result in high absolute risks; large doses to limited volumes of some sites, such as the rectum and bladder, may induce cancer; leukemia may be associated with non-sterilizing low doses received by bone marrow outside the pelvis; and ovarian damage caused by radiation may lower breast cancer risk, even among post-menopausal women. A study of women who received multiple chest fluoroscopies during pneumothorax treatment of tuberculosis indicates that repeated relatively low radiation doses pose some future risk of breast cancer, that the risk may be cumulative, that adolescence is an especially sensitive age, and that older women may be at low risk for induction of radiogenic breast cancer. Lung cancer and leukemia, however, do not appear increased. Children irradiated for benign conditions of the head and neck were found to be at high risk of developing thyroid neoplasia in several studies. Radiotherapy for ringworm of the scalp also increased the risk of brain malignancies. A study

of children irradiated for enlarged tonsils includes physical examinations, blood studies, and chromosome evaluations; preliminary analyses indicate a significant increase in chromosome aberrations in circulating lymphocytes. An increased risk of childhood malignancies was confirmed in twins exposed to prenatal x-ray. In an international study, the cumulative 25-year risk of developing a second malignancy following treatment of childhood cancer was found to be approximately 12%. Women treated with high doses of radioactive iodine for hyperthyroidism were not found to be at increased risk of dying from cancer, although a slight excess of cancer of organs with high 131-I exposure was suggested. The medical exposure studies are supported by a comprehensive dosimetry program.

Therapeutic: The International Radiation Study of Cervical Cancer is a program of studies designed to provide new insights into radiation carcinogenesis and to increase the precision of current risk estimates. These investigations include cohort studies in cancer registries and individual clinics, case-control studies, dosimetry studies, chromosome studies, and pathology evaluations. The program evolved from a WHO-sponsored investigation of 30,000 women treated for cancer of the cervix uteri in nine different countries and clinically evaluated from 1960-1970. The follow-up of most of this population is being extended to the present. However, to obtain a sample large enough to measure the effects of relatively low-dose radiation received by organs distant from the site of primary irradiation, the program has been expanded through the collaboration of 15 population-based cancer registries. Approximately 200,000 women with cervical neoplasia are being studied. The cancer registry cohort studies have been completed, and detailed case-control studies are being conducted to provide radiation dose estimates on individuals and to evaluate dose response. Thus far, preliminary cancer registry studies suggest that (1) heavily and moderately irradiated sites taken together, i.e., those likely to have received over 100 rads of radiation, show a consistent pattern of increased risk with time after exposure that is probably radiation related; (2) 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; (3) 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; (4) substantial radiation doses to the stomach, colon, liver, and gallbladder do not appear to increase risk beyond normal expectation; (5) the radiation regimens used to treat cervical cancer are not as effective in inducing leukemia as are other radiation exposures that have been studied, but a slight risk may be associated with the low-dose radiation received by bone marrow outside the pelvis; (6) radiation effects on the ovary may lower breast cancer risk at all ages of exposure, even in post-menopausal women; (7) a small excess of thyroid cancer might be associated with relatively low-dose exposure; and (8) second cancers of other sites that received relatively low doses of radiation are either not increased beyond expectation or are probably elevated due to exposures to other strong risk factors such as cigarettes or alcohol.

Several studies of childhood irradiation are being conducted. The minimal confounding effect of other carcinogenic influences, such as smoking or occupation, and the possible greater susceptibility of young people to environmental carcinogens, enhances the chances of detecting increased risks due to therapy. The study of 3,000 children treated for enlarged tonsils with radiation or surgery has continued in Boston. Physical examinations are being performed on both irradiated and surgical patients to more accurately determine the risk of thyroid nodules and to account for the potential detection bias in previous studies where only radiation-exposed persons were screened. Preliminary results suggest a two-fold risk of thyroid nodules among exposed persons. Blood studies include the evaluation of serum calcium levels and plasma thyroglobulin concentrations. Chromosome aberrations in circulating lymphocytes are also being investigated to assess exposure more accurately and to evaluate the effect of radiation in causing long-term damage in somatic cells from partial-body exposures. Preliminary results indicate a statistically significant elevation of chromosome aberrations, i.e., translocations, dicentrics and rings, among the exposed.

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.

A population-based case-control study of thyroid cancer in Connecticut indicated a high risk associated with radiotherapy for benign head and neck diseases when exposure occurred under age 10. No risk was found among persons diagnosed with thyroid cancer under age 35 in 1978, a finding consistent with the declining use of radiotherapy for benign conditions in the 1950s. A collaborative study in Israel to evaluate the risk of cancer in 10,000 children exposed to x-rays during the treatment of ringworm of the scalp, and in 15,000 comparison persons, found a high risk of thyroid cancer associated with low-dose exposures, as well as a risk of brain cancer and other head and neck malignancies. Over 9,000 persons who survived at least two years after a diagnosis of childhood cancer were found to be at high risk of developing a second cancer, reaching a cumulative risk of 12% by 25 years. The development of several second cancers, especially osteosarcoma and thyroid cancers, appeared to be associated with radiation therapy, and an on-going case-control study will evaluate the extent of this association. While sex did not appear to influence subsequent risk of developing second malignancies in these children, age at therapy did. Adolescence appeared to be a time of increased susceptibility to radiogenic osteosarcoma, possibly because of the active bone growth during this period; whereas, young children appeared especially prone to radiogenic thyroid cancer.

The Surveillance, Epidemiology, and End Results (SEER) registries were used to identify second primary cancers in persons with cancers of the breast, testes, thyroid, or salivary glands to evaluate treatment effects and generate hypotheses about common etiologies. A significant risk of developing a second breast cancer among 27,000 patients in Connecticut was found, although the increased risk was not clearly related to a particular therapy. An elevated

risk of second tumors following testis cancer is being evaluated in a case-control study. In patients with salivary gland cancer, no excess of second primary breast cancer was observed, as had been previously reported, although cancers of the lung and ovary were elevated. In patients with thyroid cancer, a significant excess of both breast and renal second cancers was observed. A similar excess of second primary thyroid cancer seen in patients with breast cancer suggests that these two malignancies may share some common etiologic factors.

Several groups of cancer patients who received radiotherapy were studied. Total-body or hemibody irradiation was associated with an increased leukemia risk in patients with non-Hodgkin's lymphoma which was radiation dose related and independent of chemotherapy duration. Radiation to the pelvis was associated with elevated rates of endometrial cancer. Radiotherapy in combination with chemotherapy did not increase the risk of leukemia in patients with ovarian cancer or gastrointestinal cancer beyond that expected from chemotherapy alone.

Diagnostic: The second mail questionnaire follow-up of women who received multiple chest fluoroscopies during pneumothorax treatment of tuberculosis between 1930-1954 reaffirms that repeated, relatively low radiation doses pose some future risk of breast cancer, that the risk may be cumulative, that adolescence is an especially sensitive age, and that older women may be at low risk of radiogenic breast cancer induction. No excess of total cancer deaths, leukemia, lung cancer, or lymphoma was seen among fluoroscopically examined women. Additional studies are being conducted in Massachusetts, Connecticut, and Denmark to further clarify the carcinogenic effect of multiple low-dose x-ray exposures in both men and women.

A case-control study was conducted in a population of over 32,000 twins born in Connecticut from 1930-1969 and followed to age 15 to evaluate cancer risk from pre-natal x-ray exposure. Twins were chosen for study because the likelihood of medical selection bias would be reduced, i.e., most mothers were x-rayed because of a suspected twin pregnancy and not for any medical condition that could conceivably predispose to childhood cancer. An increased risk of childhood malignancies was found to be associated with prenatal x-ray. Using the resources of pre-paid health plans in California and Oregon, 2,000 cases of leukemia and lymphoma and 2,000 controls are being identified and long-term histories of diagnostic x-ray exposures obtained to evaluate the possible association with radiation dose to active bone marrow.

Isotopes: To evaluate the late effects of exposure to radioactive iodine (131-I), a retrospective-cohort study was conducted of women treated for hyperthyroidism at the Mayo Clinic from 1946-1964. Mortality in 1005 women treated with 131-I was not increased overall or for specific causes including cancer. No difference in total cancer incidence, breast cancer or leukemia was observed between women treated with 131-I and 2141 women surgically treated. Although based on small numbers, an elevated risk of cancer was observed in organs that concentrate 131-I (salivary glands, digestive tract, kidney and bladder), suggesting the need for further follow-up of larger

populations. The risk of second cancers in patients with thyroid cancer treated with high-dose radioactive iodine is also being evaluated in Connecticut and Denmark.

Dosimetry: An essential part of the program of epidemiologic studies of medically irradiated populations is accurate dosimetry for specific organs. A team of medical physicists has been formed to work with the Section on dosimetry problems using physical measurements on patients, anthropomorphic phantoms, and a Monte Carlo computer code developed in collaboration with the Oak Ridge National Laboratory and the National Center for Devices and Radiological Health (FDA). Radiation dose estimates for specific organs have been obtained for tuberculosis patients repeatedly exposed to fluoroscopic x-rays, cervical cancer patients treated with intracavitary radium and external beam x-rays or gamma rays, children irradiated for enlarged tonsils, persons with leukemia who received multiple diagnostic x-rays, patients treated with high energy accelerators that produced low levels of neutron exposures, and children treated with radiotherapy for cancer who subsequently developed a second malignancy.

Serological studies: The value of cytogenetic aberration data as a biological dosimeter in persons with partial-body irradiation is being explored in three medically irradiated populations in collaboration with cytogeneticists at Oak Ridge Associated Universities. The objectives are to determine the type and frequency of somatic cell aberrations in circulating lymphocytes in order to compare dose-response relationships to those seen in A-bomb survivors with total-body exposure; and to determine the persistence of effects in relation to sex, age at exposure, dose and dose fractionation, and radiation quality. Among persons irradiated for enlarged tonsils as children, serum tests include measurements of T3, T4, TBGI, calcium, TSH and AMA.

2. Atomic Bomb Survivors. A new incidence survey through 1980 identified 564 breast cancer cases. For the first time, women who were exposed before age 10 showed a dose-related excess risk of breast cancer. An excess risk was also evident at breast doses in the range 8-16 rads, and a previously reported deficit among women irradiated at ages 40-49 disappeared. The validity of the breast cancer series was reinforced by a formal histological review. In addition, the US and Japanese pathologists also found no association between radiation dose and histological type. The temporal patterns of risk following exposure show a striking similarity between excess risk and age-specific population rates for cancers of the breast, lung, and digestive organs consistent with the relative risk model for projection of risk forward in time. Data analyses continue for case-control interview studies of breast and lung cancer. An incidence study of colorectal cancer is being combined with a case-control interview study that will address occupational, dietary, exercise and other lifestyle factors. A case-control study of thyroid cancer is planned.

A major effort was made to update the RERF breast cancer incidence series, resulting in an increase from 360 to 564 confirmed breast cancer cases, including 10 bilateral cases, diagnosed during 1950-1980. These data, which

strengthened the established connection between radiation dose and breast cancer risk, are remarkable in that, for the first time, a dose-related risk appeared among women exposed before the age of 10; that is, well before breast development. The excess in this youngest cohort appears comparable to that seen in women exposed at ages 10-19, previously thought to be the ages of maximum sensitivity for radiation-induced breast cancer. As seen for older cohorts, the excess risk did not appear until ages at which breast cancer risk normally becomes appreciable. Although no excess risk was apparent for women exposed at any age after 40, there was not, as in the 1950-74 series, a dose-related deficit of breast cancer in the 40-49 ATB (at the time of the bombings) cohort, thus removing the motivation for an earlier interpretation that radiation to the ovaries of women of perimenopausal ages may have reduced risk by disrupting hormonal output. As in previous series, the excess risk was roughly proportional to dose, within age-ATB cohorts, but the additional number of cases provided direct evidence of an excess risk at breast tissue doses as low as 8-16 rads. Data on migration from Hiroshima asnd Nagasaki and on reporting delays were used to estimate the degree of underascertainment of breast cancer in the sample.

The available histological materials for the diagnosis of breast cancer in LSS sample members through 1978 (essentially those in the Hiroshima and Nagasaki tumor and tissue registries, and those collected earlier for the 1950-1974 breast cancer series) were reviewed by a panel of pathologists from Japan and the United States. Three hundred cases were confirmed and classified by subtype. The review removed the possibility of future controversy about these particular cases, a matter of concern in view of some discrepancies among the three incidence series previously reported. No subtype differences were associated with radiation dose or with age at either diagnosis or exposure.

Over 3000 breast tissue slides were prepared from 353 autopsy cases without clinical breast cancer, including 181 with estimated radiation doses over 50 rads, and reviewed for the existence of pre-neoplastic change. A preliminary analysis indicates that dose-related differences were stronger in the survivors exposed at younger ages. Of particular interest is the comparison for ages 40-49 and 50+ ATB in which a statistically significant association was seen for the former cohort but not the latter. In combination with the results of the incidence survey, these results suggest a gradual decline in susceptibility to radiation carcinogenesis with increasing age at exposure.

A case-control interview study of breast cancer cases identified in the incidence study, and multiple controls matched by city, age ATB, and radiation dose, yielded completed interviews from 202 cases and 572 controls, 93% of those eligible. In addition, data on reproductive history, socioeconomic status, and other variables were obtained from RERF files of past studies and mail questionnaires for many of the cases and controls. One purpose of the study was to investigate risk factors other than radiation, but the primary purpose was to examine possible interactions or synergisms between radiation and other risk or host factors in the causation of breast cancer. Preliminary analyses indicate that the usual reproductive factors identified in other studies are also associated with breast cancer risk in the LSS sample (e.g.,

RR=2.0 for first full-term pregnancy after age 30, RR=0.5 for artificial menopause). Additional analyses await a quality control procedure in which data on similar questions from different sources are compared and evaluated.

Of considerable importance for future studies, the previously incomplete Hiroshima Tumor Registry was updated by the inclusion of all cancer diagnoses in the possession of Hiroshima University. This was accomplished during the case finding for the breast cancer incidence study.

Interviewing has been completed for a case-control study of lung cancer focusing on smoking history, diet, and occupational history. This study involved subject or next-of-kin interviews with over 500 lung cancer cases diagnosed during 1971-1980 and age-sex-city matched controls. Data analysis will begin this summer at NCI.

Colon and rectal cancer is being investigated using an approach similar to that for breast cancer. Incident cases diagnosed during 1950-1982 are being ascertained and reviewed. Questionnaire development and testing have been completed for a case-control interview study aimed at evaluating dietary influences, occupational exposures, and physical activity as risk factors.

A thyroid cancer incidence study has been completed at RERF as part of its regular research program. Data from this study, and from several other series from the United States and Israel, have been sent to NCI for comparative analyses of thyroid cancer risk as a function of radiation dose, sex, age at exposure, and time after exposure, in collaboration with the original investigators.

A thyroid cancer case-control study has been initiated, focusing on dietary and reproductive factors. The questionnaire was designed during a recent visit to NCI by the principal RERF collaborator.

Because the current status of radiation dose estimates for the LSS sample is in flux, and a new dosimetry is unlikely to be in place within the next two years, there is consequent uncertainty about risk estimates based on the LSS sample data. Enough is known, however, to calculate new dose estimates for persons exposed in the open or in simple shielding configurations. A collaborative effort is underway to calculate doses and cancer risk estimates for this subset of the sample, using existing mortality and incidence data.

An analysis of latent periods for leukemia, and cancers of the breast, lung, and digestive system among LSS sample members shows a clear dichotomy between leukemia and the solid cancers. The frequently cited temporal "wave" phenomenon for radiation-induced leukemia appears to be real, but depends upon histological type and age at exposure. There is also much less reason to suspect a dependence of induction period on radiation dose for radiation-induced leukemias. The temporal pattern for leukemia is in marked contrast to cancers of the breast, lung, and digestive organs, for which the distribution in time of radiation-induced cancers appears identical to that for non-radiation-induced cancers, for subjects of the same age ATB. It seems that

radiation exposure increases cancer risk, but that the additional cancers appear at ages when cancers normally occur, and are distributed over time as one might expect on the basis of age-specific population rates.

3. Occupational and Environmental Exposures. A pilot study has been successfully conducted evaluating the feasibility of studying over 170,000 x-ray technologists registered since 1926 with the American Registry of Radiological Technologists. Approximately 144,000 technologists are living with known addresses. It was determined that inactive members of the society could be located, and that radiation exposure could be characterized on the basis of length of employment, film badge readings, and questionnaire responses. The full-scale investigation is in progress.

A re-evaluation of geographic and temporal mortality in children in the western United States failed to confirm a previously reported study of excess childhood leukemia possibly associated with radioactive fallout from nuclear weapons tests.

In collaboration with the Biometry Branch, a study was completed to clarify the role of solar UV-radiation in the development of nonmelanoma 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. About 80% of skin cancers are basal cell carcinomas, males are at greater risk than females, and incidence has been increasing over calendar time.

4. Methodologic Studies. For cancer sites for which a wealth of epidemiologic data exists, attempts are made to resolve apparent inconsistencies among different studies and to strengthen inferences. This is accomplished by working in collaboration with the original investigators and by reanalyzing the basic data in parallel, using identical stratifications with respect to age at exposure, length of follow-up, and identical assumptions with respect to dose-response models and latent period. Such an approach is to be taken with respect to thyroid cancer incidence data from several exposed populations. Special problems of estimating cancer risk from low-dose exposures to ionizing radiation have been explored, including statistical power, sample size, and dose-response model assumptions. Bayesian models have been considered for incorporating information from experimental radiobiology. Random error in individual dose estimates was found to bias dose-response analyses based on grouped data. The proportional hazards method was adapted to a factorially designed, long-term, animal experiment to assess possible interactions between radiation and other carcinogens in the induction of mammary tumors. Breast cancer risk among A-bomb survivors has been explored using new models in which the temporal distribution of base-line and excess risk are compared, as well as integrated risk over the entire period of observation. Approximate statistical methods were developed to analyze interaction between radiation and other risk factors in a case-control study of breast cancer in which cases and controls were matched on radiation dose.

Because assumptions about dose response are needed to estimate cancer risks associated with low-dose exposures, and because these assumptions should be based on radiobiological considerations, the RSS has encouraged contacts between staff members and experimental radiobiologists, to the extent of participating in analyses of experimental data. One such study is a long-term animal experiment (rats) to assess possible interactions between radiation and other carcinogens for the induction of mammary tumors. The experimental design was a 2^3 factorial with tumor incidence determined by palpation and biopsy over a two-year period followed by necropsy. The unusual analytic problems presented by this design were addressed by an adaptation of the proportional hazards model.

Random errors in individual dose estimates are often cited as possible sources of bias for estimates of cancer risk. An evaluation using A-bomb survivor data indicated that such error must be very large to affect estimates based on ungrouped data or data grouped on some basis other than the dose estimates themselves. When data are grouped by estimated dose, on the other hand, random error tends to deform the apparent dose-response relationship, making the curve steeper at low doses and flatter at higher doses, and thus introduces an element of bias into analyses which seek to determine the functional form of that relationship. This bias tends to be important if random errors of a factor of 2 or more are common.

The incorporation of prior information about details of the dose-response model is subject to criticism on grounds of arbitrariness. Uncertainties about model choices should be reflected in the estimates obtained, and the estimation process should be subject to the discipline of a coherent system of inference. A Bayesian approach satisfies these conditions and, moreover, results in estimates suitable for the application of optimal decision rules.

Published cytogenetic data from the A-bomb survivor population studied by the Radiation Effects Research Foundation were analyzed using two radiation dosimetries: the current T65D system and a modified system proposed by physicists at Lawrence Livermore Laboratories. The analysis suggested that a change in dosimetry might be appropriate, although discrepancies remained even under the proposed new system.

A case-control interview study of breast cancer among A-bomb survivors required the development of a new method for estimating interaction between radiation dose and other risk factors when matching was done with respect to dose. The method is more powerful than conventional analyses in which matching does not depend on dose.

Breast cancer risk among A-bomb survivors has been explored using new models in which the magnitude of risk and its distribution over time are treated simultaneously with age at exposure. The analysis suggests that the magnitude of risk and its temporal distribution are essentially independent, but that both are heavily dependent upon age at exposure. This result confirms previous analyses that treated questions of magnitude and temporal distribution orthogonally.

Significance to Biomedical Research and the Program of the Institute:

Studies of populations exposed to ionizing radiation are 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. Epidemiologic data relating cancer incidence and mortality to radiation exposure, especially with good information on dose and timing of exposure, can also influence theories of carcinogenesis and motivate experimental research. 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. 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.

Proposed Course:

Activities will continue in all four project areas involving Radiation Studies. The aim is to assure that maximum benefit is derived from existing epidemiologic resources and to initiate studies of populations not previously evaluated, but which offer unusual potential for new information. Populations that have been identified in the past will be continually followed in order to evaluate lifetime risks associated with previous exposures. The extent of the follow-up will vary depending upon the particular population being evaluated, but could include mail questionnaire studies, national death index searches and other mortality evaluations, or record linkage with existing regional tumor registries. Populations designated for future follow-up include: (1) atomic bomb survivors, (2) tuberculosis patients in Massachusetts and Connecticut who received multiple chest fluoroscopies, (3) women given radiotherapy for cervical cancer, (4) children irradiated for lymphoid hyperplasia, and (5) x-ray technologists.

New studies that are in the planning or feasibility stage include: (1) an evaluation of persons with scoliosis who received multiple diagnostic x-rays of the spine during adolescence. Such a study would prove valuable in

evaluating the risk of breast cancer from repeated low-dose diagnostic x-ray exposures received during an especially sensitive time of life. (2) A study of the carcinogenic effects of radiation therapy for gastric ulcer is also being considered. Over 2,000 patients who were exposed between 1935-1965 have been identified, and the radiation risks for cancers of the stomach, pancreas, lung, spleen and kidney will be evaluated. Except for the lung, radiation risks for these sites are not well defined. (3) To quantify and clarify the findings of an increased risk associated with prenatal x-ray exposure in twins in Connecticut, an expanded study utilizing twin registries and cancer registries in California and Sweden is being considered. (4) A study of approximately 36,000 patients treated for hyperthyroidism with radioactive iodine and other therapies may be reactivated. Radioactive iodine is an important isotope used in medicine, a major component of fallout from nuclear weapons tests and also a major release product from nuclear power reactors. There is considerable controversy over the effectiveness of radioactive iodine in inducing malignancies, and it is felt that further studies in this area are warranted. Record linkage evaluations in Scandinavian countries and Europe are also being considered to obtain information on large numbers of patients who receive therapeutic and diagnostic doses of radioactive iodine. (5) A study of women irradiated for benign gynecological disorders (BGD) has been developed. Cancers of pelvic and abdominal organs, sites which have not been well characterized in terms of radiogenic risk, will be evaluated. In addition, the paradoxical finding of increased leukemia risk associated with low-dose exposures to the pelvic marrow in BGD patients, but not with high-dose exposures in cervical cancer patients, will be investigated, as will the unexpected reduction in breast cancer risks previously associated with radiotherapy for BGD in post-menopausal women.

Other studies under consideration include: (1) a case-control evaluation of the risk of contralateral breast cancer in women treated with radiation for primary breast cancer. The dose to the contralateral breast can be substantial, on the order of several hundred rads, and survival following breast cancer treatment is relatively good. Thus this study offers an opportunity to evaluate the risks of radiation in elderly women and the effect of interaction of radiation with an underlying host susceptibility. (2) Populations exposed to non-ionizing radiation are being considered for potential study. For example, recent reports in the literature suggest an association between leukemia and electromagnetic radiation. (3) The ability of neutrons to induce cancer in humans has not been well studied, and a study of patients who received neutron exposure from betatrons and other high-energy cancer therapy machines or neutron sources would provide new information. (4) The study of chromosome aberrations following radiation exposure may be extended to other populations such as cervical cancer patients who were treated with high-energy x-ray beams that produced a measurable amount of neutrons, persons irradiated during the first six months of life for enlarged thymus glands, adults who received radioactive iodine therapy for hyperthyroidism, persons who as adolescents received multiple x-rays of the spine for scoliosis, and patients treated with cytotoxic drugs.

Among the atomic bomb survivors, an incidence study of colorectal cancer and case-control studies of colorectal and thyroid cancer will constitute the main effort of the program. The analysis of breast cancer incidence data of the atomic bomb survivors in parallel with data from medically exposed U.S. populations provided new insights into carcinogenic risks. A second parallel analysis will incorporate new data from several sources. A similar analysis is being considered for thyroid cancer incidence data, combining atomic bomb survivor information with data from medically exposed Israeli and American populations.

There has been wide interest in evaluating health effects in U.S. nuclear power workers. However, because of the magnitude of such an investigation, coupled with the long-term commitment required, we have been cautious in embarking on such a project. A feasibility study has been considered. Radon in the home has been suggested as an important risk factor for lung cancer. A study is being considered.

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.

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CONTRACTS IN SUPPORT OF THIS PROJECT

ENERGY, DEPARTMENT OF (Y01-CP-10504)

Title: Studies on Radiation-Induced Chromosome Damage in Humans

Current Annual Level: $107,047

Man Years: 1.5

Objectives: The project was undertaken to determine the type and frequency of chromosome aberrations in circulating lymphocytes and to compare dose-response curves among three populations with respect to dose, quality of radiation, fractionation, age and sex. Its purpose is (1) to improve the usefulness of chromosome aberration frequency as a biological dosimeter for partial-body exposures, (2) to determine the persistence of radiation-induced effects, and (3) to obtain insights into a biological effect that may be similar to radiation carcinoigenesis.

Methods Employed: Chromosomal aberrations are being determined and analyzed in 450 subjects selected from among 3 populations exposed to partial-body 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 are selected as controls. Blood specimens, drawn at the hospitals where these persons were treated, are analyzed at the DOE-supported radiation cytogenetic laboratory at the Oak Ridge Associated Universities.

Major Findings: Preliminary analyses indicate a statistically significant difference in the frequency of chromosome aberrations in 35 exposed persons as compared with 42 non-exposed persons treated during childhood for enlarged tonsils (RR=1.4, p=.048). The increase appeared to be confined to translocations, dicentrics, and rings; inversions and fragments were not increased in exposed patients. The incidence of radiation-induced lesions in cervical cancer patients, based on small numbers, appears to be much higher; approximately 5 to 10 lesions per 100 cells were seen as compared with one lesion per 100 cells in exposed tonsil patients.

Proposed Course: The study is planned to be extended to other populations which have received radiation at different exposure levels and at different ages. There is a unique opportunity to draw and analyze blood from cervical cancer patients who were treated with very high energy x-ray beams that produced a measurable amount of neutrons. We would like to include a sample of persons irradiated during the first six months of life for enlarged thymus glands. Consideration is also being given to evaluate adults who were treated

with radioactive iodine (131-I) for hyperthyroidism. It may also be possible to use this approach to study patients treated with cytotoxic drugs.

PETER BENT BRIGHAM HOSPITAL (NO1-CP-11008)

Title: Cancer Following Tonsil Irradiation: Physical Examinations and Blood Studies

Current Annual Level: $34,987

Man Years: 2.0

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 thyroid and parathyroid as determined by blood tests of serum levels of TSH, TBGI, T3, T4, AMA and calcium; 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 Enviromental Epidemiology Branch has been collaborating with clinicians at the Children's Hospital Medical Center in a study of 3000 persons treated as children for lymphoid hyperplasia with either radiation or surgery. Physicians at the Peter Bent Brigham Hospital are examining 1000 exposed and nonexposed study subjects. The Brigham Medical Group provides the examination rooms, nurses, physicians, laboratories, freezers for blood storage, and all other necessary items for the conduct of the physical examinations and blood studies.

Major Findings: Project results have not been completed to date. However, preliminary results indicate that exposure to x-ray in childhood is associated with a two-fold risk of thyroid nodules as compared to surgical treatment. Three hundred serum tests have also been analyzed but not tabulated.

Proposed Course: Termination 9/29/83 unless similar studies on tuberculosis patients who received multiple chest fluoroscopies can be developed.

TEXAS, UNIVERSITY OF, M.D. ANDERSON HOSPITAL (NO1-CP-01047)

Title: Studies of Iatrogenic Cancer and Radiation Dosimetry.

Current Annual Level: $84,950

Man Years: 2.0

Objectives: To provide radiation dosimetry necessary to estimate organ doses received during radiotherapy for cervical cancer and other conditions.

Methods Employed: Physics measurements are being made for x-ray machines and intracavitary isotopes. These include orthovoltage, betatron, megavoltage x-ray machines, Van de Graaff machines, and cobalt-60 units, in addition to

radium and cesium intracavitary sources. Abstracted data from all participating radiotherapy centers in the international cervical cancer study 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. 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. Organ doses for typical cervical cancer treatments have been determined.

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 benign gynecological disorders. A five year extension has also been proposed to provide dosimetry support for epidemiologic studies of the following populations: women irradiated for breast cancer, adolescents receiving diagnostic spinal x-rays for scoliosis, children irradiated for lymphoid hyperplasia.

WESTAT, INC. (NO1-CP-01011)

Title: Support Services for Radiation Studies

Current Annual Level: $729,496

Man Years: 8.0

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) the clinical follow-up study of cervical cancer patients; (2) registry case-control studies for the cervical cancer study; (3) questionnaire preparation for the x-ray technologist study; (4) thyroid case-control interview study in Connecticut; (5) Veterans Administration

adjuvant drug study evaluations; (6) clinical trial evaluations of methyl-CCNU; (7) 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) 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: Termination 6/28/83, but project has been recompeted and multiple 5-year awards have been made.

WESTAT, INC. (NO1-CP-11018)

Title: Support Services for Radiation and Related Studies

Current Annual Level: $357,353

Man Years: 4.0

Objectives: Same as WESTAT (NO1-CP-01011), although studies under this contract are different.

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) coordinating the physical examinations and blood studies for the tonsil irradiation study; (3) TB-fluoroscopy study of males in Massachusetts; (4) case-control study of endometrial cancer following hormonal therapy for breast cancer; (5) case-control study of childhood cancer in twins associated with prenatal x-ray in Connecticut and California; (6) coordinating the chromosome study of irradiated cervical cancer patients; and (7) a study of second cancer following treatment of testis cancer in Connecticut.

Proposed Course: Same as WESTAT (NO1-CP-01011) but with termination on 9/29/83.

FOOD AND DRUG ADMINISTRATION (P00037)

Title: Radiation Dosimetry for Epidemiologic Studies

Current Annual Level: $75,000 (Interagency agreement to be awarded.)

Man Years: 1.0

Objectives: To provide on-going radiation dosimetry support for epidemiologic studies of populations exposed to ionizing radiation.

Methods to be Employed: The FDA (National Center for Devices and Radiological Health) will provide the support necessary to apply Monte Carlo computer codes

to various epidemiologic studies being conducted by the Radiation Studies Section. Specifically the FDA (NCDRH) will (1) determine the manner in which the Monte Carlo techniques can best be applied to the epidemiologic studies of interest; (2) obtain necessary modifications in the existing Monte Carlo codes to estimate the organ specific radiation doses of each study; and (3) establish and maintain working arrangements with the Oak Ridge National Laboratory for computer and code modification support.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP04500-06 EEB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Methodologic Studies of Epidemiology

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

William J. Blot, Chief, Analytical Studies Section, EEB, NCI

COOPERATING UNITS *(if any)*

Department of Biostatistics, University of Washington,
Seattle, WA

LAB/BRANCH

Environmental Epidemiology Branch

SECTION

Analytical Studies Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	2.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) Human subjects ☐ (b) Human tissues ☒ (c) Neither

☐ (a1) Minors

☐ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

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 this year, 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 review of methods to evaluate the magnitude of low-dose radiation risks.

PROJECT DESCRIPTION

Names, Titles, Laboratory and Institute Affiliations of Professional Personnel (other than the Principal Investigator) engaged on this Project:

J. H. Lubin	Senior Staff Fellow	EEB	NCI
L. W. Pickle	Senior Staff Fellow	EEB	NCI
C. E. Land	Health Statistican	EEB	NCI
J. D. Boice, Jr.	Epidemiologist	EEB	NCI
E. J. Martin	Cancer Expert	EEB	NCI
A. F. Kantor	Staff Fellow	EEB	NCI
P. Hartge	Epidemiologist	EEB	NCI
S. K. Hoar	Staff Fellow	EEB	NCI
S. G. Machado	Staff Fellow	EEB	NCI
K. P. Cantor	Epidemiologist	EEB	NCI
R. Spirtas	Biostatistician	EEB	NCI
M. Gail	Biostatistician	BB	NCI

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. Random digit dialing has been studied as an alternative to other population sampling methods, while computer-assisted telephone interviewing has been used and evaluated. Quality control techniques for large, multi-center studies have been developed, and characteristics of hospital-based, as opposed to population-based, control groups have been profiled. One report presented a method for determining the optimum sampling ratio in a case-control study.

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 to obtain unbiased risk estimates were given, while in another report it was suggested that for such sampling a matching ratio as high as 10 or 20 to 1 is occasionally necessary for precise risk estimation. An occupation-exposure linkage system was refined and its implications for use described, with a computer algorithm developed to facilitate linking exposures to job titles. The development of similar linkage systems in other countries was considered.

Several methodologic issues related to the study of radiation-induced cancers. 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. Two radiation dosimetries for the A-Bomb survivors were considered and a change suggested.

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 modelling with matched case-control data were expanded.

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.: Book Review. Statistical methods in cancer research: The analysis of case-control studies. Oncology 39: 128, 1982.

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Breslow, N. E., Lubin, J. H., Marek, P., and Langholtz, B.: Multiplicative models and the analysis of cohort data. J. Am. Statist. Assoc. 78: 1-12, 1983.

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Hartge, P., Cahill, J., West, D., Hauck, M., Austin, D., Silverman, D., Hoover, R.: Design and methods in a multi-center case-control interview study. Am. J. Public Health (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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Hoar, S. K.: Meeting highlights: Job-exposure matrices in occupational epidemiology. JNCI 69: 1419-1420, 1982.

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Hsieh, C. C., Walker, A. M., and Hoar, S. K.: Grouping occupations according to carcinogenic potential: Occupation clusters from an exposure linkage system. Am. J. Epidemiol. 117: 575-589, 1983.

Kantor, A. K.: Calculation of HLA phenotype frequencies from two- and three-locus haplotype frequencies. Hum. Hered. (In Press)

Land, C. E. and Pierce, D. A.: Some statistical considerations related to the estimation of cancer risk following exposure to ionizing radiation. In Epidemiology Applied to Health Physics. Conf-830101. Natl. Tech. Info. Service, U.S. Department of Commerce. Springfield, Virginia, 1983.

Lubin, J. H. 1982. Review of Statistics in Practice: Articles from the British Medical Journal by S. M. Gore and D. G. Altman. JAMA 248: 1647.

Lubin, J. H.: A reformulation of the SAED method for occupational mortality data. Am. J. Epidemiol. (In Press)

Lubin, J. H. and Gail M. H.: Biased selection of controls for case-control analyses of cohort studies. Biometrics. (In Press).

Morgenstern, H. and Winn, D. M.: A method for determining the sampling ratio in epidemiologic studies. Statistics in Medicine. (In Press)

Pickle, L. W., Morris, L. E., and Blot, W. J.: Information available from surrogate respondents in case-control interview studies. Am. J. Epidemiol. (In Press)

Rothman, K. J., Boice, J. D. Jr. and Austin, H.: Epidemiologic Analysis with a Programmable Calculator. Chestnut Hill, Massachusetts, Epidemiologic Resources, Inc. 1982, 197 pp.

Silverman, D. T., Hoover, R. N., and Swanson, G. M.: Artificial sweeteners and lower urinary tract cancer: Hospital vs. population controls. Am. J. Epidemiol. 117: 326-334, 1983.

Silverman, D. T., Hoover, R. N., Swanson, G. M., and Hartge, P.: The prevalence of coffee drinking among hospitalized and population-based control groups. JAMA 249: 1877-1880, 1983.

Spirtas, R. and Fendt, K.: An algorithm for linking job titles with individual exposures in occupational epidemiology studies. In Acheson, E. D. (Ed.): Job Exposure Matrices: Proceedings of a Conference Held in April 1982, at the University of Southampton. Southampton, England, Hobbs the Printer of Southampton, 1983, pp. 39-47.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP04501-06 EEB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Case-Control Studies of Selected Cancer Sites

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Robert N. Hoover, Acting Chief, Environmental Epidemiology Branch, NCI

COOPERATING UNITS *(if any)* Dept. of Health, State of New Jersey; Biometry Branch, NCI; Div. of Cancer Resources, Centers & Community Act.; Med. Branch, NCI; 28 Breast Cancer Detection Demonstration Project Centers; 10 SEER Centers in Continental U.S.; Oxford University & Birmingham University; 5 Comprehensive Cancer Centers

LAB/BRANCH

Environmental Epidemiology Branch

SECTION

Environmental Studies Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 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 *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to investigate in analytic studies the etiologies of selected cancers. Specific cancer sites and hypotheses are selected for which the need for investigation is clear but which have been difficult to study elsewhere. Case-control studies in progress include: childhood bladder cancer, bladder cancer in New England, cancer in rural Georgia, mycosis fungoides, nasal cancer, and testicular cancer in young men. These studies focus either on tumors that have not been studied analytically before (e.g., because of the rarity of the tumor) or on hypotheses that are difficult to assess (e.g., because of the prevalence of the exposure or the need to detect an effect at low levels of exposure). Since these studies are often the first or most thorough to date, they collect data on a wide range of exposures, usually from interviews and medical records.

PROJECT DESCRIPTION

Names, Titles, Laboratory and Institute Affiliations of Professional Personnel (other than the Principal Investigator) engaged on this Project:

J.F. Fraumeni, Jr.	Associate Director	FSS	NCI
W.A. Blattner	Chief, Family Studies Section	EEB	NCI
P. Hartge	Epidemiologist	EEB	NCI
L.A. Brinton	Senior Staff Fellow	EEB	NCI
T.J. Mason	Chief, Population Studies Section	EEB	NCI
K.P. Cantor	Epidemiologist	EEB	NCI
L.M. Pottern	Epidemiologist	EEB	NCI
W.J. Blot	Chief, Analytical Studies Section	EEB	NCI
A.F. Kantor	Senior Staff Fellow	EEB	NCI
S.K. Hoar	Staff Fellow	EEB	NCI
M.A. Tucker	Clinical Investigator	EEB	NCI
C. Schairer	Health Statistician	EEB	NCI
G. Gridley	Health Statistician	EEB	NCI

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 14 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 kidney cancer, 1 of testicular cancer, 2 of ovarian cancer, 1 of nasal cancer, 1 intra-ocular melanoma, and 1 of cervical cancer.

1. Breast cancer patients (1,554) identified by the Breast Cancer Detection Demonstration Project (BCDDP), women with benign breast disease (1574), 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. (See Project No. Z01CP04412-07 EEB).

2. A continuation of the breast cancer study noted above is being conducted. Breast cancer patients, approximately 2,500, identified from 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.

   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 above.

4. 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.

5. A case-control study of bladder cancer was conducted in New Hampshire and Vermont to look for environmental associations in both sexes. New Hampshire has the second highest bladder cancer mortality rates for both white men and women, according to mortality rates for 1950-1969, among the 48 contiguous states. Vermont has similarly high rates, especially for white women. Project personnel identified 364 New Hampshire and Vermont 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 using 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.

6. 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, 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.

7. Non-Hodgkin's lymphoma patients treated at the NIH Clinical Center and sibling controls were interviewed. The study consists of complete information on 91 cases and 121 controls regarding radiation exposure, occupational exposure, and past drug usage. (See Project No. Z01CP04412-07 EEB).

8. 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. 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.

9. Ovarian cancer patients (350) diagnosed between 1978 and 1981 in 25 Washington, D.C. area hospitals, and women hospitalized for other conditions (350), were interviewed in their homes to collect information about medical, family, reproductive and menstrual histories, use of exogenous estrogens, contraception, occupation, and smoking. Pathology slides have been reviewed and questionnaires mailed to subjects' physicians to collect additional data.

10. A study of ovarian cancer from the medical records of two pre-paid health plans included 510 cases and 604 controls. Data on medications, illnesses, and surgical histories were abstracted.

11. A case-control study of intra-ocular 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.

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 are 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. (See Project No. Z01CP05128-04 EEB).

13. A case-control study of childhood bladder cancer was conducted in cooperation with investigators participating in the SEER Program 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.

14. A case-control study of nasal cancer included 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, focused on occupational exposures, residential history, medical history, and smoking and alcohol usage.

Major Findings:

1. Analyses of the BCDDP breast cancer case-control data were first reported in 1981. Further explorations have shown that "minimal" breast cancers are etiologically similar to large invasive lesions for several major risk factors, including family history, age at first livebirth, bilateral oophorectomy and obesity. In situ cancers were similar to invasive cancers with respect to family history and age at first birth, but were not related to oophorectomy or obesity. Benign breast tumors were not related to the major breast cancer risk factors. Together these findings suggest that breast cancers and benign tumors have different causes, that early-life hormonal factors can effect the progression from in situ to invasive disease, and that small invasive cancers are biologically similar to large cancers.

   Analyses of interviews of breast cancer risk factors suggest that the effect of family history was modified by age at menarche, but not by age at first birth or type of menopause. Thus, familial risk may be exerted through early hormonal status.

   Detailed analyses of reproductive risk factors have been undertaken. The protection conferred by early first birth was found to be limited to full-term live births. Multiparity appeared to confer some protection, after adjustment for age at first birth. Women with multiple miscarriages before the first birth were at excess risk.

   Drug exposures were evaluated. Menopausal estrogens appeared to obliterate the protective effect of bilateral oophorectomy. Oral contraceptives did not appear to affect risk overall, nor did thyroid supplements or tranquilizers.

2. Artificial sweetener use was examined in detail to explore the possibility that it could promote bladder cancer. Overall, the data suggested no such effect.

   Hair dye use showed no effect overall and no effect according to duration, age, or frequency of dyeing or dye color. In Detroit, truck drivers and tool-and-die makers had excess risk, which was associated with duration of employment. Urinary tract infection was associated with bladder cancer risk, as was a history of bladder stones, but not kidney stones. A history of three or more urinary tract infections was strongly related to risk of squamous cell carcinoma. The association between coffee and bladder cancer was almost entirely explained by the association of tobacco and bladder cancer. Compared to those who never drank, coffee drinkers had a relative risk of 1.4, but no consistent dose response was apparent, and current drinkers had the same risk as former drinkers. There was no association apparent between snuff or chewing tobacco and bladder cancer. Pipe smokers who inhaled showed a higher risk than nonsmokers, and cigar smokers showed a slightly higher risk.

Analyses of water quality, chemical work, textile work, leather work, painting, printing, rubber work, and motor exhaust related work are in progress. Information from 1000 water utilities in the study areas was collected and merged with residential histories to create a year-by-year profile of water quality for each respondent. Nonsmokers showed excess risks if they usually drank from surface sources compared to unchlorinated ground sources. The overall relative risk was 1.3.

The 537 subjects who worked in the chemical industry showed no overall excess risk, but those who worked with organic chemicals did show an excess risk. Although the female workers in plastics and in soaps and perfumes showed excess risks, the male workers did not. No association was seen between bladder cancer risk and work in the manufacturing of drugs or paints and pigments. The 163 leather workers appeared to be at slightly elevated risk, and textile workers did not.

The 162 building painters showed an elevated risk of bladder cancer, with increased risk for those with longer durations of employment. Painters of manufactured articles showed a smaller excess, without evidence that duration was related to risk. Among the 27 artists in the study, the relative risk was 2.5. This association appears to confirm the findings of an Environmental Epidemiology Branch proportional mortality study. Female rubber workers showed excess risk, but male workers did not. Printers showed no apparent increase in risk. Elevated relative risks were seen among truck drivers, delivery men, taxi drivers, and chauffeurs, all of whom were exposed to motor exhaust.

Methodologic findings have also been presented, for example, comparing artificial sweetener use and coffee drinking in hospital and population controls.

3. Preliminary analyses of the intra-ocular melanoma study identify blue eyes and moles as risk factors. No case-control differences were seen for exposure to coal, petroleum products, or polychlorinated biphenyls. Cases more often reported radiation exposure and engineering occupations than did controls.

4. Analysis of the study of kidney cancer in rural Georgia showed no striking differences in lifestyle or occupation, but cases more frequently reported drinking coffee.

5. In the case-control study of nasal cancer, furniture workers showed an elevated risk, especially of adenocarcinoma. Female textile workers also appeared to be at excess risk. Smokers showed 2-3 fold elevated risks of squamous cell cancer. Analysis of the data is continuing.

6. Analysis of the testicular cancer study has recently begun. Undescended testes not corrected at an early age were associated with risk of cancer, but undescended testes corrected early were not. A single undescended testis was not a risk factor for contralateral testicular cancer. Groin

hernias were not associated with cancer risk. External factors influencing temperature of the testes, including type of underwear and bathing, were not associated with risk. Analysis of the mothers' interviews will begin soon.

7. Use of vinca alkaloids in the treatment of Hodgkin's disease was not associated with fatal myocardial infarction as previously reported.

8. Karyotyping of peripheral leukocytes from renal carcinoma patients did not reveal any cases of translocation previously reported between chromosomes 3 and 8. Two other constitutional abnormalities in other chromosomes and a woman with chromosomal mosaicism for Turner's syndrome were identified. HLA antigen typing revealed increased frequencies of HLA-DR8 and Bw44. Cancer developed in the offspring of two Wilms' tumor survivors (rhabdomyosarcoma, Hodgkin's disease), suggesting an excess risk of cancer among the progeny of Wilms' tumor patients.

9. Preliminary analysis of bladder cancers in childhood suggests no difference between cases and controls in early or prenatal exposure to artificial sweeteners.

10. Preliminary analysis of bladder cancer in New England suggests a relation between bladder cancer and tobacco, truck driving, textile work, and leather work. Longer duration of truck driving and textile work was associated with greater risk. Self-reported exposure to diesel fumes was associated with risk, but no dose response was seen. No association with pulp or paper mill exposure, coffee, or bracken fern was apparent.

Significance to Biomedical Research and the Program of the Institute:

The case-control methodology provides a rapid, relatively inexpensive, yet scientifically valid 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 from 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 intra-ocular 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 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:

Baker, L. H., Carlile, T., Milbrath, J. R., Threatt, B., Gilbert, F. I., Jr., Hoover, R. N., Prorok, P. C., Brinton, L. A., Howard, J., Byrd, B. F., Jr., Seidman, H., Garfinkel, L. and Hutter, R. V. P. (BCDDP Data Management Advisory Group): Breast cancer detection demonstration project: Five year summary project. CA-A Cancer Journal for Clinicians 32: 194-225, 1982.

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Brinton, L. A.: Re: Risk factors for benign breast disease. The first author replies. Am. J. Epidemiol. 115: 796-797, 1982.

Brinton, L. A., Hoover, R. and Fraumeni, J. F., Jr.: Epidemiology of minimal breast cancer. JAMA 249: 438-487, 1983.

Brinton, L. A., Hoover, R. and Fraumeni, J. F., Jr.: Interaction of familial and hormonal risk factors for breast cancer. JNCI 69: 817-822, 1982.

Brinton, L. A., Hoover, R. and Fraumeni, J. F., Jr.: Reproductive factors in the etiology of breast cancer. Br. J. Cancer (In Press)

Cantor, K. C., Kofler, F. C., Hoover, R. and Strasser, P.: Cancer epidemiology as related to chemicals in drinking water. Toxicol. Environ. Chem. Rev. 4: 47-65, 1981.

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DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP04779-07 EEB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Field Studies in High Risk Areas

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

William J. Blot, Chief, Analytical Studies Section, EEB, NCI

COOPERATING UNITS *(if any)* Univ. of North Carolina; Univ. of Minnesota; Lehigh Univ.; Louisiana State Univ.; Univ. of Texas; Medical University of South Carolina; Cancer Institute, Chinese Academy of Medical Sciences; Shanghai Cancer Institute.

LAB/BRANCH

Environmental Epidemiology Branch

SECTION

Analytical Studies Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
8.5	7.5	1.0

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The objectives of this project are to identify and describe environmental and host determinants of cancer in areas at high risk of cancer through the use of analytical epidemiologic techniques, particularly case-control studies of specific cancers. Completed during the year were analyses of a) kidney cancer in Minnesota, where ethnic factors, cigarette smoking, and (among women) high relative weight were associated with renal adenocarcinoma, and cigarette smoking was found to be the major cause of renal pelvis cancer; b) oral cancer among women in North Carolina, where, in addition to an increased risk associated with snuff use, an association with mouthwash use was observed and risk was inversely related to diets high in fruits and vegetables; and c) lung cancer in Pennsylvania, where increased risks were found among long-term steel workers and with residence in proximity to a zinc smelter. Interviewing continued for case-control studies of respiratory cancer in New Jersey and coastal Texas; lung, stomach, and pancreas cancers in Louisiana; bladder cancer in rural New England; and esophageal cancer in coastal South Carolina. Pilot studies were initiated in areas of China at high risk for lung and esophageal cancer and choriocarcinoma to test the feasibility of large-scale epidemiologic investigations.

Project Description

Names, Titles, Laboratory and Institute Affiliations of Professional Personnel (Other than the Principal Investigator) engaged on this project:

J. F. Fraumeni, Jr.	Associate Director	FS&S	NCI
R. N. Hoover	Acting Chief	EEB	NCI
T. J. Mason	Chief, Population Studies Section	EEB	NCI
B. J. Stone	Mathematician	EEB	NCI
L. Pickle	Senior Staff Fellow	EEB	NCI
L. Brinton	Senior Staff Fellow	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	Senior Clinical Investigator	EEB	NCI
A. Kantor	Senior Staff Fellow	EEB	NCI
A. Ershow	Staff Fellow	EEB	NCI
W. DeWys	Associate Director	DRCCA	NCI
P. Greenwald	Director	DRCCA	NCI

Objectives:

To identify and describe the environmental determinants of cancer in areas 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 Tidewater, 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 1940s 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. Further study of respiratory cancer in the south, where mortality rates are highest, was continued in coastal Texas in collaboration with the University of Texas School of Public Health. Interviewing to obtain detailed information on characteristics of cancer patients was completed in Louisiana, as part of a collaborative (with the EPA and Louisiana State University) case-control interview study for lung, pancreatic, and stomach cancers. Initial analyses show an increased risk of lung cancer associated with Cajun/Acadian ancestry, in part due to differential patterns in tobacco use, including the use of hand-rolled cigarettes. 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 the focus of a study begun in rural New England to evaluate the usually high rates in both sexes in this area of the country.

A case-control study of lung cancer in eastern Pennsylvania showed a significantly increased risk 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 previously recognized. Lung cancer risk was also inversely related to distance of residence from the smelter, and analyses of soil samples revealed the highest concentrations of arsenic, cadmium, and zinc in proximity to the smelter. Although the numbers of neighborhood residents with lung cancer was small, the findings suggest that further research is warranted to evaluate metallic air pollutants as risk factors for lung cancer.

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. This year a case-control interview study of cases diagnosed in Virginia and North Carolina over the past ten years was completed. A 5-fold excess of nasal adenocarcinoma was found to be associated with wood-working occupations. For the first time, cigarette smoking was shown to be related to nasal cancer, but only to squamous cell types.

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. Review of dietary data found a gradient in oral cancer risk with decreasing consumption of fruits and vegetables, suggesting that these foods or the nutrients they contain may be protective. Study of esophageal cancer and diet also continued among blacks in coastal South Carolina, where rates have been high at least since the 1940s.

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. The area's high rates of renal adenocarcinoma appear to be at least in part related to ethnic factors, as increased risks were associated with German and Scandinavian background, the major ethnic groups in this metropolitan center. One of the strongest risk factors was weight-for-height, but only among women. Cigarette smoking was associated with about a 60% increased risk of renal adenocarcinoma in men and a 90% increase in women. Separate analysis showed that the relative risk for smoking exceeded 5-fold in both sexes for transitional cell cancers of the renal pelvis. Long-term phenacetin use was also associated with excess risk of renal pelvis cancer and, to a lesser extent, renal adenocarcinoma. Genetic factors for renal cancer were assessed by searching for unusual frequencies of HLA antigens in 35 patients with a young age-at-onset, a family history of renal cancer, or a bilateral tumor. Certain HLA antigens (DR5, BW44) were in excess and most pronounced among patients of German or Scandinavian descent. A balanced reciprocal translocation between chromosomes 3 and 8 had been previously reported in a family prone to kidney cancer, but was not observed in Minnesota.

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.

Epidemiologic studies were also initiated in high-risk areas outside the United States. In China the rates of esophageal cancer in Linxian exceed $100/10^5$ in both sexes, and elevated lung cancer rates are found among females in Shanghai, even though few smoke. Pilot studies of these cancers, and of choriocarcinoma in Beijing, were begun in collaboration with the Cancer Institute, Chinese Academy of Medical Sciences, to test the feasibility of conducting full-scale case-control studies. In addition, a pilot nutrition intervention trial involving supplementing diets with multiple micronutrients was launched in Linxian. The investigation will assess practical issues in

the conduct of such an experimental study, which offers potential for evaluating the role of specific nutritional factors in the late stages of cancer in an area of the world where cancer rates are exceptionally high and diets are low in intake of several nutrients.

Analyses of data from a case-control study in Cuba, where lung cancer rates are high by Latin American standards, showed the smoking of dark tobaccos was responsible. Review of interview information from a case-control study of lung cancer in seven European centers was also undertaken. The exceptionally large size of the study (7,500 cases; 15,000 controls) enables precise estimates of risk associated with various cross-classifications of smoking variables, such as duration, amount, filter type, and cessation interval.

Significance to Biomedical Research and the Program of the Institute:

These studies allow the testing of hypotheses regarding the etiology of cancer. 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.

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 interviewing coming to completion in South Carolina, 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. To follow up the leads regarding mouthwash, electronics manufacture, and diet, a new case-control study of oral cancer will be launched in several areas of the country. Should the pilot studies of lung, esophageal, and choriocarcinoma in China prove successful, full-scale investigations of these and perhaps other cancers will be begun to take advantage of unique opportunities for etiologic research in the high-risk areas. A case-control study of gastric cancer in several areas of Italy, including Forli where rates are among the highest in the world, may be begun in collaboration with epidemiologists from Italian cancer institutes should an on-going preliminary investigation show that a multicenter study is feasible.

Publications:

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Kantor, A. F., Blattner, W. A., Blot, W. J., Fraumeni J. F., Jr., McLaughlin, J. K., Schuman, L. M., Lindquist, L. L., Wang, N., and Hozier, J. C.: Hereditary renal cell carcinoma and chromosomal defects. N. Eng. J. Med. 307: 1403-1404, 1982.

McLaughlin, J. K., Blot, W. J., Mandel, J. S., Schuman, L. M., Mehl, E. S., and Fraumeni J. F., Jr.: Etiology of cancer of the renal pelvis. JNCI (In Press)

McLaughlin, J. K., Mandel J. S., Blot, W. J., Schuman, L. M., Mehl, E. S., and Fraumeni, J. F., Jr.: A population-based case-control study of renal cancer. JNCI (In Press)

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Winn, D. M., Ziegler, R. G., Pickle, L. W., Gridley G., Blot, W. J., Hoover, R.: Diet in the etiology of oral and pharyngeal cancer among southern women. Cancer Res. (In Press)

CONTRACTS IN SUPPORT OF THIS PROJECT

WESTAT, INC. (NCI-CP-01044)

Title: Support Services for Epidemiologic Studies.

Current Annual Level: $1,500,000 (total for all support services, including services in addition to those in high risk areas of the U.S.)

Man Years: 45

Objective: To provide technical, managerial, and computer support for epidemiologic studies of cancer, including those in high risk areas.

Major Contributions: Field studies were completed for oral cancer in North Carolina, lung cancer in Florida, and renal cancer in Minnesota. Interviewing and/or computational support were conducted for studies of bladder cancer in New England, nasal cancer in North Carolina and Virginia, esophageal cancer in South Carolina, 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.

LEHIGH UNIVERSITY (NO1-CP-81038)

Title: Support Services for Epidemiologic Studies of Lung Cancer in Communities with Non-ferrous Smelters.

Current Funding Level: Funding completed.

Man Years: 2.8

Objectives: To provide interviewing, data preparation, and environmental measurement support services for a case-control study of lung cancer in eastern Pennsylvania.

Major Contributions: An interviewing and medical records abstract team was established which carries out the field data collection phase of this case-control study and it assembled information on area environmental pollutants.

Proposed Course: This contract expired on April 30, 1981, but results were still being analyzed in FY83.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01CP05128-04 EEB

PERIOD COVERED

October 1, 1982 to September 30, 1983

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Diet and Nutrition in Cancer Etiology

PRINCIPAL INVESTIGATOR *(List other professional personnel on subsequent pages.)*

(Name, title, laboratory, and institute affiliation)

Regina G. Ziegler, Expert, EEB, NCI

COOPERATING UNITS *(if any)*

National Center for Health Statistics; National Institute on Aging, California Tumor Registry; University of Hawaii; University of Southern California; Kaiser Health Plan of Oregon; Kaiser Health Plan of Northern California

LAB/BRANCH

Environmental Epidemiology Branch

SECTION

Environmental Studies Section

INSTITUTE AND LOCATION

NCI, NIH, Bethesda, Maryland 20205

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.6	3.3	0.3

CHECK APPROPRIATE BOX(ES)

☒ (a) Human subjects ☐ (b) Human tissues ☐ (c) Neither

☐ (a1) Minors

☒ (a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

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, folacin, and trace minerals; general nutritional status; anthropometry; biochemical indices, such as serum cholesterol and serum vitamin A; and storage and cooking practices. Cancers being studied include those of the colon, rectum, breast, esophagus, pharynx, oral cavity, lung, cervix, pancreas, stomach, kidney, larynx, and chorion. Case-control studies have been initiated in high risk areas with unusually high cancer mortality conceivably related to diet, and among migrants whose changing cancer rates appear related to new lifestyles, such as among Oriental-Americans. Selected cohorts with relevant dietary or biochemical data already collected, such as HANES I participants, are being followed. Data from HANES I and II 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 determinants of nutrient intake.

PROJECT DESCRIPTION

Names, Titles, Laboratory and Institute Affiliations of Professional Personnel (other than Principal Investigator) engaged on this Project:

A.G. Ershow	Staff Fellow	EEB	NCI
G. Gridley	Health Statistician	EEB	NCI
L.W. Pickle	Senior Staff Fellow	EEB	NCI
R.N. Hoover	Acting Chief	EEB	NCI
W.J. Blot	Chief, Analytical Studies Section	EEB	NCI
A.E. Blair	Chief, Occupational Studies Section	EEB	NCI
D.M. Winn	Staff Fellow	EEB	NCI
L.M. Brown	Epidemiologist	EEB	NCI
L.A. Brinton	Senior Staff Fellow	EEB	NCI
C.E. Land	Health Statistician	EEB	NCI

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 epidemiologic studies. Such hypotheses may concern food groups, food items, macronutrients or micronutrients, general nutritional status, food additives or contaminants, cooking or processing practices, biochemical measures related to diet, or anthropometric parameters. Cancer may be initiated, promoted, or inhibited by such exposures.

2. To test systematically for the existence of associations between diet and specific cancers, and to generate hypotheses about the nature of any relationships detected.

3. To develop and utilize national nutrition data resources that might contribute to cancer epidemiology.

4. To develop and validate methods for nutritional epidemiology, including dietary questionnaires, protocols for laboratory tests, and analytic approaches.

5. 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, 4, 5, 6, 7, 8, 9, 10, 11, and 12 are the nutrition components of studies described in more detail in Project Z01CP04779-07 EEB, Field Studies in High Risk Areas. Studies numbered 18 and 19 are the nutrition components of studies described in more detail in Project Z01CP04481-07 EEB, Studies of Radiation Induced Cancer. The study numbered 16 is the nutrition component of the study described in more detail in Project Z01CP04412-07 EEB, Carcinogenic Effects of Therapeutic Drugs. The study

numbered 17 is the nutritional component of the study described in more detail in Project Z01CP04480-07 EEB, Studies of Occupational Cancer.

A. Studies seeking to explain distinctive geographic patterns in cancer risk, such as those revealed by the U.S. cancer maps, and assessing nutrition as one of several possible reasons:

1. A case-control study of oral and pharyngeal cancer was conducted among women in North Carolina, since the U.S. cancer maps revealed high female mortality rates for these two cancers among white women in the Southeast United States. Interviews were completed for 232 cases, who were diagnosed (or died) in 1975-1978, and 410 controls, individually matched on age, race, county of residence, and source of ascertainment. Next-of-kin provided 59 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.

2. Another case-control study suggested by the U.S. cancer maps focused on an unexpected "hot spot" of colon cancer in several rural farming counties in eastern Nebraska, (unexpected since colon cancer is generally associated with an affluent diet and urbanization). Many people of Czechoslovakian ancestry live in this area; and Czechoslovakians, when compared with other immigrants to the U.S., show elevated rates of colon cancer. Since their traditional ethnic foods might be involved etiologically, emphasis was placed on assessing diet. Interviews were completed for 58 colon cancer and 28 rectal cancer cases, all diagnosed during 1970-1977, 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, and wine and beer consumption.

3. A population-based, case-control study of lung cancer 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. The study was supplemented with a dietary component designed to assess whether carotene, retinol, total vitamin A, or the broader related food groups, or a non-nutritional correlate, was associated with reduced risk. Vitamin A has been postulated to protect against cancer primarily on the basis of experimental studies of pharmacologic doses of retinoids. Carotene has been postulated to protect against cancer primarily because of its chemical properties and the responsiveness of serum carotene to daily diet. The few relevant epidemiologic studies have demonstrated less specific associations: high fruit and vegetable and/or dairy product intake is found among those at reduced risk of certain epithelial cancers.

   In the interview, usual adult frequency of consumption prior to 1975 of 44 food items and a history of vitamin pill usage were collected. Approximately 920 male lung cancer cases diagnosed in 1980-81, and 1043 population controls of comparable age, race, and residence were

interviewed; 38 percent of the interviews were with next-of-kin. The study should also be able to elucidate the interaction between diet and smoking, occupation, and residential history in the development of lung cancer.

4. A parallel case-control study of lung cancer with a dietary component 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. Approximately 945 male and female lung cancer cases diagnosed in 1976-82 and 955 population controls of comparable age, sex, race, and residence--as well as 209 white male laryngeal cancer cases and 250 population controls of comparable age and residence--were interviewed. Approximately 18 percent of the interviews with the lung cancer cases and all 73% of the interviews that could be conducted directly with the laryngeal cancer cases included questions on the usual frequency of consumption, four years earlier, of 37 food items and usual vitamin pill usage.

5. The case-control study of lung cancer in New Jersey conducted during 1980-81 included primarily white men. Comparable population-based case-control studies of lung cancer were initiated among black men and white women in the high risk areas of New Jersey in 1982. The relative risks and exposure rates for the pertinent dietary factors will be compared for the three populations, and the ability of poor diet to explain the rapid rise in lung cancer among U.S. blacks will be evaluated. The dietary component of the interview, expanded to assess other nutrients possibly correlated with retinol and/or carotene intake, like vitamin C, includes the usual adult frequency of consumption of 59 food items and a history of vitamin pill usage. Approximately 300 black male and 900 white female cases of lung cancer are expected to be ascertained for the study.

6. 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 1250 lung, 400 stomach, and 350 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 27 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, and alcohol consumption.

7. 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.

8. A case-control study of esophageal cancer in males was started in 1982 in Charleston, Savannah, and Jacksonville, three major cities with elevated esophageal cancer mortality along the Southeast coast. Black males, in particular, have especially high rates of esophageal cancer in this area. Approximately 50 cases and 50 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 includes 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.

9. Chinese investigators, aware of the extremely high rates of esophageal cancer in Lin County (Linxian), have developed a number of hypotheses concerning the etiology of this cancer, but they have not yet been able to identify any definitive risk factors. A pilot study to test the feasibility of a large case-control study of esophageal cancer was started in 1983 in Lin County. All new cases of confirmed esophageal cancer diagnosed in the early months of 1983 (50-70 persons) and an equal number of age- and sex-matched controls, or their next-of-kin, are being interviewed. The questionnaire focuses on dietary patterns and inquiries about frequency of consumption of moldy or spoiled foods, alcoholic beverages, hot and cold beverages, and a wide variety of locally available foods. Information is sought about two time periods: recent years (circa 1979) and before 1959, a time at which the region underwent rudimentary economic development. Subjects are asked whether they personally experienced famine conditions.

10. The city of Shanghai experiences some of the highest lung cancer rates in the world, particularly for women. The low rate of smoking among Chinese women suggests that other risk factors must be considered. A pilot case-control study of lung cancer in the urban area of Shanghai was begun in 1983 and included a dietary component. The frequency of consumption of retinol-and carotene-rich foods and exposure to cooking oil fumes is receiving special attention. The pilot study has enrolled 50-70 female lung cancer cases and an equal number of controls.

11. Choriocarcinoma, a rare cancer in the United States, occurs more frequently in China and other Far Eastern countries. Apart from hydatidiform mole, no other major risk factors have been defined for this disease. The association of higher incidence rates with poverty and warm climate suggests that poor nutrition may play a role in its etiology. A dietary component was incorporated into a pilot study of choriocarcinoma started in 1983 in Beijing. Twenty-five cases, 25 population controls, and 25 hospital controls are being interviewed. The questionnaire inquires about the frequency of ingestion of sources of high-quality protein, vitamin A, and folic acid. Special dietary habits during

pregnancy are asked about, as well as the consumption of unrefined cottonseed oil, a source of the contraceptive chemical gossypol.

12. A pilot study to test the feasibility of a large-scale, long-term intervention trial to prevent esophageal cancer was initiated in Lin County, Hunan Province, China in January 1983. At that time 1,028 residents of rural Chinese villages in a region with extremely high rates of esophageal cancer were assigned by village to one of three dosage regimens: daily multivitamin pill, weekly multivitamin pill, or weekly placebo. The pills were presented to the subjects in coded blister packs. Pill counts and urinary riboflavin levels will be used to assess compliance. Pill-taking began May 1, 1983 and will continue for 28 weeks.

    Nutritional assessments were carried out on a subsample (N=200) of the study population before the pills were distributed. The same subjects will undergo a second nutritional assessment during the fourth month of the study. The nutritional assessment includes anthropometric measurements, a dietary interview, and biochemical measures of nutritional status. The dietary interview includes a 24-hour recall, food frequency questions, and questions on food preservation techniques used in the home. Special attention is paid to seasonal changes in diet and nutritional status. The biochemical assessment component will determine the prevalence of nutritional deficiency states before and after supplementation with the Recommended Dietary Allowances of a variety of vitamins. The subjects will be assessed for status of retinol, beta-carotene, tocopherol, thiamin, riboflavin, niacin, ascorbic acid, copper, and zinc.

    The intervention trial will be deemed feasible if compliance with pill-taking is good, if the administrative arrangements run smoothly, and if adequate quality control in data collection can be ensured. The results of the nutritional assessment component will be used to determine the composition of the supplement for the full-scale trial.

B. Studies primarily focused on nutritional hypotheses

13. A death certificate-based, case-control study of colorectal cancer was initiated in the three regions of Florida with high rates of inmigration from the Northeast and North Central States. The U.S. cancer maps had shown 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, a reduction that could not be explained by differences in income or population density. Close examination of the age-specific cancer mortality rates for those counties in Florida where many Northerners move at retirement revealed that colorectal cancer rates in those counties were as low as in Southern counties of comparable population and did not rise toward the Northern rates at older ages. This study seeks to explore the characteristics of this apparent reduction, quantify it, and to see whether it might be due to some change in

lifestyle, (e.g., eating more fruit and vegetables or drinking different water), or whether it might be due to the migrants being a self-selected healthy subset of Northerners. The final study population, drawn from the 1979 Florida mortality tape, consists of 935 colon cancer cases, 165 rectal cancer cases, 845 controls dying of other cancers, and 496 controls dying of causes other than cancer. Both control series were frequency-matched to the case series on age, sex, and usual county of residence (only whites were selected). Next-of-kin were interviewed by phone. Questions focused on residential history, medical history, social, economic, and demographic characteristics, and a few indicators of general dietary pattern (usual adult frequency of consumption of 11 food items, before and after migration to Florida).

14. A population-based case-control study of breast cancer in young Oriental-Americans is being started in 1983 in Los Angeles, San Francisco, and Oahu; 450 cases are expected to be or have been diagnosed during 1981-1986. When Oriental women migrate to the U.S., their low rates of breast cancer rise toward American rates over a period of several generations as they adopt a more westernized diet. In this study, diet should be sufficiently heterogeneous to permit the identification of strong associations of diet with breast cancer risk. The study subjects will be 55 years or younger so that they, as well as many of their mothers, can be interviewed about their childhood and adolescent diet. Thus the hypothesis that diet is operative on breast cancer risk primarily during these 2 periods of the lifespan can be evaluated. This study will also permit evaluation in Oriental-Americans of the standard breast cancer risk factors and an estimation of the difference in Oriental and Caucasian breast cancer rates attributable to various risk factors. Serum will be collected from the cases, after diagnosis, and the controls for lipoprotein, tocopherol, retinol, and carotene determinations. In order to study the interrelationships of ethnicity, dietary patterns, and hormonal levels, but to avoid the hormonal changes induced by breast cancer and its therapy, urine and serum will be collected for hormonal assays from the controls only.

15. It has been noted in several longitudinal studies of heart disease that serum cholesterol levels were reduced among those who later developed cancer, particularly colon cancer. A cohort study of serum cholesterol levels and subsequent cancer at any site is being conducted among the 200,000 members of the Kaiser Health Plan of Northern California who participated in multiphase screening, a much larger group than any analyzed so far. The more detailed and accessible records of the Kaiser Health Plan of Portland are being utilized for a case-control study of colon cancer and serum cholesterol that will consider issues such as time elapsed between the cholesterol determination(s) and cancer diagnosis, medical reasons for the cholesterol determination, relationship of multiple cholesterol values, if available, and the exact site, staging, and outcome of the cancer.

C. Analytic studies of special cancers or special populations where nutritional questions are part of the total justification for the study

16. A case-control study involving 500 newly diagnosed cases of invasive cervical cancer, a sample of cases of in situ cervical cancer, and 1,000 neighborhood controls, matched by age and race to the invasive cases, was initiated in 1982 in five Comprehensive Cancer Centers with especially large numbers of cervical cancer patients (Philadelphia, Chicago, Miami, Birmingham, and Denver). 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 postulated to increase the risk of cervical dysplasia, cervical cancer, or cancer in general. The folacin hypothesis is specific for cervical cancer. Moreover, poor nutritional status may partially explain the predominance of cervical cancer in women of low socioeconomic status.

    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 are being collected with which to measure serum levels of retinol, carotene, vitamin C, folacin, and tocopherol and red blood cell folate, and possibly serum cholesterol and ferritin. In addition, serum will be stored for immunologic assays of relevant infectious agents, such as herpes virus type II. Blood is being collected from the cases several months after completion of treatment, when appetite, diet, and metabolism have had an opportunity to revert to patterns existing prior to disease. Blood is also being collected, whenever possible, prior to treatment.

17. Pattern-makers in the auto industry have a 2- to 3-fold excess risk of colorectal cancer, possibly due to occupational exposures, or possibly due to an "affluent" diet related to their high payscale. A screening program run by the pattern makers' union and the Workers' Institute for Safety and Health (WISH) has revealed a particularly high prevalence (approximately 18%) of intestinal polyps among union members. These polyps may be precursor lesions for colorectal cancer. A case-control study of intestinal polyps is being initiated in pattern-makers who have undergone the screening. A self-administered questionnaire asks about usual adult dietary habits. Blood specimens will be obtained for analysis of serum retinol, carotene, tocopherol, serum selenium, total serum cholesterol, and the LDL/HDL cholesterol ratio. Since the cases will have only polyps, not clinically detectable cancer, serum levels will not be altered by the dietary and metabolic changes induced by colon cancer, surgery, and therapy.

18. Exposure to radiation markedly increases the risk of thyroid cancer, but few other risk factors for this disease have been elucidated. The high rates of thyroid cancer in Japanese persons exposed to the atomic bomb during World War II allow for investigation of the interaction of other

risk factors with radiation. Approximately 166 cases of thyroid cancer in residents of Hiroshima and Nagasaki diagnosed since 1959 and 322 age, sex, city, radiation dose and vital status matched controls will be interviewed. A dietary section has been included in the questionnaire. Special attention is paid to frequency of consumption of sources of iodine, dietary goitrogens, retinol, and carotene. An index of "Westernization" of the diet will be constructed.

19. The risk of colon cancer and rectum cancer is elevated two-fold in persons exposed to the atomic bombs of World War II. In addition, the incidence rate for this disease has been rising in the Japanese population, and "Westernization" of diet is considered a prime suspect. Risk factors for colon cancer and rectal cancer will be evaluated separately. Approximately 303 cases diagnosed between 1960-1980 and 606 controls matched on age, sex, radiation dose, city, and vital status will be interviewed. The dietary section of the interview inquires about consumption of "Western" foods, traditional Japanese foods, meat, fiber, retinol, carotene, and fat. An additional component will evaluate recall of diet 20 years in the past by comparing results with those of a dietary interview undergone by the same subjects during the early 1960s.

D. Studies to develop and utilize national nutrition data resources

20. HANES I, the U.S. Health and Nutrition Examination Survey conducted in 1971-74 by the National Center for Health Statistics, collected dietary, biochemical, clinical, and anthropometric information on a national sample of 23,000 people. 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 can 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 are higher in Northeast and North Central states than in the South, and the difference cannot 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.

21. With the serum vitamin A data collected in HANES I for 14,000 adults, possible determinants of serum vitamin A levels are being evaluated, including, sex, race, age, region, poverty status, health status, and diet. Two prospective studies, in the U.S. and England, have recently shown that mean serum vitamin A levels were lower prior to disease, among those that eventually developed cancer. Yet it is generally believed that within a population as well-fed as in the U.S., serum vitamin A levels are not reflective of daily vitamin A intake but are maintained at a constant high level by adequate liver stores.

22. In a third HANES study reliable predictors of age at menarche are being sought in the data on food group consumption, macronutrient intake, and anthropometry. Approximately 100 women between the ages of 12 and 18 were

examined in this study. In international correlation studies and in case-control studies, a young age at menarche is associated with an elevated risk of breast cancer. Internationally, a young age at menarche is also correlated with certain broad dietary patterns. It is possible that within the U.S. population young age at menarche may be an informative indicator of dietary patterns that promote breast cancer.

23. Using the HANES I 24-hour dietary recalls, individual food items were ranked by their contribution to total vitamin A intake for various age-sex-race-region subpopulations. The 1689 different food items reported were combined into 485 food items based on their mean vitamin A content per serving and their generic nature. Case-control interview studies examining the association between vitamin A and cancer at various sites have been hampered by the restricted time available for interview. In the past, interviews have included different abbreviated lists of food items, or even broad food groups, thus limiting the comparability of results.

24. In 1982-85 the Environmental Epidemiology Branch (EEB), in cooperation with the National Institute on Aging, other NIH Institutes, and the National Center for Health Statistics, will trace and re-interview, if still living, the 14,000 adults examined in HANES I 8-14 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 evaluated. Once these people are traced, their social security numbers will be obtained so that further cancer mortality can be monitored with the National Death Index. A comprehensive dietary section, designed to assess exposure to those food groups, food items, nutrients, additives, and cooking practices now suspected of being related to cancer, was drafted by the EEB and incorporated into the re-interview. Analysis of these new data should provide useful descriptive information on dietary practices and dietary variation within the U.S. In addition, the expanded dietary section should facilitate future analyses of diet and cancer relationships within this cohort.

Major Findings:

1. In the study of oral and pharyngeal cancer among North Carolina women, risk was associated with low fruit and vegetable intake within the entire population and within all subgroups examined. Those in the lowest quartile of fruit and vegetable consumption had a risk of 1.9 relative to those in the highest quartile; the trend over quartiles was statistically significant. Thus, vitamin C, carotene, fiber, or some other factor concentrated in fruits and vegetables, could be protective. There was no evidence that the underlying risk factor was poor nutritional status. Low bread and cereal intake was also associated with increased risk, as was high meat and fish intake, possibly a result of meat and fish being prepared or preserved in an unusual way. Neither relationship was as consistently noted within all subgroups as was the fruit and vegetable association. All the dietary associations were independent of each other, and they were not confounded by the other major risk factors in this

population, (snuff, cigarette, or alcohol use), nor by socioeconomic status or dental health.

2. In the study of colorectal cancer in rural Nebraska, increased risk was noted among persons of Czechoslovakian ancestry, with Bohemians and Moravians predominating in the study area. Among Bohemians risk was associated with diets high in fat (meat and dairy products) and sweets. Colon cancer risk was elevated among beer drinkers regardless of their ethnic background, Bohemians being particularly heavy consumers. Risk was also associated with intestinal polyps, reported more often by Moravians, and with familial occurrence of gastrointestinal and other cancers. Since 1950-1969, the period for which the U.S. cancer maps identified a "hot spot" of colon cancer in this region, the mortality and incidence rates for colon cancer have declined, presumably as a consequence of acculturation of the American-born descendants of Czech immigrants.

3. In the study of lung cancer among white men in New Jersey, preliminary analysis shows that risk, adjusted for smoking, increases with decreased intake of carotene. The intake of retinol and total vitamin A are not associated with risk, but fruit and vegetable consumption shows as strong an inverse association as carotene intake. Both fruit and vegetable consumption and carotene intake seem to be protective primarily among moderate and heavy smokers.

4. In the study of lung cancer in Louisiana fruit and vegetable intake was inversely associated with risk in whites and blacks of both sexes. Those in the lowest quartile of fruit and vegetable consumption in each sex-race group had from 1.5 to 1.9 times the risk of those in the highest quartile. Adjustment for alcohol and cigarette use and Cajun lifestyle did not markedly reduce these associations. No other consistent findings with diet were noted in all sex-race groups.

5. In the study to identify vitamin A indicator foods, the index of vitamin A contribution (considering frequency of consumption, portion size, and vitamin A density (IU per 100 mg)), was used to rank the food items in various subpopulations. A comparison of these ranks identified certain fruits and vegetables whose relative contribution to vitamin A intake varied by sex-race group, season of interview, and region of the country. Age and poverty level had little effect on the food rankings. The major contributing foods for any subpopulation included both retinol (dairy products, liver) and carotene (certain fruits and vegetables) sources of vitamin A, and included items (e.g., mixed tomato dishes) not usually considered. The top 50 foods were adequate to classify correctly 80%-90% of the individuals into low, moderate, and high consumption categories.

Significance to Biomedical Research and the Program of the Institute:

Specific foods and food groups, nutrient levels, general dietary patterns, overall nutritional status, cooking and storage practice, and food consumption of additives and contaminants, are being recognized as possible causes of

cancer. Certain diets and foods seem able to initiate carcinogenesis, others seem to promote it, while still others seem to reduce cancer risk. 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/or regulate. Epidemiologic 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, clinical observations, and descriptive epidemiology. In addition, exploratory nutritional epidemiology can suggest associations between dietary patterns and cancer, which then serve as the basis for further laboratory research and analytic epidemiology.

Proposed Course:

Analysis will continue on recently completed studies and will be started on studies where data are currently being collected. The recently completed studies include male lung cancer in New Jersey, lung and laryngeal cancer along the Texas Gulf Coast, lung, stomach, and pancreas cancer in Louisiana, kidney cancer around Minneapolis-St. Paul, and colorectal cancer among Florida migrants. Now in the field are case-control studies of lung cancer among white women and black men in New Jersey, male esophageal cancer along the South Carolina-Georgia coast, breast cancer among young Oriental-Americans, colorectal and thyroid cancer in Japan, invasive cervical cancer at five cancer centers, and intestinal polyps among pattern-makers.

Additional emphasis will also be placed on international studies, since many of the provocative high risk areas identified within the U.S. (by the cancer maps) have been investigated. International studies in high risk areas include three case-control studies planned for China: esophageal cancer in Linxian, lung cancer among women in Shanghai, and choriacarcinoma in Beijing. An ambitious intervention trial of multivitamin supplements among 1600 individuals with severe esophageal dysplasia, and possibly 20,000 other individuals residing in the high risk province of Linxian, is also being considered. The protocols for these four studies are currently being tested for feasibility. Also, a large population-based, case-control study of stomach cancer is being designed for several provinces near Florence, Italy, where gastric cancer rates are among the highest in the world.

The results from the studies of colorectal cancer among Florida migrants and breast cancer among young Oriental-Americans will determine the direction in which the nutrition research on these major cancers will go. If the death certificate-based study of colorectal cancer in Florida migrants shows a reduction in risk upon migration from the Northeast-North Central states to the South, and defines the demographics of the subpopulation in whom the risk reduction is greatest, an incident case-control study of colorectal cancer will be initiated in Florida. This study might use both Northern and Southern controls, to ascertain the specific dietary and other lifestyle patterns associated with the reduction in risk. If the case-control study of breast cancer in young Oriental-Americans shows a strong association of Western diet,

(measured by any dietary parameter) with risk, and defines the period of life during which sensitivity to diet is most pronounced, an additional study will be initiated in Oriental populations. Dietary questions will be directed toward the relevant period of life, to try to identify the characteristics of a Western diet that influence breast cancer risk. Also under consideration is a large case-control study of endometrial cancer, a cancer for which there is strongly suggestive, but not direct, evidence of an association with a Western, high-fat diet.

Analytic studies of cancers of special interest will continue to include diet as a potential risk factor. The assessment of diet will be, to some extent, comparable from study to study so that any associations found can be said to be specific for the cancer site, and not a function of the interview instrument, the method of analysis, or selective recall. If certain cancers have no persuasive associations with diet, that, too, will be reported. Currently being planned for the U.S. are analytic case-control studies of biliary tract, salivary gland, oral, and pharyngeal cancer. Major case-control studies of pancreatic, liver, and prostate cancer in the U.S. are being contemplated, and will address the nutritional hypotheses for these cancers; but the final decisions await the results of studies by other investigators. As our experience in developing short, reliable interviews that can distinguish broad dietary patterns increases, more of the Branch's studies of cohorts and families of special interest will incorporate a brief dietary component.

HANES I will continue to provide data for descriptive, methodologic, and hypothesis-testing studies. Other national nutrition data resources becoming available include HANES II, similar to HANES I but conducted during 1976-80 on another large U.S. sample, HANES III, which was concentrated in Spanish-speaking areas of the U.S., and the 1978 USDA Food Consumption Survey, which oversampled in Alaska, Hawaii, and Puerto Rico and collected 24-hour recalls over several consecutive days. The EEB will continue to collaborate with other Federal agencies in the design of national nutrition surveys, just as it had the NCHS in the development of the HANES I follow-up and the HANES III instrument. The logistics of analyzing the data from the HANES I follow-up are now being discussed with the NCHS and the other participating Institutes. Morbidity and mortality results are expected in the next 2-3 years.

Other methodologic and descriptive reports will be based on our experience in fielding and analyzing nutritional epidemiology studies. For example, the dietary interviews for the lung cancer studies, systematically designed by a nutritionist, should provide useful descriptive information on vitamin pill consumption among representative healthy adult men and women, and on the need to distinguish between seasonal and non-seasonal consumption of fruits and vegetables, in order to develop reliable measures of the intake of such nutrients as carotene.

The analytic study of invasive cervical cancer was the first attempt by the EEB to introduce a biochemical component into a large case-control study. Results should provide guidance for incorporating laboratory measures into

future nutritional epidemiology studies, clearly an important trend within Branch activities. This study should provide practical information on the impact of cancer diagnosis and treatment on serum nutrient levels, the variability inherent in non-fasting serum nutrient measurements, the correlation of serum and dietary measures of the same nutrient, and viable collection, storage, and nutrient assay procedures for extensive field studies.

Publications:

Pickle, L. W., Greene, M. H., Ziegler, R. G., Toledo, A., Hoover, R., Lynch H. T. and Fraumeni, J. F., Jr.: Colorectal cancer in rural Nebraska. Cancer Res. (In Press)

Pickle, L. W. and Hartman, A. M.: Indicator foods for vitamin A assessment. Nutr. Cancer. (In Press)

Winn, D. M., Ziegler, R. G., Pickle, L. W., Gridley, G., Blot, W. J. and Hoover, R.: Diet in the etiology of oral and pharyngeal cancer among Southern women. Cancer Res. (In Press)

Ziegler, R. G.: Assessing diet in case-control studies of cancer. In Zervos, C. (Ed.): Proceedings for a Conference on Exposures Assessment: Problems and Prospects. Bethesda, MD, Sept. 13-14, 1982. (In Press)

ANNUAL REPORT OF

THE CARCINOGENESIS EXTRAMURAL PROGRAM

NATIONAL CANCER INSTITUTE

October 1, 1982 through September 30, 1983

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, cooperative agreements, interagency agreements, and contracts.

The CEP was established to integrate the management and coordination of these diverse activities. The Program contains three branches: the Biological Carcinogenesis Branch, the Chemical and Physical Carcinogenesis Branch, and the Special Programs Branch. It has a current on-board staffing level of 27 full time permanent positions and has a budget of $125 million in Fiscal Year 1983.

Technical review of all research proposals (contract, cooperative agreement and grant) is now conducted by either the Division of Research Grants (NIH) or the Division of Extramural Activities (DEA) utilizing peer review groups whose members are drawn from the outside scientific community. In the past year, steps have been taken to also include the technical review of research support and resource contracts within DEA. For contracts, review for relevance, priority and need are performed by the senior staff of the Division of Cancer Cause and Prevention (DCCP). All new initiatives require concept approval by DCCP's Board of Scientific Counselors, an external peer review group, before steps are taken to implement the activity by the issuance of a Request for Applications (RFA) or Request for Proposals (RFP).

The figures appearing at the end of this discussion are an attempt to present graphically some summary information on the CEP program. These figures illustrate the trends in our activities over the past five years as well as the distribution of our resources for the current fiscal year. The data for past years are "actuals" while data for this fiscal year are estimates based on our best projections at the time of report preparation (June 1983). It should be pointed out that, as a result of early preparation, some discrepancies will occur throughout the report, when numbers of research grants or total support levels are discussed. In general, discussions of program areas are restricted to those research grants active in the period October 1, 1982 through June 1, 1983; additional research grants will be funded during the remaining four months of the fiscal year but their individual focus and exact support level is uncertain at this time. Based on past experience, we are able to estimate their impact on budget at the Branch level, but not their impact on individual programs within a Branch.

A number of trends, evident from these figures, are worthy of note. From the first figure, the decreased level of contract support within CEP is evident. It is also clear that many of the funds made available by this reduction have been utilized to increase support for research grants. It should also be noted that in-house costs remain low relative to the total budget being managed, and the cooperative agreement is now being utilized as an additional instrument of support. The second figure illustrates the fact that the reduction in contract support has fallen proportionately in each of the branches. It also suggests that little more funding for the support of research grants can be made available from this source. The third figure reinforces the effectiveness of our efforts to reduce our utilization of the contract mechanism and that the great majority of the funding made available in this way has come from the research contracts category. We have also been successful in reducing the costs of providing resources to the scientific community by converting many resource contracts to a "pay-back" system, but the magnitude of the recovered funding is small compared to that made available by the phasing out of research contract support.

The fourth, fifth and sixth figures illustrate the posture of the program with respect to "assistance funds." The use of this term is made necessary by our use, during this fiscal year, of the cooperative agreement (U01). This, like the research grant, is an assistance instrument used to support investigator-initiated research. It differs from a grant in that there is substantial involvement on the part of the awarding agency in the conduct of the activity. CEP has made use of this instrument for the support of certain studies focusing on the acquired immunodeficiency syndrome (AIDS) where it was felt appropriate for institute staff to have a significant role in coordinating the studies, facilitating information exchange, consideration of proposed treatment protocols and making sure that optimal use is made of biological samples which become available from the individual initiatives.

It is clear from these figures that all of the CEP Branches are increasing their support of grants and cooperative agreements, that the vast majority of available research grant funds (and an increasing proportion) are committed to the funding of non-competing renewals, and that more than 95% of available assistance dollars are used for the support of traditional R01 and P01 research grants.

Significant changes for fiscal year 1983: We have continued to reduce the level of contract support used in providing resources to the research community in general, and have introduced cost recovery mechanisms into most of our resource provision activities. These modifications have significantly reduced the cost of providing resources as well as resulting in the elimination of some 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, in the opinion of the members of DCCP's Board of Scientific Counselors, are inadequately covered by grants. A number of Requests for Grant Applications 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 have 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.

A major emphasis, during this fiscal year, has been placed on investigation of the emerging epidemic of acquired immunodeficiency syndrome (AIDS). Since June of 1981 investigators have identified an epidemic of this condition in the United States. As of May 9, 1983, 1410 U.S. cases of the syndrome had been reported to the Centers for Disease Control. Although the prepondence of the reports have come from the U.S., an additional 103 cases had been reported from 16 other countries. Since the recognition of this entity, the rise in new cases has been exponential with high case fatality rate within two years of onset. The underlying immune defect in these patients is complicated by the advent of Kaposi's sarcoma (KS) and/or any of a variety of opportunistic infections, the most common of which is pneumocystis carinii pneumonia.

The NCI became concerned with this problem very early because of the unusual nature of the tumor being reported. Prior to the emergence of this epidemic, KS had been reported infrequently in this country, usually as an indolent lesion of elderly males. Suddenly there were reports of KS in young males, superimposed on an immune defect, where the lesion was rapidly progressing. This Division, in collaboration with the Division of Cancer Treatment, issued an RFA seeking applications on the epidemiology, diagnosis, treatment and immunology of this condition. By the time the RFA was advertised, interest on the part of the extramural scientific community was great and increasing. The response to the RFA was excellent, a process of expedited review was initiated (including a mail ballot of the National Cancer Advisory Board) and awards were made early in this fiscal year. Because of the dramatic public health importance of the issue, both of the initiating divisions made additional funding available for the support of applications, the Division of Cancer Biology and Diagnosis freed additional funds to support applications in the area of basic immunology and the National Institute of Allergy and Infectious Diseases (NIAID) committed funds for the support of approved applications which could not otherwise have been funded.

By the time the responses had been evaluated, it had become clear that we were dealing with an emerging epidemic and major collaborative efforts between Institutes were initiated. The epidemic pattern observed to date suggests an infectious etiology for the underlying syndrome with transmission by sexual contact or by exposure to blood or blood products. The case fatality rate is high, survival after the onset of symptoms is short and no known therapeutic measures have been effective. The Biological Carcinogenesis Branch, CEP, has recently issued (May 1983) an RFA entitled "Infectious Etiology of Acquired Immune Deficiency Syndrome and Kaposi's Sarcoma" in collaboration with NIAID. An additional RFP was also recently issued (April 1983) entitled "The Natural History of Acquired Immune Deficiency Syndrome in Homosexual Men," again in collaboration with NIAID. It is anticipated that awards will be made under one or both of these advertisements during the current fiscal year.

During this fiscal year individuals have been identified (in the Division of Resources, Centers and Community Actitivies [DRCCA]) who will be responsible for the overall coordination of all NCI efforts in the areas of: 1) nutrition, 2) chemoprevention and 3) smoking and health. Major efforts have been made to develop long-term plans for the further development and conduct of the programs. CEP staff members have been extensively involved in these planning initiatives and the plans developed either have been presented to the divisional Boards of Scientific Counselors and the National Cancer Advisory Board, or will be presented in the near future.

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 studies on the etiology of the Acquired Immune Deficiency Syndrome have implicated the human T-cell leukemia-lymphoma virus (HTLV) as a possible agent associated with the disease. Nineteen of 75 AIDS patients, including 60 homosexual males, had antibodies directed against cell membrane antigens associated with HTLV. Six of 23 homosexual male patients with lymphadenopathy prodrome were also seropositive for HTLV. More intensive efforts are now being made to ascertain the role of HTLV in AIDS.

Some cancers are consistently characterized by specific chromosomal translocations. Study of translocations in human Burkitt lymphomas (BL), whether carrying the Epstein-Barr virus or not, has provided a firm molecular basis for the oncogene-activation hypothesis. The human cellular analogue of the v-myc oncogene c-myc, has been localized on the distal segment of the normal chromosome 8 and is transposed to the 14q+ chromosome in BL cells in all cases examined. Detailed studies on c-myc oncogene expression are ongoing to determine its role in malignant transformation.

The ability to detect and diagnose cancer at an early stage of disease increases the probability of successful treatment. Results from a current multi-institution study indicate that certain anti-Epstein-Barr virus antibodies are valuable markers for clinicians for the diagnosis of undifferentiated types of human North American nasopharyngeal carcinoma including occult primary tumors. The IgA anti-VCA antibody response is the most specific for this disease and of the greatest diagnostic value when used alone or in combination with the anti-EA test.

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.

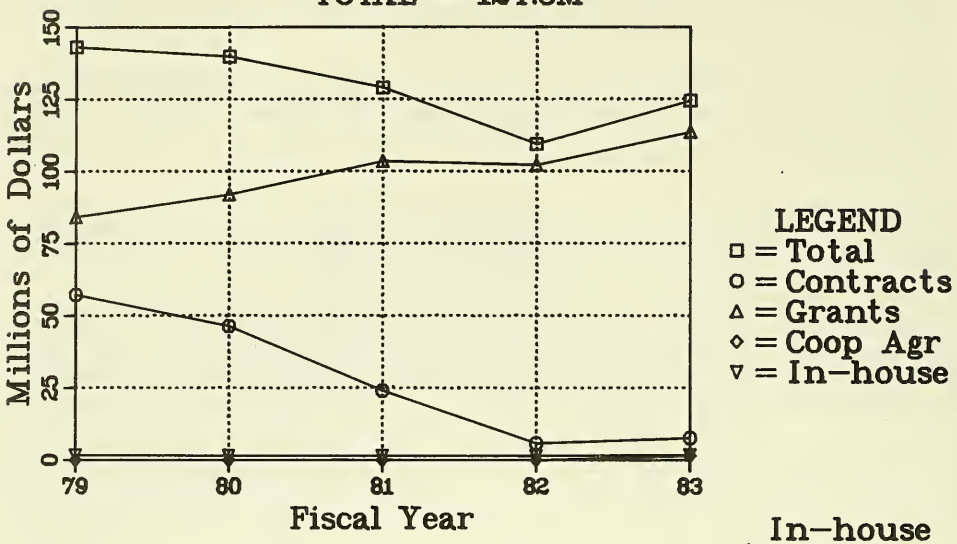
A disturbing finding has emerged from studies in the chemoprevention area during this fiscal year. A substance, shown in a number of prior studies to

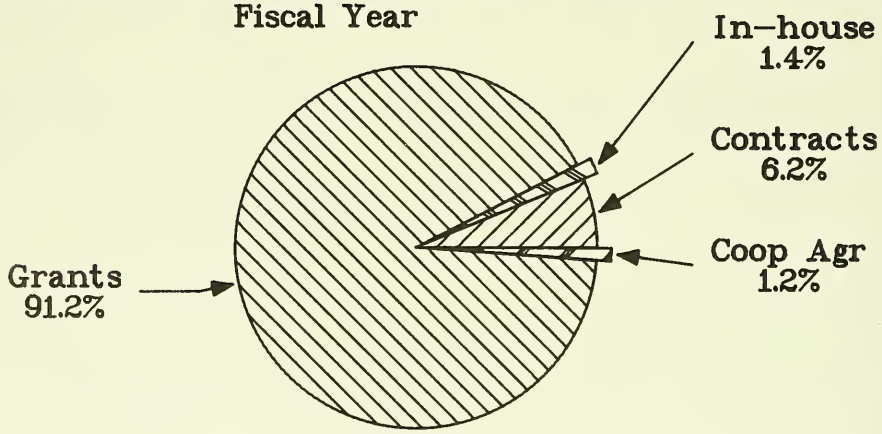
have an anti-carcinogenic effect against liver tumors, now appears to have a promoting effect on tumors of another site (bladder). The finding, if substantiated, suggests the need for extreme caution in attempting to apply findings from a single model system to other situations.

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 single most dramatic finding in the Epidemiology Program in 1982 was the death of a 7 year old Taiwanese child from primary hepatic cancer. He was born of a carrier mother, was free of disease in early infancy, contracted hepatitis by his seventh month, became chronically infected and subsequently died of hepatoma. This occurrence documents that childhood liver cancer deaths as well as adult liver cancer deaths, can be attributed, at least in part, to hepatitis B infection. Much research in this area is being supported in CEP. In the long term, it seems probable that means will be found to reduce the cancer burden related to this tumor in the population.

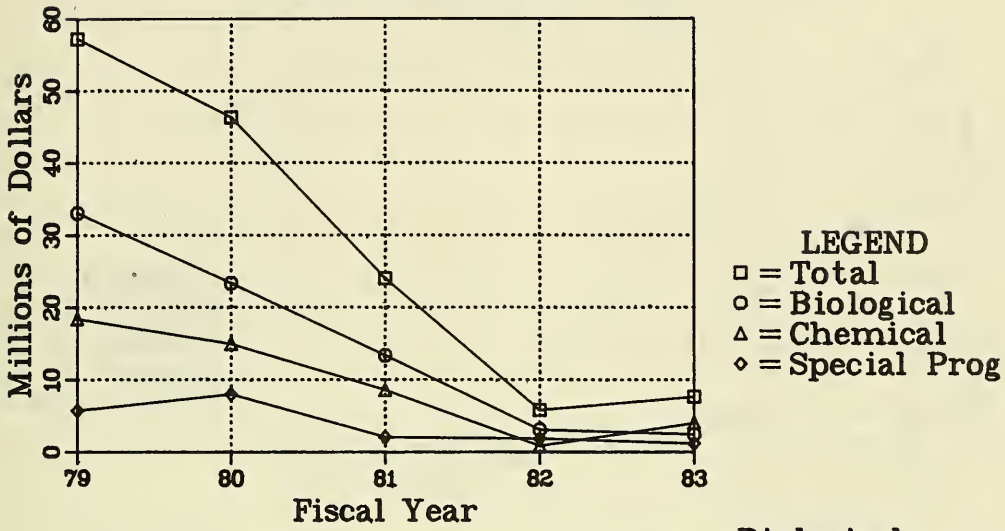
CARCINOGENESIS EXTRAMURAL PROGRAM
TOTAL = 124.5M

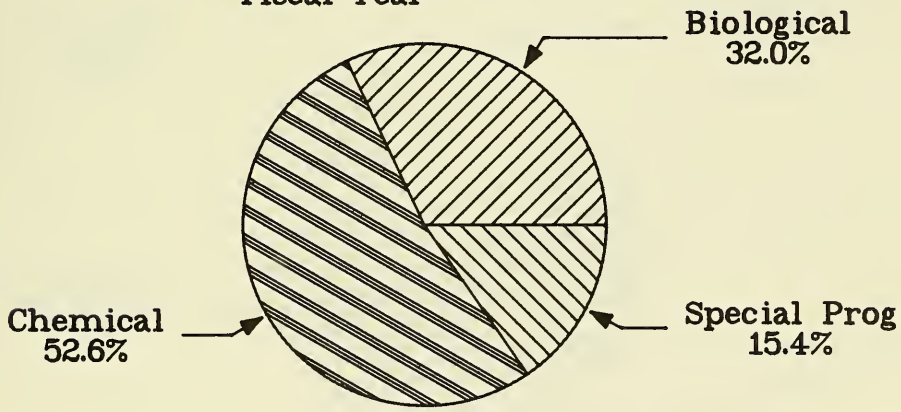




FISCAL YEAR 1983 ESTIMATE	$ (Millions)	%
Contracts	7.66	6.2
Grants	113.60	91.2
Cooperative Agreements	1.46	1.2
CREGs/RFA	(8.72)	(7.0)
Subtotal	122.72	98.6
In-House	1.77	1.4
TOTAL	$124.49	100%

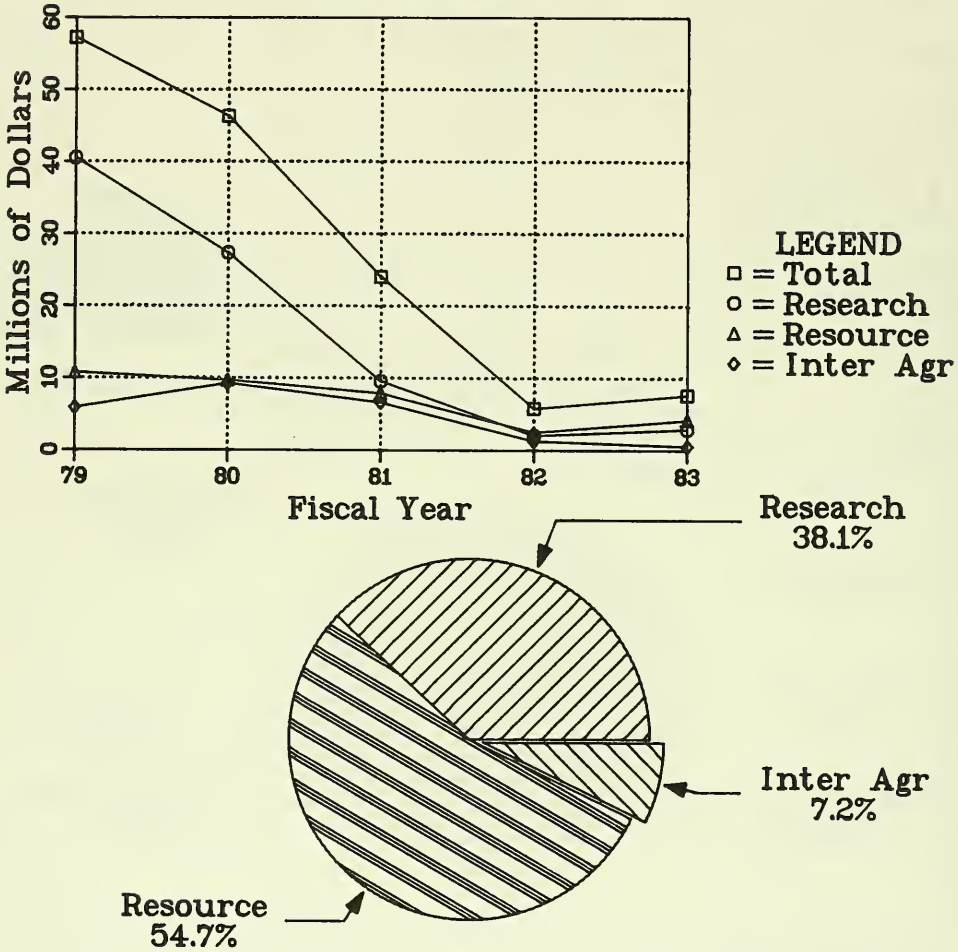
DISTRIBUTION OF CONTRACT FUNDS BY CEP BRANCH



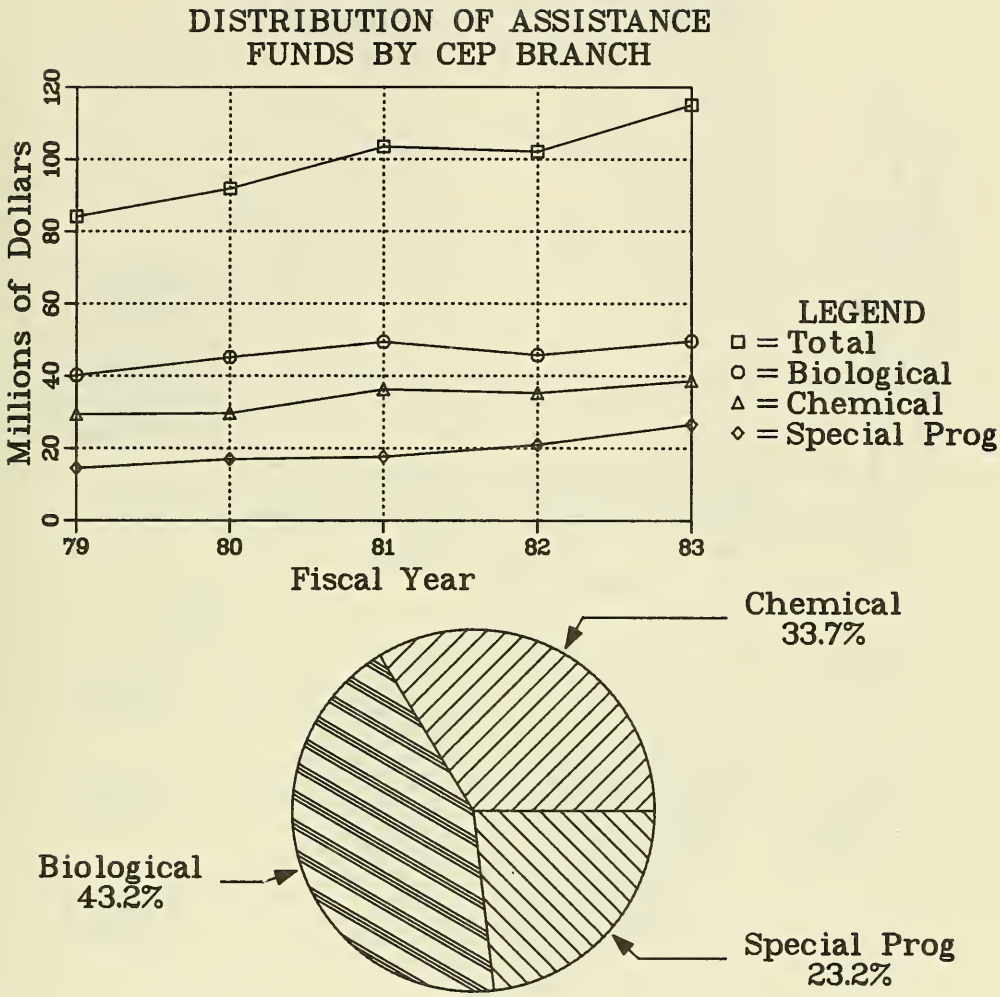


FISCAL YEAR 1983 ESTIMATE	$ (Millions)	%
Biological Carcinogenesis Branch	2.45	32.0
Chemical and Physical Carcinogenesis Branch	4.03	52.6
Special Programs Branch	1.18	15.4
TOTAL	$ 7.66	100%

DISTRIBUTION OF CONTRACT FUNDS BY CATEGORY

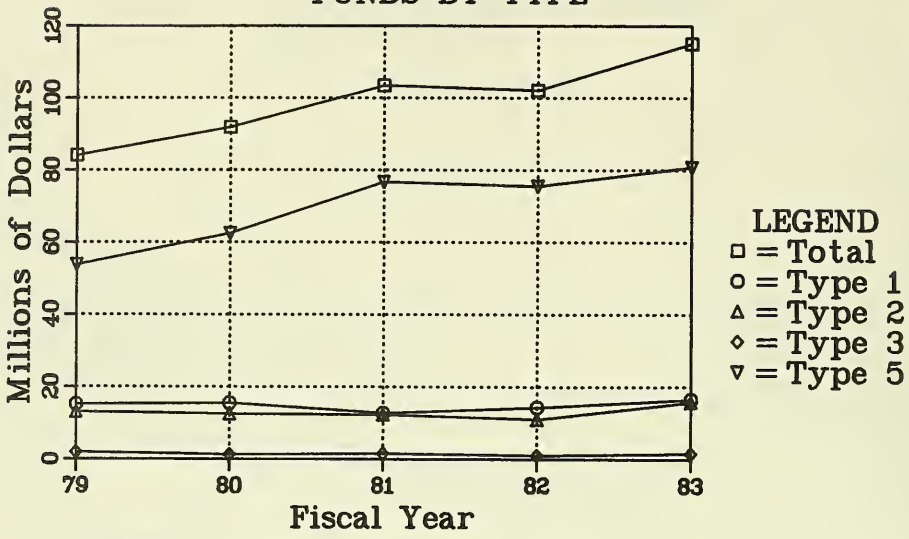


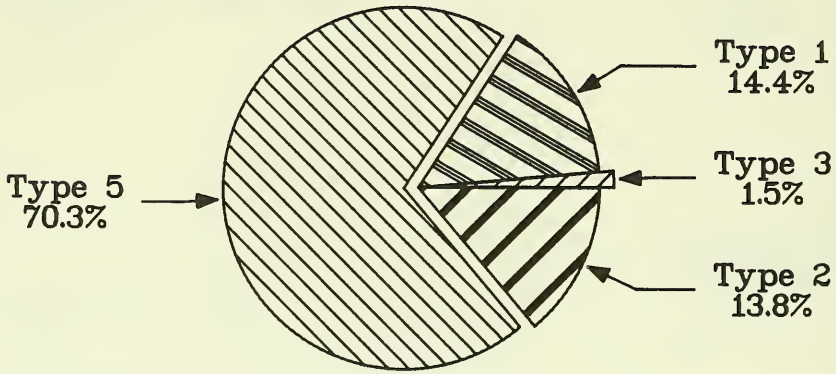
FISCAL YEAR 1983 ESTIMATE	$ (Millions)	%
Research Contracts	2.92	38.1
Resource Contracts	4.19	54.7
Interagency Agreements	.55	7.2
TOTAL	$ 7.66	100%



FISCAL YEAR 1983 ESTIMATE	$ (Millions)	%
Biological Carcinogenesis Branch	49.68	43.2
Chemical and Physical Carcinogenesis Branch	38.72	33.7
Special Programs Branch	26.66	23.1
TOTAL	$ 115.06	100%

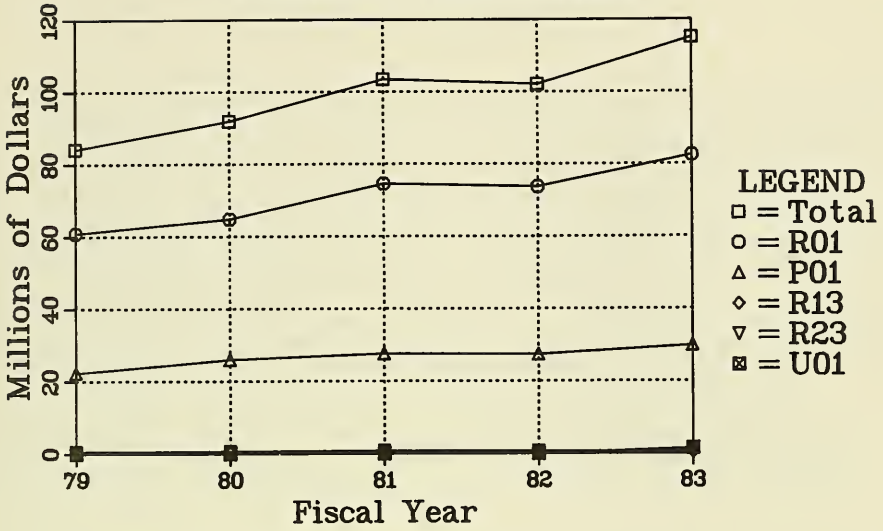
DISTRIBUTION OF ASSISTANCE FUNDS BY TYPE

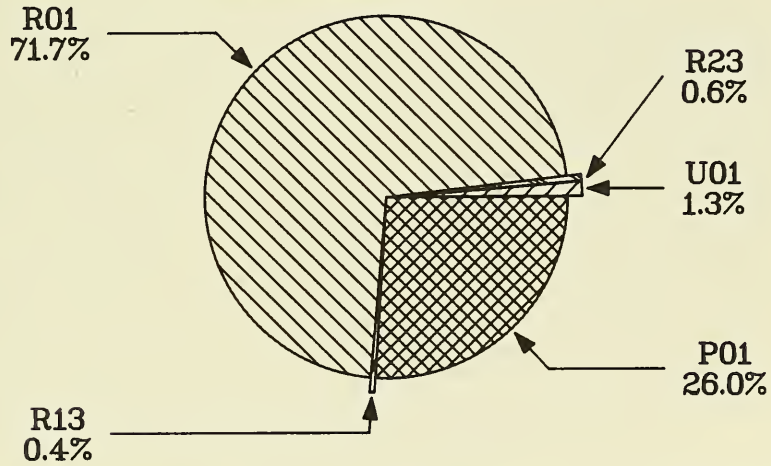




FISCAL YEAR 1983 ESTIMATE	$ (Millions)	%
New (T1)	16.62	14.4
Competing Renewals (T2)	15.89	13.8
Supplement (T3)	1.68	1.5
Continuation (T5)	80.87	70.3
TOTAL	$ 115.06	100%

DISTRIBUTION OF ASSISTANCE FUNDS BY INSTRUMENT





FISCAL YEAR 1983 ESTIMATE	$ (Millions)	%
Traditional Research Grants (R01)	82.52	71.7
Program Project Grants (P01)	29.93	26.0
Conference Grants (R13)	.50	.4
Young Investigator Grants (R23)	.65	.6
Cooperative Agreements (U01)	1.46	1.3
TOTAL	$ 115.06	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 the 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 350 research activities with an annual budget of approximately 56 million dollars. The research projects of the branch divide into three main categories: (1)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 DNA Virus Studies. (2)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. (3)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. The Office of the Branch Chief serves as the focal point for cooperative agreements concerning virological studies on the Acquired Immune Deficiency Syndrome (AIDS) and the issuance of an RFA for cooperative agreements to search for the etiological agent(s) of AIDS.

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. In last year's report, the resources

payback system was described in detail. There are currently six resource contracts functioning in the payback mode. These include two for production of viruses, three for animal resources, and one for specialized testing services. Overall, the payback system is performing as expected. The demand for high quality biological reagents not readily available from commercial sources has remained fairly constant. Reimbursement for full or partial costs of services has led to more careful use of costly resource reagents, with a subsequent reduced level of effort in several resource contracts resulting in significant savings in DCCP expenditures.

Table I focuses on mechanisms of support of extramural research and related activities in the area of biological carcinogenesis. The total budget in FY83 is estimated to be about one million dollars less than the FY82 budget. The primary reason for the decreased funding was the programmed termination of approximately 16 contracts. Table II provides an estimate of grant and contract support, respectively, in each of the four Branch components and the Office of the Branch Chief described above. The Branch administers 16 contracts and 356 grants.

Two RFAs were published this year: one for traditional research grant applications and the other for cooperative agreements. The traditional grant RFA with a set aside of $900,000 per year for four years seeks to encourage research to determine: (a) whether or not hepatitis B virus is a complete carcinogen in humans; (b) the molecular mechanisms underlying the viral transformation of hepatocytes to malignant cells in human and model systems; (c) the characteristics of model systems already developed in terms of their suitability for studying the development of hepatocellular carcinoma and establishing their relevance, if any, to human disease; (d) whether or not any of the gene products of the hepatitis B virus are transforming proteins.

Up to $2,000,000 per year for up to five years was set aside by the NCI and NIAID for research on the infectious etiology of AIDS and Kaposi's sarcoma (KS). The purpose of this RFA is to stimulate studies aimed at a direct microbiological approach to the problem. It is designed to encourage studies on the search for the isolation, and characterization of the biological agent(s) which may be the primary causative factor(s) in AIDS and KS. The studies are to encompass not only the classical microbiological technologies for isolation and characterization of the agent, but also the contemporary technologies of immunology, cytogenetics, and molecular biology.

The BCB sponsored two workshops in May 1983 at NIH in Bethesda. The objective of both workshops was to determine what areas of research needed increased efforts to resolve specific problems. The "Workshop on Bovine Leukemia Virus" was chaired by Dr. M. Baluda and had as an additional objective the determination of need for resources and services useful in bovine leukemia virus (BLV) research. A major topic of discussion concerned the relationship of BLV with the human T-cell leukemia-lymphoma virus. A workshop on "Chronic Carriage of Hepatitis B Virus: Neoplastic Sequelae and Possibilities for Intervention-Prevention" was chaired by Dr. M. Essex and Dr. W. Rutter. The primary topics discussed concerned secondary prevention, immunology of hepatitis B virus (HBV) and primary hepato- cellular carcinoma (PHC), and immunotherapy of HBV-PHC. The recommendations and discussions from both workshops are being evaluated for possible new initiatives.

Investigations carried out in the Biological Carcinogenesis research program have continued to yield valuable insights on cause and prevention of cancer. The highlights of the past year are presented here and in greater detail in the section reports which follow.

Recent studies on the etiology of AIDS have implicated the human T-cell leukemia-lymphoma virus (HTLV) as a possible agent associated with the disease. Nineteen of 75 AIDS patients, including 60 homosexual males, had antibodies directed against cell membrane antigens associated with HTLV. Six of 23 homosexual male patients with lymphadenopathy prodrome were also seropositive for HTLV. More intensive efforts are now being made to ascertain the role of HTLV in AIDS.

Some cancers are consistently characterized by specific chromosomal translocations. Study of translocations in human Burkitt lymphomas (BL), whether carrying the Epstein-Barr virus or not, has provided a firm molecular basis for the oncogene-activation hypothesis. The human cellular analogue of the v-myc oncogene, c-myc, has been localized on the distal segment of the normal chromosome 8 and is transposed to the 14q+ chromosome in BL cells in all cases examined. Detailed studies on c-myc oncogene expression are ongoing to determine its role in malignant transformation.

The ability to detect and diagnose cancer at an early stage of disease increases the probability of successful treatment. Results from a current multi-institution study indicate that certain anti-Epstein-Barr virus antibodies are valuable markers for clinicians for the diagnosis of undifferentiated types of human North American nasopharyngeal carcinoma including occult primary tumors. The IgA anti-VCA antibody response is the most specific for this disease and of the greatest diagnostic value when used alone or in combination with the anti-EA test.

The cellular oncogenes ras^H and ras^K are members of a family of related cellular genes. The involvement of two different members of a gene family in human bladder, lung, and colon carcinomas suggests the possibility that they may also be involved in other human carcinomas. The transforming activity of ras^K genes in human lung and colon cells is activated by mutations of the ras^K which alter the structure of the expressed p21 proteins.

The oncogenicity of Abelson murine leukemia virus is attributed to expression of a viral transforming gene v-abl which encodes a protein with tyrosine-specific kinase activity. However, oncogenesis may not be a single-step consequence of v-abl expression. Recent results suggest that v-abl expression may induce early events in the neoplastic process and that secondary activation of a distinct cellular transforming gene is necessary for progression to neoplasia.

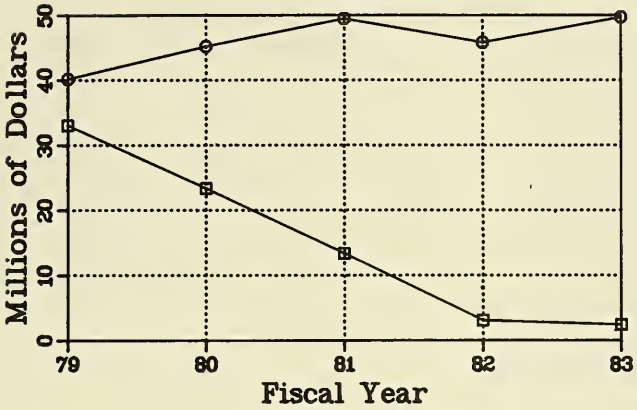
Although new oncogenic avian retroviruses continue to be isolated, their oncogenes (or long terminal repeats) do not deviate from the general patterns already established for these genes or sequences in terms of their modes of action and their mechanisms of insertion into the viral genome and their expression. Many investigators are now seeking intracellular targets (substrates) for oncogene products, most of which are protein kinases. While

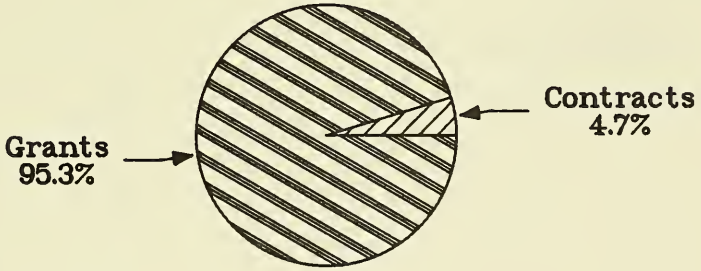
a number of putative targets have been found, no single protein or any combination of identified proteins have been reported which are sufficient to maintain the transformed phenotype.

In studies of HBV and PHC, the chimpanzee, which is the only animal other than man which becomes infected with HBV, was shown not to have integrated viral DNA in its liver cells. This is true in animals which have been chronic carriers for at least five years, and may explain why these animals do not develop PHC. In contrast, HBV DNA has been found to be integrated in unique sites in the liver cells of PHC patients.

A patent application has been filed for a serologic test for the diagnosis of non-A non-B hepatitis in humans. This may be important since non-A non-B hepatitis resembles hepatitis B virus both in its tendency to establish chronic infections or carrier states and perhaps in its potential for causing liver tumors as well.

BIOLOGICAL CARCINOGENESIS BRANCH





FISCAL YEAR 1983 ESTIMATES	$ (Millions)	% of Program
Contracts	2.45	4.7
Grants	49.68	95.3
Total	$ 52.13	100.0

TABLE I
BIOLOGICAL CARCINOGENESIS BRANCH
(Extramural Activities - FY 1983 - Estimated)

	No. of Contracts/Grants	$ (Millions)
Research Contracts	6	0.41
Research Grants	356	54.20
Traditional Project Grants (324 grants; $37.86 million)		
Conference Grants (4 grants; $0.08 million)		
New Investigator Research Grants (4 grants; $0.19 million)		
Program Project Grants (21 grants; $15.27 million)		
Cooperative Agreements (3 grants; $0.80 million)		
Research Resources Contracts	10	1.89
TOTAL	372	56.50

TABLE II
BIOLOGICAL CARCINOGENESIS BRANCH
(Contracts and Grants Active During FY 1983)

	FY 83 (Estimated)			
	CONTRACTS		GRANTS	
	No. of Contracts	$ (Millions)	No. of Grants	$ (Millions)
DNA Virus Studies	4	0.41	160	26.08
RNA Virus Studies I	2	0	99	14.98
RNA Virus Studies II	0	0	93	12.32
Office of the Branch Chief	0	0	4	0.82
Research Resources	10	1.89	0	0
TOTAL	16	2.30	356	54.20

OFFICE OF THE BRANCH CHIEF

GRANTS ACTIVE DURING FY83

Investigator/Institution/Grant Number	Title
HIRSCH, Martin S. Massachusetts General Hospital 1 U01 CA 35020-01	Viruses, Acquired Immunodeficiency and Kaposi's Sarcoma
MULLINS, James I. Harvard University 1 U01 CA 34975-01	Malignancy Associated Genetic Changes in Kaposi's Sarcoma and AIDS
VOLBERDING, Paul A. University of California (SF) 1 U01 CA 34980-01	Studies of Acquired Immune Deficiency Syndrome
YOHN, David S. Ohio State University 2 R13 CA/TW 30226-02	XIth International Symposium on Comparative Leukemia Research

SUMMARY REPORT

DNA VIRUS STUDIES

In the DNA Virus Studies component, extramural research is supported by two funding instruments. The grant instrument provides $25,700,446, total cost, for 160 grants. These include the traditional research grants, program project grants, conference grants, and new investigator grants. The major research grant emphasis lies in mechanisms of transformation which includes genome structure, function, and expression (50%); and virus-cell interactions (35%). In terms of the viruses being studied, 35% concern the herpesviruses (herpes simplex virus [HSV], 17%; Epstein-Barr virus [EBV], 12%); and 65% concern the better known smaller DNA viruses, the adenoviruses and papovaviruses. The contract mechanism provides $522,234, total cost, for four contracts. The research conducted within these contracts concerns the development of methods for diagnosis, prognosis, and intervention for neoplastic diseases associated with DNA viruses. The major efforts have centered on the EBV and nasopharyngeal carcinoma (NPC), and cytomegalovirus (CMV) and EBV diseases in renal transplant patients.

Specific chromosomal translocations are regularly associated with human Burkitt lymphomas (BLs). Most BLs carry a 14q+ marker and an extra band, joined to the distal part of one chromosome 14, was derived from the terminal part of chromosome 8. Chromosome 14 was known to carry the human immunoglobulin heavy-chain locus. This suggests that 8;14 translocation in BL may be homologous with the murine plasma cytoma (MPC) associated 12;15 translocation, and that it may act through the same presumptive oncogene-activation mechanism. The discovery of two BL-associated variant translocations lends strong support to this notion. They appear in about 10% of the cases, with approximately equal frequencies. The same fragment of chromosome 8 becomes transposed as in the typical translocation, distal to band q24, but either chromosome 2 or 22 serves as the recipient. The consistent involvement of the same segment of chromosome 8 was in line with the postulated oncogene localization. Subsequently, it was demonstrated that chromosome 2 carries the kappa and chromosome 22 the lambda gene, supporting the presumptive activating role of the functionally active chromosome region. The potential importance of the translocations for the pathogenesis of BL is emphasized by the fact that all adequately analyzed cases of confirmed BL, whether carrying EBV or not, carry one of three characteristic chromosomal markers, generated by translocation of the distal fragment of chromosome 8 (breakpoint:q24) to one of three Ig-gene-carrying chromosomes. No other variants have been encountered so far. The breakpoint on each recipient chromosome is in the same region as the Ig locus. The human IgH locus was localized by in situ hybridization to band q32 of chromosome 14, corresponding to the breakpoint. The kappa gene was localized to band 13 on the short arm of chromosome 2, whereas the lambda gene is near band 22q11. These locations correspond to the variant translocation breakpoints. In somatic hybrids between the BL line Daudi and a mouse myeloma, the major portion of the IgH cluster, including the C_H and part of the V_H region, segregated with the 14q+ marker, whereas the 5'-terminal end of $^{H}V_H$ segregated with 8q-. This

means that the break in 14q32 has cut right across the IgH gene, and confirms that the translocating is reciprocal, as previously suggested. The corollary--that the translocation must have affected the allelically excluded chromosome 14--was directly confirmed in the same segregating somatic hybrid series, showing an association between the secretion of human mu chains and the presence of the normal 14, but not the translocated 14q+, chromosome. Definite evidence for which of the two reciprocally translocated chromosomes carries the activated oncogene came from the continued analysis of the Daudi-murine hybrids. The human c-myc oncogene was localized on the distal segment of the normal chromosome 8 and became transposed to 14q+ in the BL cell. In BL lines with a rearranged c-myc, the simultaneous presence of both reciprocal translocation products was demonstrated. The high level of transcription of c-myc is maintained in interspecies hybrids between BL and MPC cells after they have lost the majority of the human chromosomes. The myc oncogene was first identified as a cell-derived transforming sequence carried by the acute avian leukemia virus MC-29. Later it was found in the genomes of four independent isolates of avian myelocytomatosis virus. When carried by the MC-29 virus, v-Myc can induce various hemopoietic tumors, carcinomas and some sarcomas. c-Myc can be activated in chicken B-cell lymphomas induced by avian leukemia virus by the insertion of the retroviral promoter-carrying (LTR) region in its immediate neighborhood. c-Myc is amplified and highly transcribed in the myelogenous leukemia line, HL60, but its transcription is switched off when differentiation is induced (27, 72).

EBV, the causative agent of infectious mononucleosis, has been implicated as a factor in the etiology of NPC throughout the world. These associations are now supported by a wealth of data from biological, biochemical, virologic, epidemiologic, and immunologic investigations on patients from different parts of the world. The immunologic investigations have also resulted in the identification of antibodies to certain EBV-associated antigens which appear to be of potential clinical value for the diagnosis and clinical management of patients with this disease. The most important of these appear to be the antibody response to the diffuse (D) component of the EBV-induced early antigen (EA) complex and the IgA antibody titers to viral capsid antigens (VCA) and to a lesser extent to D. A cooperative study was initiated to determine the possible clinical value of EBV serology in the diagnosis and clinical management of North American patients with NPC. The design of the study was prospective instead of retrospective. Most previous studies that examined these questions in non-American populations were done retrospectively. In this study of 124 American patients with histologically confirmed NPC from a variety of institutions in the US, it was found that antibodies to different EBV antigens were present more frequently and at higher titers than in control populations. The controls were primarily age-matched patients with benign disorders or other types of malignant diseases in the head and neck region. A previous study had established that the IgA anti-VCA pattern in patients with different types of lymphomas was similar to normal populations and significantly different from those with NPC. Anti-EBV studies on sera from 150 patients with different types of lung cancer showed that their EBV serologic profiles were also similar to normal populations. The most specific anti-EBV antibody response in these NPC patients was IgA antibody to VCA. Sera from 69% of the North American

patients with NPC contained IgA antibodies to VCA as compared to 9-15% of the controls and their titers were significantly higher in the NPC patients than controls. This specificity was similar to that noted previously in other studies. It is interesting that 5% of the individuals with benign diseases of the head and neck area were also positive for IgA anti-VCA titers, some at elevated levels. Five of these 21 patients had benign, hyperplastic lymphoid tissue in the nasopharynx. Although random biopsies did not reveal tumor in these masses, these patients are under observation to determine if indeed they do have occult NPC as suggested by their serologic profiles. Similarly, the so-called normal individuals with elevated IgA anti-VCA and IgG anti-EA antibodies are being observed for the development of NPC. Such follow-up should provide the information needed to determine whether the IgA antibody marker can be used to screen populations for individuals who are likely to develop NPC. The major objective of this study was to determine whether these EBV serologic patterns were related to all histopathologic forms of NPC. The results show quite clearly that sera from patients with nonkeratinizing carcinomas (WHO 2) and undifferentiated carcinomas (WHO 3) frequently contained antibodies to EBV antigens and generally at high titers. This was not true for patients with squamous cell carcinomas (WHO 1); generally, their EBV serologic profiles were similar to those found in patients with squamous cell carcinomas anywhere in the upper aerodigestive tracts and the other control populations. These differences between the WHO groups were particularly evident in the anti-EA and IgA anti-VCA antibody responses. Eighty-six percent of the sera with nonkeratinizing and undifferentiated carcinomas were positive for anti-EA antibody, mainly anti-D as previously reported, in contrast to 38% of the sera from patients with squamous cell carcinomas. Eighty-three percent were positive for IgA anti-VCA antibodies as opposed to only 19% of sera from patients with well-differentiated tumors. In addition, antibody to both antigens were generally elevated in these two groups since 62% of the patients with the less differentiated carcinomas had high antibody titers by both tests as opposed to 1-7% of controls and patients with well-differentiated carcinomas. Because of the specificities of these antibody responses, they appear to be of potential value as aids to the clinical diagnosis of NPC, particularly when used in combination, and of help in the histopathological subclassification of the more undifferentiated forms of NPC. The most important single antibody response of value for the diagnosis of NPC is the IgA anti-VCA antibody because of the low percent of positive responses which were generally present at low levels in control populations. However, the use of the IgA anti-VCA and IgG anti-EA tests in combination appears to be of greatest diagnostic value for this disease. This specificity has also been noted with biopsy specimens from these different histopathologic types of NPC. Out of 20 NPC biopsies so far examined for EBV by DNA hydridization techniques and/or for the presence of the EBV-induced nuclear antigen (EBNA), 15 have been positive by one or another of these methods. All positive biopsies with one exception were from biopsies with WHO 2 or WHO 3 histopathologic findings. The results on the other five biopsies which were also undifferentiated carcinomas were equivocal because of the small sizes of the biopsy specimens. These preliminary results also indicated, therefore, that EBV was associated specifically with these two histopathological types of NPC. Because of the specificity of the IgA antibody response, this test has now been applied to patients with metastatic cancer to the neck from an occult

primary to determine whether it might be useful for the detection of occult NPC. Results from previous studies suggested that this test was potentially useful for detecting occult NPC. In this study, sera from 76 patients with metastatic carcinoma from unknown primary sites were tested at the time the patient first presented for examination. Twenty-four of the sera were positive for IgA antibodies to VCA, some at elevated levels. An NPC primary has now been identified in 12 of these patients. Ten of these patients also had elevated IgG antibodies to EA. In one patient with a high titer of IgA antibody but no antibody to EA, a parotid primary was identified by biopsy. The remaining 11 patients are being followed. Of the 55 IgA anti-VCA negative sera, an NPC primary has been identified in two patients to date, one of whom had a squamous cell carcinoma while the other was diagnosed with a poorly differentiated carcinoma. Taken together, these data support the belief that the IgA anti-VCA test when used either alone or in combination with the anti-EA test is of potential value to the clinician for identifying occult NPC. These antibody responses can provide direction not only to the biopsy procedure but also to treatment planning in the patients with cervical metastatic undifferentiated or nonkeratinizing carcinomas. External radiation therapy is the mainstay of treatment in patients with proven nasopharyngeal carcinoma. The nasopharynx and base of skull are always included with the neck in the radiation fields when the primary can be identified microscopically in the biopsy material from the nasopharynx. Sometimes the nasopharynx is included in the radiation fields even though a primary cannot be identified there; however, the decision to include the nasopharynx and base of skull in the radiation fields is made empirically when the primary cannot be identified. If anti-EBV antibody titers are elevated, particularly the IgA anti-VCA and IgG anti-EA titers, then the radiotherapist can be reasonably certain that the primary cancer is in the nasopharynx, and the decision to include the nasopharynx and base of skull in the radiation fields is made objectively, using the serologic data even though tumor cannot be found in random biopsies of the nasopharynx. The question about the relationship of stage of disease to antibody titers is still under investigation. Other studies have reported that anti-EBV titers, particularly anti-EA and IgA antibody titers, increased with stage of disease using one staging systems. The North American patients being studied in this project are being clinically staged by five different systems. To date, the numbers of patients in the different stages in the various staging systems under study are too small to draw any definitive conclusions (164, 162, 163, 58, 95).

As discussed in the previous paragraph, NPC patients develop a characteristic spectrum of EBV-specific antibodies to EBV-coded antigens. Recently, an EBV-specific DNase was demonstrated in Raji cells after their superinfection with the virus. It was reported, furthermore, that the great majority of sera (94%) from mostly Chinese NPC patients neutralized well over 6 units of EBV DNase, whereas sera from seropositive patients with other diseases or healthy donors often lacked this antibody. In order to determine whether the anti-EBV DNase activity might afford another criterion for assessment of the prognosis, serial sera from juvenile cases of NPC were examined for their capacity to neutralize the enzyme. The results revealed that NPC patients who became long-term survivors (LTS) without evidence of the disease either never possessed significant levels of antibodies to the enzyme or showed a gradual

decline in the number of EBV DNase units neutralized from an elevated level at diagnosis to a insignificant figure several years later. All the 10 LTS neutralized less than four, and some neutralized less than two units of the enzyme three or more years after the initial diagnosis. In contrast, serial sera from juvenile patients who died of NPC neutralized over 10 and as many as 25 units of EBV DNase either persistently until death occurred or with transient declines during unmaintained remissions. Rises and declines in the neutralizing activity were, with few exceptions, accompanied by corresponding changes in the titers of IgA and IgG antibodies to EBV VCA and to the diffuse component of the early antigens. Although the number of juvenile NPC cases available for study was small, the observations suggest that the EBV DNase neutralization test may serve to provide information on the prognosis of the patients (95, 58, 45).

Herpesvirus infections are a major cause of morbidity in patients who have received renal transplants. HSV and varicella-zoster virus appear to have a predominantly localized effect on skin and mucous membranes, whereas CMV and EBV have a more systemic impact. In addition to producing various infectious disease syndromes (mononucleosis, pneumonia, and hepatitis), CMV and EBV have other important clinical effects. CMV appears to predispose the host to potentially lethal superinfections and to be associated with a distinct form of allograft glomerular injury. EBV may be responsible for a B-cell lymphoproliferative syndrome. The mechanisms by which CMV and EBV produce these effects are not yet fully understood, but direct immunomodulating properties of these viruses may be involved. Previous studies of immunologically normal patients with acute mononucleosis have demonstrated that primary infection with CMV or EBV is associated with profound alterations in T-lymphocyte subpopulations in the peripheral blood. Prior investigations of T-lymphocyte subsets in groups of patients who have received renal or bone-marrow transplants have focused on the relation between these subsets and the probability of allograft rejection or graft-versus-host disease. The relations between T-lymphocyte subsets and viral infection in immunosuppressed patients have not yet been examined in great detail. The studies discussed here concern alteration in T-lymphocyte subsets observed in association with primary or reactivated herpesvirus infections in recipients of renal transplants and changes correlated with clinical disease, particularly with the risk of opportunistic infection and the development of cytomegalovirus-associated glomerulopathy. Previous studies have shown that primary infection with CMV or EBV in otherwise normal persons is associated with lymphocyte hyporesponsiveness. The results of investigations using monoclonal antibodies to T-lymphocyte surface antigens suggest that virus-induced alterations in T-lymphocyte subsets may contribute to this hyporesponsiveness. It was shown that primary or reactivated infections with CMV or EBV in recipients of renal allografts are associated with alterations in T-lymphocyte subsets that are similar to the changes previously reported only in association with primary infections from CMV or EBV. During the first three months after transplantation, a decrease in the OKT4 lymphocyte subset and an increase in the OKT8 subset were seen in association with all primary or reactivated infections with CMV or EBV in recipients of cadaveric allografts, whether or not antithymocyte globulin had been administered. These changes usually persisted for more than three months after the initial detection.

In recipients of grafts from living related donors the OKT4/OKT8 lymphocyte-subset ratio was inverted in association with symptomatic CMV disease, but no changes

were observed in conjunction with transient, asymptomatic CMV excretion or with localized orolabial HSV infection. All the patients in this group who were intensively studied for herpesvirus infections had quantitatively normal lymphocyte subsets before transplantation, and persistent inversions in the OKT4/OKT8 lymphocyte-subset ratio were not observed in the absence of demonstrable viral infection. Although the association between herpesvirus infection and alterations in the T-lymphocyte subsets were strong in this group of patients, this association does not prove causation, and it is likely that the alterations were associated with other factors as well. An interesting aspect of these observations is the apparent increased risk for opportunistic infection in patients with an inverted OKT4/OKT8 ratio; a pattern that other workers have correlated with decreased helper-cell function and increased suppressor-cell function. The inverted OKT4/OKT8 ratio appears to be a marker for an increased risk of opportunistic infection and may also be an important pathogenetic factor. Furthermore, this relation may be operative in the recently recognized syndrome of acquired immunodeficiency and opportunistic infections in the homosexual male population. The correlation made between systemic herpesvirus infection and inverted OKT4/OKT8 lymphocyte ratios during the first three months after transplantation has several practical implications. Previous studies have shown that patients with normal or increased ratios of OKT4/OKT8 lymphocyte subsets in whom allograft dysfunction develops are likely to have a classic rejection that will respond to increased immunosuppression. In contrast, biopsy specimens obtained from patients with allograft dysfunction associated with an inverted OKT4/OKT8 ratio are more likely to show the glomerular lesion in association with CMV infection. Unlike the classic form of rejection, this lesion responds poorly to increased immunsuppression. Graft dysfunction associated with a high or normal ratio of OKT4/OKT8 T-lymphocyte subsets has been reversed by increased immunosuppression in more than 90% of episodes. Allografts were lost by 72% of patients in whom renal dysfunction developed in association with an OKT4/OKT8 ratio of less than 1.0. The glomerulopathy observed in the patients correlated well with the presence of CMV infection; only patients with CMV infection had the characteristic lesion. Since inverted ratios of the OKT4/OKT8 lymphocyte subsets are also correlated with an increased risk of opportunistic infection, sequential monitoring of the T-lymphocyte subsets should help in selecting the immunosuppressive regimen that is appropriate for the individual patient. Because of the association between an inverted OKT4/OKT8 ratio and herpesvirus infection, a presumptive diagnosis of viral infection can now be entertained days to weeks before most conventional virologic and serologic techniques yield useful information. As antiviral chemotherapy becomes more efficacious, a diagnostic approach that includes monitoring of the T-lymphocyte subsets may be useful for early initiation of therapy. Finally, it has been suggested that antithymocyte globulin has a more profound effect on the OKT4 lymphocyte subset than on lymphocytes of the OKT8 phenotype. These observations, coupled with previous data on the effects of antithymocyte globulin on herpesvirus infection in renal-transplant recipients, suggest that the presumed selective effect of antithymocyte globulin on T-lymphocyte subsets may be mediated by its effects on viral reactivation. In addition, it has recently been reported that renal-allograft recipients treated with cyclosporine have an increase in the OKT8 lymphocyte subset. It is possible that this effect is a manifestation of the increased activity of the EBV observed in patients treated with cyclosporine

and suggests that careful attention should be paid to viral activity in studies of pharmacologic agents and circulating T-lymphocyte subsets (161, 60, 58).

The presence of papillomaviruses in epithelial-derived cancers from several animal species has led to the speculation that these viruses may also have a pathogenic role in the development of certain human carcinomas, particularly those associated with the anogenital tract. Recently, human papillomavirus (HPV) DNA has been detected in epithelial-derived cancers, both cutaneous and metastatic, from patients exhibiting the rare, chronic flat wart disease, epidermodysplasia verruciformis (EV). Except for patients exhibiting this chronic wart syndrome, the association of HPV genomes with human epithelial cancers has not been demonstrated. The possible association and involvement of papillomaviruses with human anogenital neoplasias was studied. The Southern blot hybridization procedure was used to search for HPV DNA in three types of anogenital tumors: verrucous carcinoma, which is a form of invasive squamous cell carcinoma; carcinoma in situ; and Bowenoid papulosis, which is histologically identical to but biologically distinct from carcinoma in situ. From the Southern blot hybridization analysis, it was clear that HPV-specific nucleotide sequences were present in several different anogenital tumor samples. The capability to detect as few as six copies of heterologous HPV genome equivalents per cell genome, using conditions that facilitate hybridization with heterologous HPV types, enhanced detection of HPV sequences. At present it is unclear what, if any, is the specific involvement of HPV in the development of these anogenital tumors. The 12 patients analyzed in this study exhibited three clinical syndromes each characterized by either benign, premalignant or malignant transformation of anogenital skin. The presence of HPV DNA in these tumors suggests but does not dictate a pathogenic role for papillomaviruses. Since several patients had a previous history of wart disease it is possible that latent virus or viral DNA remained in the skin of these patients after eradication of the wart disease. In support of this contention is the identification of HPV DNA in normal skin of some patients with EV disease. On the other hand, the history of previous wart disease was not a criterion for choosing the patients. The original interest was in patients with multifocal tumors which clinically resembled warts. These studies, therefore, also included patients that exhibited no previous history of wart disease, and HPV DNA was similarly found associated with their tumors. It is conceivable that either these patients may have had small lesions that were undetected or they were infected subclinically. Nevertheless, the studies indicated that the entire spectrum of anogenital tumors from benign to malignant, can carry free HPV DNA genomes. More patients must be studied to understand the prevalence of this association. Since many patients with anogenital wart disease do not develop genital malignancies, other factors may be involved. Host immunity, co-carcinogens and infection with other virus groups may also have a pathogenic role with HPV in the process of malignant conversion (37, 155, 47).

The thesis that the continuum of disease leading to the development of uterine cervical carcinoma is associated with prior infection by HSV type 2 has recently been tested at the molecular level by the use of in situ cytological hybridization. The use of ^{3}H-labeled viral DNAs as probes to detect

virus-specific RNA in cervical tissue has resulted in general agreement, based on published reports, that at least 30% of all cervical intraepithelial neoplasia (CIN) and cervical carcinoma tissues contain cells that bind a HSV-2 (^{3}H) DNA probe but not probes representing other viral DNAs, indicating the presence of HSV-specific RNA. The majority of these in situ hybridizations have been conducted with whole genomic DNA as the probe. This report summarizes studies using cloned subgenomic fragments of HSV-2 DNA as hybridization probes to define regions of the virus genome from which the detected RNA species are transcribed and correlate the findings with attempts to identify viral antigen(s) expressed in cervical carcinoma cells. There have been numerous reports that antibodies to HSV-specific proteins are found more frequently, or at higher titer, in women with CIN or cervical carcinoma than in controls and that HSV-specific antigens can be detected in the neoplastic cells by immunofluorescent or other methods. Among the abundance of sometimes confusing data, two series of recent reports were of interest with respect to in situ hybridization results. First, antiserum to the major HSV-2 binding protein was shown to react with human cervical carcinoma cells in two separate studies. The tumor antigens of other DNA viruses have been shown, without exception, to have DNA-binding properties and a herpes simplex tumor antigen might be expected to exhibit a similar function. Second, it was demonstrated that sera from patients with cervical carcinoma precipitated two HSV-2 polypeptides (with molecular weights 38,000 and 118,000) more frequently than did sera from controls. The 38,000-dalton protein was of interest because it has been shown that a protein of this size is encoded within Bgl II fragment N of HSV-2 DNA which has been reported to have transforming activity in rodent cells. Rodent cells transformed in vitro by HSV-2 have been reported to express a number of HSV-specific antigens including VP143, ICP10, thymidine kinase, membrane glycoproteins gA/gB, and CP-1. HSV-2 transformants were therefore examined in parallel with specimens of cervical carcinoma in an attempt to find a protein universally present in cells transformed in vitro and possibly in vivo by HSV-2. The results of this study showed that two of the three viral parameters sought in DNA virus-transformed cells were identified in the same samples of neoplastic uterine cervical tissue. That at least in some instances the virus-specific RNA detected represents the information for a virus-specified protein can be deduced from the results after hybridization with the cloned HSV-2 DNA probe pDG304 and immunoperoxidase staining with ICSP 11/12. Studies of the properties of a temperature-sensitive mutant of HSV-1 have shown that ICP8, which corresponds to ICSP 11/12, maps within the coordinates representing the HSV-2 DNA cloned in pDG304. Thus, those tumor specimens positive for hybridization with the pDG304 cloned DNA and for the ICSP 11/12 (VP143, ICP8) protein provide direct evidence for herpesvirus-specific RNA and protein being encoded within a known region of DNA. These results also provide some resolution to the difficulties presented by the use, in the literature, of different nomenclatures for the same major DNA-binding protein. A report describing cytoplasmic and perinuclear staining with anti-VP143 antiserum in HSV-2-transformed rodent cells is now seen to be consistent with the finding that ICSP 11/12 is present in cervical carcinoma and in vaginal carcinoma tissues and that antibodies to this protein were detected in the sera of cervical cancer patients. Thus, some progress has been made toward defining a role for HSV-2 in the etiology of this disease, but many questions remain. The detection of ICSP 11/12 in

neoplastic cells is not, by itself, sufficient to implicate HSV-2 because it has been shown that at least five different herpesviruses induce proteins that cross-react with ICSP 11/12 and that cytomegalovirus-infected human cells are also positive with antibody to ICSP 11/12. The two regions of HSV-2 DNA described as containing transforming genes map between positions 0.4 and 0.63 on the genome and do not contain the coding sequences for the major DNA-binding protein. Sequences mapping between 0.30 and 0.45 on the HSV-1 genome, which does include the ICP8 coding sequence, have been shown to have transforming ability, and DNA mapping within this region has been shown to persist in cells transformed by UV-inactivated HSV-2. Those transformants are positive by immunofluorescence for VP143 (ICP8, ICSP 11/12). The Bgl II region N of HSV-2 also persists in these cells but no evidence of the 38,000-dalton protein encoded by the Bgl II region N sequences was found. Other experiments have shown that monoclonal antibodies A6 and H11 react with two proteins translated in vitro from RNA selected with Bgl II fragment N of HSV-2. The coding sequences for these proteins have been shown to be partially co-linear and, for the larger protein, to be also encoded in part within Bgl II fragment C. Cells transformed in vitro with Bgl II N sequences alone do not appear to express the 38,000-dalton protein. It is possible that Bgl II region N products are present in amount too low to be detected or that they may only be required for initiation. It is interesting that, even though cervical carcinoma cells are negative for this protein, it has been shown that cervical cancer patients may have significantly increased levels of antibody to the 38,000-dalton protein. Except for one report, attempts to detect HSV DNA in cervical carcinoma tissues have failed. The demonstration that viral RNA and protein are present in about 30% of these cases has stimulated investigation of more tumors at the DNA level by hybridization with the cloned DNA probes containing transforming sequences from HSV-1 and HSV-2. These segments of DNA provide greater sensitivity than was available for the earlier studies. Cloning of DNA from tumor tissues to provide libraries that can be screened for herpesvirus sequences will also enable more sensitive detection of viral DNA (84, 41, 104, 127, 25).

Human cytomegalovirus (HCMV) is a herpesvirus capable of establishing permissive, persistent, and latent infections in human cells. The virus also has oncogenic properties in vitro and may be associated with several human neoplasms, including Kaposi's sarcoma. Knowledge of the events leading to virus production in the permissive infection will aid our understanding of the other virus-cell interactions observed. For these reasons the transcription pattern of HCMV strain AD169 in permissively infected human fibroblasts was studied. The HCMV genome is a large double-stranded DNA molecule 240 kb in length and consists of two covalently linked unique segments, each bordered by inverted terminal repeats. The long unique segment (174 kb) and adjacent long repeat sequences (12 kb each) comprise 83% of the genome while the short unique segments (38 kb) and short repeat sequences (2 kb each) make up the remaining 17%. In a given population of virus, all four of the possible genome orientations can be found. The large size and complex structure of the genome have made analysis of transcription difficult. Comigrating and submolar bands in viral DNA digests have complicated studies using virion DNA. The EcoRl restriction endonuclease fragments representative of the entire genome of HCMV strain AD169 have been mapped and cloned. The cloned EcoRl

fragments do not include the termini. However, the terminal sequences are included in the region which spans the junction of the long and short segments of the genome. The three terminal EcoRl fragments are designated: fragment W, contained entirely within the long repeat region at both ends of the long segment (0.00-0.018 and 0.804-0.822 map units); fragment L, which spans part of the short unique segment and all of the short repeat sequences at 0.962-1.00 map units; and fragment N, which includes a separate part of the short unique segment and all of the short repeat sequences at 0.822-0.854 map units. The cloned AD169 library contains the L-S junction fragment H. In this configuration, fragments W and N are fused, forming a single EcoRl fragment containing all of the information of the two separate fragments. When the short segment is in the opposite orientation, a new fusion product is formed. This W and L fusion product is designated fragment F. The use of this complete set of molecularly cloned subgenomic fragments has allowed detailed examination of HCMV gene expression. Permissive infection of fibroblasts by HCMV strains AD169 could be divided into four main phases of RNA transcription. The immediate early RNAs are those specified immediately after infection and are synthesized in the absence of protein synthesis. Major immediate early RNAs are transcribed from restricted portions of the genome with major species mapping to three regions in the long unique segment. A second early phase of RNA transcription occurs before viral DNA replication if protein synthesis is permitted and represents a larger fraction of the genome. Midpoint RNA is synthesized at the time of viral DNA replication and also represents a greater fraction of the genome. Late RNA is synthesized in abundance after viral DNA replication and is transcribed from most or all of the genome. Similar transcription patterns have been reported for the Davis and Towne strains of HCMV and for HSV. In the case of HSV, however, the major immediate early RNAs are transcribed from the terminal repeats and adjacent sequences. These transcriptional mapping studies provide a framework for studying the underlying mechanisms of viral gene regulation during the permissive infection. In addition, they establish a method for analyzing viral transcription patterns associated with HCMV persistent and latent infections and with HCMV oncogenesis (129, 29, 54, 113, 152).

The Epstein-Barr virus (EBV)-determined nuclear antigen (EBNA) was discovered by Reedman and Klein by anticomplement immunofluorescence (ACIF). Recently, EBNA has been purified to homogeneity. The native EBNA has a molecular weight of 180-200,000 daltons. It contains 48K subunits that form complexes with a 53K protein of cellular origin. Acid-fixed nuclear binding (AFNB), complement fixation (CF) and ACIF inhibition tests showed that the 48K component but not the 53K component binds anti-EBNA antibodies. In order to study cell-mediated immune reactions to EBNA and other EBV determined antigens, an EBV-specific leukocyte migration inhibition (LMI) test was recently developed. LMI and macrophage migration inhibition (MMI) are regarded as the in vitro correlates of delayed hypersensitivity. In both cases lymphocyte effector molecules (lymphokines) are released in the presence of specific antigens. The lymphokines can inhibit the migration of polymorphonuclear leukocytes (LMI) or macrophages (MMI). Significant LMI was found when leukocytes of EBV-seropositive but not seronegative donors were confronted with extracts of EBV-genome-carrying cells. Corresponding extracts of EBV-negative lymphoma cells had no LMI effect. Subsequent dissection of this reaction showed that

EBV-non-producer cells, known to contain EBNA as the only serologically demonstrable EBV product, gave significant EBV-specific LMI reactions. This suggested that EBNA alone may cause LMI. This was subsequently confirmed with partially purified EBNA that contained both the 48K and 53K subcomponents. It was not clear, however, whether the host was sensitized to the EBNA-specific 48K subunit and/or the associated cellular 53K protein, or to a complex of the two. In view of the widespread occurrence of 53K proteins in transformed cells this question is of considerable interest. It is known that tumor-bearing but not normal mice can form antibodies to the 53K component of chemically induced tumors. In contrast, anti-53K antibodies in tumor-bearing human patients could not be demonstrated. The LMI test provides an alternative method of examining the question whether the human host can respond to 53K, as presented in the EBNA complex formed by the EBV-transformed cell. This question was approached by confronting the leukocytes of EBV seronegative and seropositive donors with purified 48K and 53K, alone and in combination, in the LMI test, and measured their migration inhibitory activity. A concentration of 10 micrograms/ml was still effective while 5 micrograms/ml had no detectable effect. EBNA-associated cellular 53K protein had no effect by itself, but it potentiated the effect of 48K, even if the latter was added at the subliminal concentration of 5 micrograms/ml. The related 53K protein, isolated from EBV-negative human lymphoma cells, was also effective, whereas the corresponding murine-tumor-associated 53K had no potentiating effect. Immunization of mice with an extract of DNA-binding proteins from EBV-carrying Raji cells, known to contain both 48K and 53K, induced a significant macrophage migration inhibition response to both human 48K and 53K. Murine 53K was ineffective, however. Human but not murine 53K increased the migration inhibitory activity of subliminal concentrations of 48K in the murine macrophage system as well. These findings suggest that human but not murine 53K may reconstitute with 48K (EBNA) to form a highly immunogenic complex (71).

The plasmid form of the EBV genome is a covalently closed circular molecule that exists only intracellularly. Since its discovery, an understanding of the mechanism whereby the EBV plasmid is maintained in cells has remained an attractive but elusive objective. The replication of the EBV plasmid is strictly regulated in synchrony with the cell cycle; the plasmid replicates only during the S phase, and the copy number is constant and remains relatively low. The plasmid is situated in the nucleus and exhibits a nucleosomal arrangement. Circular EBV genomes are found both in cell lines and in fresh tissue samples of Burkitt lymphoma and nasopharyngeal carcinoma. The plasmid is the only form of the EBV genome found in nonvirus-producing cell lines, but plasmids are also found in the nonproductive cellular fraction of virus-producing lines. One of the intriguing aspects of EBV plasmids is that they are believed to be replicated by host DNA polymerase rather than virus-induced DNA polymerase. The viral enzyme is responsible for replication of the linear form of the EBV genome which is encapsidated in virions; the enzyme is not detectable in the nonvirus-producing Raji cell line. In addition, the linear form of the EBV genome replicates independently of the host-cell cycle, and the relative number of virus genomes fluctuates over a wide range. The controlled, limited replication of the EBV plasmids, together with the absence of viral polymerase, suggests that this EBV DNA form is

replicated by host DNA polymerase. The existence of specific sites at which the genomes of numerous prokaryotes and several eukaryotic viruses initiate DNA synthesis is accepted fact, and these sites have been termed origins of replication. However, in eukaryotes the existence of such defined origins of replication, while assumed, has not been demonstrated clearly except in a limited number of small extrachromosomal DNAs such as mitochondrial DNA in which initiation occurs at only one site for the entire molecule or from each strand. Cellular and complex viral genomes (e.g., herpesviruses) may have some similarities in their replication; therefore, viruses of this type may provide a more useful system in which to study eukaryotic-like origins of replication. The EBV plasmid in particular is likely to have and indeed may be the best candidate to contain eukaryotic origins. Recent advances in molecular cloning have produced a system in yeast analogous to replication systems in prokaryotes that can be utilized to screen eukaryotic DNA segments for their ability to replicate autonomously. Those DNA sequences that replicate in this manner have been referred to as autonomously replicating sequences (designated ars) and are presumably functional eukaryotic origins of replication. Several yeast genes coding for various amino acids and DNA precursors paired with their corresponding yeast auxotrophs have been isolated and used as the basic selective system to analyze for the presence of ars in eukaryotic DNAs. One of these systems utilizes the URA3 gene of S. cerevisiae for selection. The plasmid of interest, YIp5, is a hybrid plasmid consisting of pBR322 and the URA3 gene of S. cerevisiae. Therefore, YIp5 can be utilized in a yeast selective system and can be grown and recovered in an Escherichia coli system as well. In addition, YIp5 lacks a functional yeast origin of replication and cannot transform the corresponding URA auxotroph of S. cerevisiae unless an exogenous ars is incorporated into the plasmid. This system then provides the necessary screening system for the detection of ars within unrelated DNAs. Recent work from several laboratories has shown the validity of this yeast vector system when used in this capacity. This selection system is used in experiments designed to determine the number and location of ars in the genome of EBV. Cloned EBV DNA EcoRI restriction fragments, A, B, and DIJ_{het}, were judged to function in this capacity by their ability to convert YNN27 cells to the uracil phenotype after transformation with each EBV-specific fragment ligated into YIp5. Additional analyses to confirm and to specify further the location of the ars were performed by cleavage of EcoRI fragments A and B into smaller BamHI fragments, which were subsequently cloned in YIp5 and tested for their ability to function as ars. BamIII fragment X (obtained from EcoRI fragment A) and BamHI fragment R (obtained from EcoRI fragment B) showed ars behavior. The successful recovery of the appropriate virus DNA segments in plasmid form from transformed yeast cells and the ability of these yeast cells to be propagated further substantiated the ars capability of the three EBV fragments. The most important aspect of the experiment will be to determine if those EBV DNA sequences containing putative origins of replication will allow a stable extrachromosomal form of the virus DNA to become established. The results of such experiments should help to provide answers to the question of how the EBV plasmid replicates and is maintained in cells (95).

Cellular transformation by DNA tumor viruses is characterized by the integration of the viral genome into the host DNA. In rat cells transformed by

polyoma virus (Py), the most common type of integration is a head-to-tail tandem arrangement of viral genomes, and an active Py large T-antigen has recently been shown to be required for the formation of such structures as well for a high efficiency of transformation. The primary integration pattern is not always stable. Excision of Py genomes from the viral insertion can take place at a high rate, together with the production of free viral DNA molecules, which originate from the integrated sequences. Also, amplification of integrated viral sequences can determine an increase in the number of viral genomes within the original insertion. In cell lines transformed by the ts-a mutant of Py, which produces a thermolabile large T-antigen, these phenomena have been observed only when cells are propagated at temperatures permissive for this viral protein. These studies, in addition to showing a requirement for a functional Py large T-antigen, also showed that both excision and amplification involved the mobilization of an integral number of genomic "units." This suggested the involvement of homologous intramolecular recombination events, made possible by the presence, in all of the cell lines studied, of complete or partial tandem insertions of Py molecules. To verify this hypothesis, excision, amplification, and free viral DNA production in Py ts-a-transformed rat cell lines carrying single viral insertions with a complete early region and no duplication at their termini were studied. The results showed that none of the cell lines studied were able to undergo excision or amplification of Py genomes with the same characteristics described before, irrespective of the presence or absence of large T-antigen function. Two cell lines showed a very low frequency of rearrangements of the viral insertion which probably involved host DNA sequences. None of the cell lines studied spontaneously produced free viral DNA. After fusion with mouse 3T3 fibroblasts, some of the cell lines produced a low amount of heterogeneous free DNA molecules which, again, contained viral and host sequences. These results suggest that, in addition to an active Py large T-antigen, the presence of regions of homology in the integrated sequences is required for excision and amplification of integrated Py genomes. In the absence of viral DNA homology, regions of partial homology at the level of host flanking sequences can probably be used and might explain the events observed at low frequency in two cell lines (4, 5, 7).

Transformed cells and tumors induced by polyoma virus do not always contain the entire coding region for the viral large tumor antigen (T antigen). Both transformants and tumors can be obtained by introducing segments of polyoma DNA that span only the proximal portion of the early region. In contrast, all simian virus 40 (SV40)-transformed rodent cells examined so far contain and express at least one complete copy of the early viral gene which encodes the T antigen. Transformed rodent cells that contain and express fragments of T antigen, but always in the presence of an apparently intact early gene, have been identified. SV40 and polyoma virus are not strictly comparable because their early regions are organized differently. Nevertheless, the polyoma-derived results raise the possibility that a portion of the SV40 early region encoding only the NH_2 - terminal half of T antigen might facilitate transformation. This has been found to be the case. An assay was used that required only that secondary rat embryo cells continue to grow after transfection with recombinant plasmids containing segments of the SV40 genome. The immortalized cells exhibited phenotype ranging from nearly "normal" to

fully transformed. The generation of a range of growth phenotypes implies a significant but non-uniform contribution to the growth potential of the immortalized lines by cellular gene products (123).

Promoters and terminators are potential sites at which gene expression can be controlled. In prokaryotes, RNA polymerases, with and without accessory factors, specifically recognize promoter and terminator sequences. In eukaryotes, although sequences which constitute promoters have been partially characterized, the mechanism of termination of RNA polymerase B transcription is not known. In prokaryotes, transcription termination sites are located within as well as at the end of the operons. The internal termination sites cause premature termination of the transcripts and quantitatively regulate the level of gene expression by selectively reducing the transcription of distal portions of the operons. The mechanism of regulation has been termed attenuation. In contrast to termination at the end of the operon, attenuation is not a complete termination process. It can be overcome by regulatory proteins, as in bacteriophage, or in response to changes in physiological conditions, as in the case of amino acid operons in bacteria. The sequences at the termination and attenuation sites share common features. The DNA immediately preceding the site of termination is GC-rich and often possesses dyad symetry. The 3' terminus of the transcript typically contains a series of uridine residues. The role of these and other features, such as secondary structure in the transcript, distal DNA sequences, protein factors involved in termination and read through and RNA-RNA or RNA-DNA interactions is not fully understood. In eukaryotes, an abundant population of promoter-proximal RNA chains has been observed and studied, mainly in whole nuclear RNA and in SV40. On the basis of these studies it has been suggested that a premature termination process resembling attenuation in prokaryotes occurs in eucaryotes. Moreover, these studies have shown that adenosine and the analog 5,6-dichloro-1-B-d-ribofuranosylbenzimidazole (DRB) enhance premature termination, but their mode of action is not understood. Although the use of the term attenuation in eukaryotes follows closely the original usage in prokaryotic systems, there is no experimental basis to suggest that the mechanism of attenuation in eukaryotes is similar to that in prokaryotes. Furthermore, there is no experimental evidence that DRB enhances premature termination at the attenuation site. The determination of the complete nucleotide sequences of SV40 and the localization of the major and minor initiation sites for late transcription provide means for elucidating the nucleotide sequences involved in the attenuation mechanism. A site was localized at which transcription of SV40 late RNA is attenuated in vitro. The DNA sequences where the RNA synthesis stops are strikingly similar to the termination signal in prokaryotes. DRB enhances premature termination at the same region, and proflavine, a drug known to interfere with RNA secondary structure, allows read through. On the basis of these observations, it is suggested that the eukaryotic RNA polymerase B can respond to a transcription termination signal similar to that to which the prokaryotic polymerase responds, and in which attenuation and mRNA modulation in a feedback control mechanism quantitatively regulate SV40 gene expression. The suggested mechanism which resembles models for gene regulation in bacteria and bacteriophage, opens up approaches to the investigation of attenuation and

mRNA modulation as a possible mechanism whereby eukaryotes may regulate transcription in a variety of circumstances (1, 137, 28, 153).

The oncogenic potential of human adenoviruses (Ad) varies over a broad range from "nononcogenic" to "highly oncogenic." The tumorigenic potential of cells transformed with a given adenovirus serotype may also show great variability, which does not correlate with a number of in vitro traits expressed in these cells. Very important for the ultimate outcome of the tumor-host interaction are host factors. The protective effects of immunization and the existence of adenovirus-specific tumor-specific transplantation antigen (TSTA) have been established. It has been recently demonstrated that both the cytolytic T-cell response and complement-dependent antibody-mediated cytotoxicity in syngeneic animals are directed against a product of the adenovirus E_1 early gene block in both Ad12 and Ad2 transformed cells. These results, together with an examination of mutants of Ad5 for their ability to induce group Ad group C TSTA and demonstration of protective effects of immunization with Ad12 transformed cell lines indicate that product(s) of the E_1 region is a major component of adenovirus TSTA. An early glycoprotein product of the E_3 region, which forms a ternary complex with the major histocompatibility antigen on the plasma membrane of transformed rat cells, may be a minor component of adenovirus transplantation antigen. Immunity against tumor cells has been thought to be mediated by three types of effector cells: specific immune T cells, antibody-dependent cytotoxic cells, and activated macrophages. In recent years it has been recognized that an additional mechanism is important: natural cell-mediated cytotoxicity. There is a considerable body of evidence that the sensitivity or resistance of individual transformed cell lines to killing with natural killer (NK) cells in vitro may play an important role in their tumorigenic potential in vivo, particularly under circumstances of impaired T-cell immunity. Recent studies indicate that NK activity in LIS rats corresponds to that in other studied rat strains. It is absent in newborn animals, but is fully developed in weanling rats. This activity appears to remain essentially unchanged from weaning through 36 weeks of age. The tissue distribution of NK activity also corresponds to that found in other rat strains. The five adenovirus-transformed LIS lines studies showed significant differences in sensitivity to syngeneic NK cells. The highly tumorigenic Ad12-transformed cells are resistant to syngeneic NK cells. The sensitivity of Ad2-transformed cells shows an inverse relation to their tumorigenic potential in vivo: tumorigenic 5OA cells show an intermediate degree of sensitivity; T_2C_4 cells tumorigenic in immunosuppressed newborn rats are more sensitive; and the highest sensitivity is seen in nontumorigenic A_2T_8 cells. Although some variability of absolute cytotoxicity values was detected between individual experiments, the pattern of relative sensitivity of five transformed cell lines was highly reproducible in over 20 separate experiments. The differences in sensitivity of the Ad2-transformed lines to rat NK cells are reflected in their ability to induce tumors in nude mice. The dose of cells required to induce tumors in 100% of animals was $2X10^4$ for A_2T_8 cells, while with 5OA cells only $1.9x10^2$ cells were required. As indicated by competition and absorption assays all tested Ad-transformed cells appear to be recognized by the same subpopulation(s) of NK cells. All these cells thus appear to share antigenic specificities recognized by the NK cells irrespective of the transforming virus. Such specificities were not detected

on established syngeneic fibroblastic cell line LIS-U, which is itself sensitive to LIS NK killing. Such antigenic specificities, present on the studied Ad-transformed cell lines, however, were also detected on some allogeneic or xenogeneic cells. The finding of a rather limited repertoire of antigenic specificities recognized by NK cells is not surprising and has been previously noted with both human and rat lymphoid cells. It is obvious that the different sensitivity to NK killing does not correlate with the ability to bind the NK cells in the absorption assays, although the more sensitive cells were more efficient competitors in the cold competition assays. The sensitivity of different cell lines cannot be explained by general differences in sensitivity to immune cytolytic stimuli. In parallel experiments, these cell lines do not show great differences in sensitivity to killing by syngeneic secondary cytolytic T cells. The cytolytic T-cell response is highly specific and is directed against products of the E_1 region of the adenovirus genome. Cytolytic T cells raised against Ad2-transformed cells do not lyse syngeneic Ad-12 transformed cells and vice versa. In vitro generation of secondary cytolytic T cells in this system is not associated with generation of a significant subset of NK cells called "anomalous killers" characteristically associated with allogeneic stimulation of murine lymph node cells in culture. Although differences were seen in cytolytic T-cell activity in tumor-bearing rats, results indicate that the ability to elicit cytolytic T cells in mature rats does not significantly differ between non-tumorigenic and tumorigenic Ad-transformed cells. The antigens recognized by the NK cells in Ad-transformed cells have not been identified and exact explanations for great differences in sensitivity to NK killing of tumorigenic and nontumorigenic cell lines have to be established. It has been shown that sensitivity to killing by NK cells may be dependent on a number of factors, including interferon production or mycoplasma infection. Based on studies of tumorigenic potential of Ad- and SV_{40}-transformed hamster cells, four distinct tumorigenic phenotypes have been defined by host cellular immune-tumor cell interactions. Cells used in these studies would correspond to their phenotypes I (A_2T_8 and T_2C_4), II (50A), and III (RFC_1 and RT_2). Results on sensitivity of rat cells of different tumorigenicity to syngeneic NK cells are in general agreement with preliminary observation that Ad12-transformed hamster embryo cells of phenotype III (tumorigenic in both newborn and adult syngeneic animals) are significantly more resistant to hamster NK cells than less tumorigenic phenotypes I and II. The ultimate outcome of host interaction with the transformed cell load in the rat thus depends on at least two defense mechanisms: the rapid natural immunity of limited specificity, which is represented by NK and other cells, and slower, but highly specific induced immunity, best represented by cytolytic T cells. The latter mechanism appears to play a central role in protective effects related to adenovirus TSTA. In nonimmunized animals the tumorigenic potential of a given cell line is determined by its sensitivity to both of these immune mechanisms. The data suggest, however, that differences in sensitivity or resistance to killing by NK cells, rather than differences of immunogenicity correlate with oncogenic potential of studied Ad-transformed cells. This pattern of relation of tumorigenicity and sensitivity to NK cell killing was observed with a number of additional Ad-transformed cell lines, irrespective of the strain origin of the rat. It is interesting that all tested highly tumorigenic cell lines transformed with isolated EcoRI-C fragment of Ad12 DNA

are resistant, while marginally tumorigenic cells transformed with isolated HindIII-G fragment of Ad12 DNA are considerably more sensitive to NK killing. Studies to determine the role of expression of adenovirus genome in induction of resistance or sensitivity of transformed cells to NK cell killing are in progress (106, 156).

All known DNA polymerases require a primer for initiating the synthesis of DNA chains during replication. Commonly, DNA synthesis at replication origins employs self-priming by the template DNA or priming by short RNA or DNA molecules. Unusually, initiation of adenovirus DNA replication involves priming by a deoxynucleotide that is covalently linked to a protein. Studies of adenovirus DNA replication have been greatly facilitated by the development of cell-free extracts capable of initiating and completing one round of DNA replication. This development was essential to the understanding of the "protein-priming" mechanism and will enable the factors involved in replication to be purified and their functions determined. The initiation of adenovirus DNA replication occurs at either end of the linear DNA molecule, within an inverted terminally repeated sequence. Replication then proceeds unidirectionally from either origin toward the other end of the molecule, displacing the nontemplate DNA as a single strand which is subsequently replicated by a process not yet reproduced in vitro. A deoxycytidine residue, which eventually becomes the 5'-terminal base in the DNA, is covalently bound to the virus-coded 80 kilodalton (kd) terminal protein precursor (pTP) in a reaction that requires specific DNA sequences at the origin. The pTP-dCMP complex acts as the primer for DNA synthesis, and the 80 kd protein remains covalently bound to the newly synthesized DNA strands both in vitro and in vivo. The precursor protein (pTP) is cleaved to the 55 kd terminal protein (TP) late in the infection, probably during morphogenesis of the virions. The the 55 kd TP is found covalently linked to virion DNA. The pTP has been purified in a functional form from adenovirus-infected cell extracts. It is an essential component of a fractionated replication system and copurifies with a 140 kd protein of hitherto unknown origin. The purified protein fraction contains an aphidicolin-resistant DNA polymerase activity in addition to the ability to covalently bind dCMP to the pTP. The 140 kd protein possesses DNA polymerase activity and is required for the dCMP binding reaction. Another virus protein involved in the elongation of adenovirus DNA replication is the 72 kd single-strand DNA-binding protein encoded by the E2A region of the adenovirus genome. This protein has also been purified in a functional form by many different procedures and can complement for replication the thermolabile cell-free extracts from Ad5ts 125-infected cells. The mRNA for the pTP maps in a large, early transcription unit of the adenovirus genome, designated region E2B. Within the DNA sequence of the E2B region, the open translation reading frame encoding pTP lies between coordinates 29 and 23.4, but, significantly, does not overlap the locus of the DNA negative mutants of complementation group N. These conditional mutants, including Ad5ts149 and Ad5ts36 are unable to replicate virus DNA at the nonpermissive temperature, although they are competent at this temperature for production of mRNA from several early transcription regions. Another interesting phenotype of these mutants is their inability to transform rat embryo cells at the nonpermissive temperature. This lesion affects initiation, rather than maintenance, of the transformed phenotype. Since these

mutants do not map within the region encoding pTP, there was the strong possibility that they define a third virus-coded protein that functions in DNA replication. A 140 kilodalton (140 kd) protein which complements these defective extracts and contains DNA polymerase activity has been purified from HeLa cells infected with wild-type Ad2. It is tightly associated with the 80 kd pTP in a replication complex. Both of these proteins are products of the E2B region of the adenovirus genome, and the 140 kd protein coding sequences lie immediately downstream from those encoding the 80 kd protein. These results demonstrate that adenovirus encodes a novel DNA polymerase that is required for priming of DNA synthesis at the origin of replication. This protein may also function in the initiation of transformation of cultured cells (118, 90, 61, 107, 156)..

DNA VIRUS STUDIES

GRANTS ACTIVE DURING FY83

Investigator/Institution/Grant Number	Title
1. ALONI, Yosef Weizmann Institute of Science 5 RO1 CA 14995-09	Control of Gene Expression in Tumor Viruses and Cells
2. ALWINE, James C. University of Pennsylvania 1 RO1 CA 33656-01	Control of Late Gene Expression In DNA Tumor Viruses
3. ALWINE, James C. University of Pennsylvania 5 RO1 CA 28379-03	Regulation of DNA Tumor Virus Gene Expression
4. BASILICO, Claudio New York University 5 RO1 CA 11893-13	Cellular and Viral Control of Oncogenetic Transformation
5. BASILICO, Claudio New York University 5 PO1 CA 16239-09	Biosynthesis in Normal and Virus-Transformed Cells
6. BAUM, Stephen Yeshiva University 5 RO1 CA 10945-12	Interaction of Oncogenic Viruses (Adenovirus and SV40)
7. BENJAMIN, Thomas L. Harvard University 2 RO1 CA 19567-07	Mechanism of Cell Transformation by Polyoma Virus
8. BENJAMIN, Thomas L. Harvard University 5 RO1 CA 25390-05	Effects of HR-T Mutations on Polyoma Gene Expression
9. BERG, Paul Stanford University 5 RO1 CA 31928-02	Transduction of Genetic Information Related to Cancer
10. BERK, Arnold J. University of California (LA) 5 RO1 CA 25235-05	Biosynthesis of Adenovirus Early RNAs
11. BOTCHAN, Michael R. University of California (Berkeley) 5 RO1 CA 30490-03	Transformation of Cells by SV40 Virus

12. BROCKMAN, William W.
University of Michigan
5 RO1 CA 19816-07

Role of SV40 Gene A in Cellular Transformation

13. BROKER, Thomas R.
Cold Spring Harbor Lab
1 R13 CA/AI/TW 32922-01

International Conference on Papilloma Viruses

14. BUTEL, Janet S.
Baylor College of Medicine
5 RO1 CA 22555-06

Biological Properties of SV40 Early Proteins

15. BUTEL, Janet S.
Baylor College of Medicine
5 RO1 CA 25215-05

Tumor Virus Effects on Mouse Mammary Epithelial Cells

16. CALNEK, Bruce W.
Cornell University
5 RO1 CA 06709-21

Avian Leukosis Complex

17. CARMICHAEL, Gordon G.
University of Connecticut
1 RO1 CA 32325-01

Regulation of Polyoma Early Gene Expression

18. CARROLL, Robert B.
New York University
5 RO1 CA 20802-07

Biochemical and Functional Properties of SV40 T Antigen

19. CHINNADURAI, G.
St. Louis University
5 RO1 CA 31719-02

Genetic Analysis of AD2 Early Genes

20. CHINNADURAI, G.
St. Louis University
9 RO1 CA 33616-04

Adenovirus in Locus: Role in Oncogenic Transformation

21. CLOUGH, Wendy G.
University of So California
2 RO1 CA 23070-06

EBV DNA Synthesis in Transformed Lymphocytes

22. CONLEY, Anthony J.
St. Louis University
1 RO1 CA 33101-01

Regulatory Functions of Herpes Simplex Virus Gene Expression

23. CONSIGLI, Richard A.
Kansas State University
5 RO1 CA 07139-19

Studies in Polyoma Transformed Cells--Virion Proteins

24. COOPER, Neil R.
Scripps Clinic
5 RO1 CA 14692-10

Humoral Immunity to Viruses and Virus-Infected Cells

25. COURTNEY, Richard J.
University of Tennessee
5 RO1 CA 24564-06

Studies of Purified Herpes Simplex Virus Glycoprotein

26. COURTNEY, Richard J.
University of Tennessee
5 RO1 CA 27870-03

Proteins of HSV-Infected and Transformed Cells

27. CROCE, Carlo M.
Wistar Inst of Anatomy & Biology
5 RO1 CA 16685-08

Mapping of Tumor Virus Genomes in Transformed Cells

28. DARNELL, James E., Jr.
Rockefeller University
5 RO1 CA 16006-10

RNA and Growth Control in Animal Cells

29. DE MARCHI, Jeanette M.
Vanderbilt University
5 RO1 CA 20806-06

Induction by Cytomegalovirus of Cell DNA Synthesis

30. DE PAMPHILIS, Melvin L.
Harvard University
5 RO1 CA 15579-09

Tumor Virus DNA Replication: A Probe into Oncogenesis

31. DEROSIERS, Ronald C.
Harvard Medical School
5 RO1 CA 31363-02

Molecular Basis for Herpesvirus Saimiri Oncogenicity

32. DI MAYORCA, Giampiero
College of Med & Dent, NJ
3 RO1 CA 25168-04

Transformation Genes of Simian Virus 40

33. DI MAYORCA, Giampiero
College of Med & Dent, NJ
3 RO1 CA 25169-03S2

BK Virus, A Human Papovavirus

34. ECKHART, Walter
Salk Institute for Bio Studies
5 RO1 CA 13884-11

Viral Gene Functions and Regulation of Cell Growth

35. EGGERDING, Faye A.
University of California (LA)
5 RO1 CA 25545-02

Regulation of Adenovirus 2 Transcription

36. FALK, Lawrence A., Jr.
Harvard University
5 RO1 CA 27225-03

Study of Human and Simian Lymphotropic Herpesviruses

37. FARAS, Anthony J.
University of Minnesota
5 RO1 CA 25462-05

Human Papilloma Viruses and Malignant Disease

38. FLUCK, Michele M.
Michigan State University
5 R01 CA 29270-03
Control of Gene Expression on Viral Transformants

39. FOLK, William R.
University of Michigan
2 R01 CA 13978-11
Mammalian Cell Transformation by Oncogenic Viruses

40. FRIEDMANN, Theodore
University of California (SD)
2 R01 CA 24288-04
Cellular and Papovaviral Gene Expression

41. GALLOWAY, Denise A.
Fred Hutchinson Cancer Res Ctr
5 R01 CA 26001-04
Herpesvirus Expression in Transformation and Latency

42. GAYNOR, Richard B.
University of California (LA)
5 R23 CA 30981-02
Adenovirus 5 Mutants in Transforming Functions

43. GHOSH, Prabhat K.
Yale University
1 R01 CA 32799-01
Regulation of Simian Virus 40 Transcription

44. GINGERAS, Thomas R.
Cold Spring Harbor Laboratory
5 R01 CA 27275-03
DNA Sequence and Computer Analysis of a Tumor Virus

45. GLASER, Ronald
Ohio State University
5 R01 CA 29066-03
Epstein-Barr Virus DNA In Transfected Cells

46. GRALLA, Jay
University of California (LA)
5 R01 CA 19941-07
Regulation of Transcription by DNA-Protein Complexes

47. GREEN, Maurice
St. Louis University
5 R01 CA 28689-03
Human Papillomaviruses

48. GREEN, Maurice
St. Louis University
5 R01 CA 29561-25
Biochemistry of Animal Virus Multiplication

49. GREEN, Maurice
St. Louis University
5 R01 CA 21824-06
Replication of RNA Tumor Viruses

50. GURNEY, Elizabeth T.
University of Utah
2 R01 CA 21797-06
Growth Control and Viral Gene Expression

51.	GUTAI, Mary W. New York State Department of Health 5 RO1 CA 28250-03	SV40 DNA Replication and Recombination in Animal Cells
52.	HAGER, Lowell P. University of Illinois (Urbana) 5 RO1 CA 17619-08	Biochemical Studies on T Antigen and Transformed Cells
53.	HARTER, Marian L. College of Med & Dent, NJ 5 RO1 CA 28414-03	Functions of Early Proteins Encoded by Adenovirus
54.	HAYWARD, Gary S. Johns Hopkins University 5 RO1 CA 22130-06	Structural Organization of Herpesvirus Genomes
55.	HAYWARD, Gary S. Johns Hopkins University 5 RO1 CA 28473-03	Cellular Transformation by DNA of Human Herpesviruses
56.	HAYWARD, S. Diane Johns Hopkins University 5 RO1 CA 30356-02	EBV Genome Expression: Localization of Specific Functions
57.	HELD, William A. New York State Dept Health 5 RO1 CA 27647-03	TK-Mutants of Herpes Virus and Their Suppression
58.	HENLE, Werner Children's Hospital (Philadelphia) 1 RO1 CA 33324-01	EBV Serology In Human Cancers And Immune Deficiencies
59.	HINZE, Harry C. University of Wisconsin (Madison) 5 RO1 CA 21195-04	Vaccination Against an Oncogenic Herpes Virus
60.	HIRSCH, Martin S. Massachusetts General Hospital 5 RO1 CA 12464-12	Immune Reactivity and Oncogenic Virus Infections
61.	HORWITZ, Marshall S. Yeshiva University 5 RO1 CA 11512-14	Adenovirus DNA Synthesis and Polypeptide Assembly
62.	HOWETT, Mary K. Pennsylvania State Univ (Hershey) 5 RO1 CA 25305-05	In Vivo Cocarcinogenesis of Chemicals and Viruses
63.	HUANG, Eng-Shang University of NC (Chapel Hill) 5 RO1 CA 21773-04	Cytomegaloviruses and Human Malignancy

64. HUNTER, Anthony R.
Salk Institute for Bio Studies
5 RO1 CA 28458-03
Viral Transforming Proteins

65. HYMAN, Richard W.
Pennsylvania State Univ
Hershey Medical Center
5 RO1 CA 16498-09
Malignancy and DNA Homology Among the Herpesviruses

66. ISOM, Harriet C.
Pennsylvania State Univ
Hershey Medical Center
5 RO1 CA 23931-05
Regulation of Differentiation in Hepatocytes in Vitro

67. KASAMATSU, Harumi
University of California (LA)
5 RO1 CA 21768-05
Centriolar Antigens and Involvement in Cell Proliferation

68. KETNER, Gary W.
Johns Hopkins University
5 RO1 CA 21309-05
Transformation and Gene Regulation by Adenoviruses

69. KIEFF, Elliott D.
University of Chicago
5 RO1 CA 17281-08
EBV Interaction with Lymphoblasts In Vitro & In Vivo

70. KIT, Saul
Baylor College of Medicine
5 RO1 CA 06656-20
Biochemical Aspects of Viral Carcinogenesis

71. KLEIN, George
Karolinska Institutet
1 RO1 CA 28380-02
Epstein-Barr Virus Determined Nuclear Antigen (EBNA)

72. KLEIN, George
Karolinska Institutet
5 RO1 CA 30264-02
Immune Effector Mechanisms in EBV-Carrying Patients

73. KNIPE, David M.
Harvard University
2 RO1 CA 26345-04
Genetics of Herpesvirus Transformation

74. KOWALSKI, David
Roswell Park Memorial Institute
5 RO1 CA 23996-03
Role of DNA Relaxing Enzyme in SV40 DNA Replication

75. LANCASTER, Wayne D.
Georgetown University
5 RO1 CA 32603-02
Role of Papillomavirus DNA in Cell Transformation

76. LANCASTER, Wayne D.
Georgetown University
5 RO1 CA 32638-02
Papillomavirus DNA and Antigens in Human Neoplasms

77. LEBOWITZ, Jacob
University of Alabama (Birmingham)
5 RO1 CA 17077-09

Circular DNA Implications for Cancer and Drug Resistance

78. LEHMAN, John M.
University of Colorado Med Ctr
5 RO1 CA 16030-09

Pathology of Neoplastic Transformtion

79. LEVINE, Arnold J.
State Univ of NY (Stony Brook)
5 RO1 CA 28127-03

SV40 Cellular Antigens and Host Range

80. LEWIS, James B.
Fred Hutchinson Cancer Res Ctr
5 RO1 CA 29600-02

Functions of Adenovirus Proteins in Transformation

81. LIVINGSTON, David M.
Sidney Farber Cancer Institute
5 RO1 CA 15751-09

Structure and Function of SV40 Non Virion Proteins

82. LIVINGSTON, David M.
Sidney Farber Cancer Institute
5 RO1 CA 24715-05

Isolation and Function of Small SV-40 T Antigen

83. LUFTIG, Ronald B.
University of South Carolina
5 RO1 CA 28078-04

Viral Interaction of Microtubules in Cancer Cells

84. MC DOUGALL, James
Fred Hutchinson Cancer Res Ctr
5 RO1 CA 29350-03

The Biology of Transformation Herpesviruses

85. MANN, Kristine E.
University of Alaska
5 RO1 CA 26048-03

SV40 Tumor Antigen in SV40 Nucleoprotein Complexes

86. MARTIN, Jonathan
Tulane University
5 RO1 CA 29631-03

Molecular Analysis of JC Virus Interaction with Cells

87. MILLER, I. George, Jr.
Yale University
5 RO1 CA 12055-12

Studies of Epstein-Barr Virus

88. MILLETTE, Robert L.
Wayne State University
2 RO1 CA 21065-07

Herpes Simplex Virus Gene Regulation

89. MUNNS, Theodore W.
Washington University
2 RO1 CA 27801-04

Characterization of RNA/DNA in Oncogenic Systems

90. NATHANS, Daniel
Johns Hopkins University
5 PO1 CA 16519-08

Program on Molecular Biology of Viral Tumorigenesis

91. NONOYAMA, Meihan
SHOWA University
Research Institute
5 RO1 CA 31949-02

Marek's Disease Virus: Transformation and Oncogenesis

92. NONOYAMA, Meihan
SHOWA University
Research Institute
5 RO1 CA 31950-02

Oncogenicity of Epstein-Barr Virus

93. NOONAN, Christine
Baylor College of Medicine
5 RO1 CA 30925-02

Interaction of Human Wart Virus With Cultured Skin Cells

94. OZER, Harvey L.
Hunter College
5 RO1 CA 23002-07

Host Functions Related to Tumor Virus Infection

95. PAGANO, Joseph S.
University of North Carolina
5 PO1 CA 19014-06

DNA Virus Genomes, Oncogenesis and Latency

96. PEARSON, Gary R.
Mayo Foundation
2 RO1 CA 20679-07

Biochemistry of Herpes Virus-Induced Membrane Antigens

97. PEARSON, GARY R.
Mayo Foundation
1 R13 CA/TW 33410-01

Fourth International Symposium on Nasopharyngeal Carcinoma

98. PEARSON, George D.
Oregon State University
2 RO1 CA 17699-08

Replication Map of an Oncogenic Virus

99. POLLACK, Robert E.
Columbia University
5 RO1 CA 25066-05

Oncogenic Virus Gene Control of Cell Growth and Shape

100. POLLACK, Robert E.
Columbia University
1 PO1 CA 33620-01

Directed SV40 Mutation: Cell and Molecular Consequences

101. PRIVES, Carol L.
Columbia University
5 RO1 CA 26905-04

Function and Expression of SV40 Viral Tumor Antigens

102. PRUSOFF, William H.
Yale University
2 RO1 CA 05262-22

Iododeoxyuridine, IODO-DNA, and Biological Activity

103. RAAB-TRAUB, Nancy
University of NC (Chapel Hill)
1 R01 CA 32979-01A1
EBV Transcription in Nasopharyngeal Carcinoma

104. RAPP, Fred
Pennsylvania State University
5 P01 CA 27503-04
Herpesviruses and Neoplasia

105. RAPP, Fred
Pennsylvania State University
1 R01 CA 34479-01
Latency and Transformation by Herpesviruses

106. RASKA, Karel, Jr.
Rutgers Medical School
5 R01 CA 21196-06
Low Molecular Weight RNA in Adenovirus Infections

107. REKOSH, David M.
State Univ of NY (Buffalo)
5 R01 CA 25674-04
Adenovirus Early Gene Function and DNA Replication

108. RICCIARDI, Robert
Wistar Institute
5 R01 CA 29797-03
Organization and Expression of Adenovirus Genes

109. ROBERTS, Bryan E.
Harvard University
5 R01 CA 27447-05
Organization and Expression of Genes in Viral DNAs

110. ROBERTS, Thomas
Sidney Farber Cancer Institute
5 R01 CA 30002-02
Isolation of Polyoma T Antigens Synthesized in E Coli

111. ROEDER, Robert G.
Washington University
5 R01 CA 16640-08
Regulation of Adenovirus Gene Expression

112. ROEDER, Robert G.
Rockefeller University
1 R01 CA 34223-01
Regulation of Adenovirus Transcription

113. ROIZMAN, Bernard
University of Chicago
5 R01 CA 08494-18
Mechanisms of Viral Infection in Relation to Cancer

114. ROMAN, Ann
Indiana Univ (Indianapolis)
5 R01 CA 29318-02
Regulation of Papovavirus Replication

115. ROSENTHAL, Ken S.
Northeastern Ohio Universities
5 R23 CA 28342-03
Herpes Simplex Virus Glycoproteins and Infection

116. ROTHSCHILD, Henry
Louisiana State University
5 RO1 CA 27943-03
Structure and Function of an SV40 Thermoinducible Mutant

117. RUNDELL, Mary K.
Northwestern University
5 RO1 CA 21327-05
Structural Analysis of the SV40 Tumor Antigen

118. SAMBROOK, Joseph
Cold Spring Harbor Laboratory
5 PO1 CA 13106-12
Cold Spring Harbor Laboratory Cancer Research Center

119. SCHAFFER, Priscilla A.
Sidney Farber Cancer Institute
5 RO1 CA 20260-07
Transforming Genes of Herpes Simplex Virus

120. SCHAFFHAUSEN, Brian
Harvard Medical School
1 RO1 CA 34722-01
Products of the Transforming Genes of Polyoma Virus

121. SCHIERMAN, Louis W.
University of Georgia
5 RO1 CA 30109-03
Immunogenetic Study of a Herpes Virus Induced Lymphoma

122. SHAH, Keerti V.
Johns Hopkins University
5 RO1 CA 13478-11
Investigation of SV40-Related Infections of Man

123. SHENK, Thomas E.
State Univ New York (Albany)
5 RO1 CA 28919-04
Structure and Function of DNA Tumor Virus Genomes

124. SILVERSTEIN, Saul J.
Columbia University
5 RO1 CA 17477-09
Molecular Biology of Herpes Virus

125. SIMMONS, Daniel T.
University of Delaware
5 RO1 CA 25942-03
Origin and Function of SV40 Specific TAU Antigens

126. SOMPAYRAC, Lauren
University of Colorado
1 RO1 CA 34072-01
SV40 Deletion Mutants: Oncogenic Proteins

127. SPEAR, Patricia G.
University of Chicago
2 RO1 CA 21776-06
Herpesvirus Gene Expression in Transformed Cells

128. SPECTOR, David J.
Pennsylvania State University
1 RO1 CA 34381-01
Regulation of Adenovirus E1b Gene Expression

129. SPECTOR, Deborah H.
University of California (SD)
1 RO1 CA 34729-01
Human CMV, Cell-Related DNA, Oncogenes and Kaposi Sarcoma

130. SPELSBERG, Thomas C.
Mayo Foundation
5 RO1 CA 25340-03
A New Class of Epstein-Barr Virus Nuclear Antigen

131. ST. JEOR, Stephen C.
University of Nevada
5 RO1 CA 28089-04
Herpesvirus-Induced Malignancy

132. STEINBERG, Mark L.
New York University
2 RO1 CA 27869-04
Viral Transformation of Human Keratinocytes

133. STROMINGER, Jack L.
Sidney Farber Cancer Institute
5 PO1 CA 21082-07
Molecular Basis of Viral Oncogenesis

134. STROMINGER, Jack L.
Sidney Farber Cancer Institute
5 RO1 CA 24926-04
Study of Epstein-Barr Nuclear Antigen

135. SUMMERS, William C.
Yale University
5 RO1 CA 13515-10
Genetic Study of Animal Viruses

136. TACK, Lois C.
Scripps Clinic & Res Fdn
1 RO1 CA 33979-01
SV40 Chromosome: T-Antigen Complexes and Viral Function

137. TAMM, Igor
Rockefeller University
5 RO1 CA 18608-23
Virus Induced Alterations in Animal Cells

138. TEGTMEYER, Peter J.
State Univ of NY (Stony Brook)
5 RO1 CA 18808-08
Mechanisms of Viral Oncogenesis: SV40 Gene Function

139. TEGTMEYER, Peter J.
State Univ of NY (Stony Brook)
5 PO1 CA 28146-03
Tumor Virus-Host Interaction

140. TENEN, Daniel G.
Sidney Farber Cancer Institute
5 R23 CA 26018-03
SV40 T Antigen Binding Sites in Cellular DNA

141. TEVETHIA, Mary J.
Penn State Univ Hershey Med Ctr
2 RO1 CA 24694-05
Mutagenesis of Specific Regions of the SV40 Genome

142. TEVETHIA, Satvir S.
Penn State Univ Hershey Med Ctr
5 RO1 CA 25000-05
Biology of SV40 Specific Transplantation Antigen

143. THEIS, Gail A.
New York Medical College
5 RO1 CA 18904-06
Responses of Lymphocytes to an Oncogenic Herpes Virus

144. THORLEY-LAWSON, David
Tufts University
7 RO1 CA 31893-01
Epstein-Barr Virus Membrane Antigen

145. TIBBETTS, Clark J.
Vanderbilt University
2 RO1 CA 34126-02
Adenovirus Genome Expression/ Assembly/Transfection

146. TJIAN, Robert
University of CA (Berkeley)
5 RO1 CA 25417-05
The SV40 Tumor Antigen

147. TJIAN, Robert
University of CA (Berkeley)
1 RO1 CA 34724-01
Autoregulation of SV40 Early Gene Expression

148. TROY, Frederic A.
University of CA (Davis)
5 RO1 CA 17327-08
Membrane Bound Enzymes, Tumor Antigens and Malignancy

149. ULTMANN, John E.
University of Chicago
5 PO1 CA 19264-07
Viral Oncology Research Center Program

150. VARSHAVSKY, Alexander
Massachusetts Institute of Technology
5 RO1 CA 30376-02
Studies on the SV40 Virus Structure & Replication

151. VILLARREAL, Luis P.
Univ of Colorado Medical Center
2 RO1 CA 25819-04
Gene Expression of a Small DNA Tumor Virus: SV40

152. WAGNER, Edward K.
University of California (Irvine)
2 RO1 CA 11861-14
Viral RNA Synthesis Control in Herpes Virus Infection

153. WEISSMAN, Sherman M.
Yale University
PO1 CA 16038-09
Program on the Molecular Basis of Viral Transformation

154. WENTZ, William B.
Case Western Reserve
1 RO1 CA 31973-02
Sexually Transmitted Disease in Uterine Carcinogenesis

155.	WETTSTEIN, Felix O. University of California (LA) 2 RO1 CA 18151-07	Analysis of the Shope Papilloma-Carcinoma System
156.	WILLIAMS, James F. Carnegie-Mellon University 2 RO1 CA 21375-06	Genetic Analysis of Adenoviruses
157.	WILLIAMS, James F. Carnegie-Mellon University 1 RO1 CA 32940-01	Study of Adenovirus Trans-formation - Defective Mutants
158.	WILSON, John H. Baylor College of Medicine 2 RO1 CA 15743-10	Oncogenic Mechanisms: SV40 and Host Genetic Analysis
159.	WOLD, William S. St Louis University 5 RO1 CA 24710-05	Adenovirus 2 Coded Early Glyco-protein
160.	ZIMMER, Stephen G. University of Kentucky 1 RO1 CA 33434-01	Analysis of Defined AD2 Transformed Cell Revertants

CONTRACTS ACTIVE DURING FY83

Investigator/Institution/Contract Number		Title
161.	HIRSCH, Martin Massachusetts General Hospital NO1-CP4-3222	Clinical Trials of the Role of Interferon in Preventing Activation of Potentially Oncogenic Viruses in Organ Transplant Patients
162.	HYAMS, Vincent J. Armed Forces Institute of Pathology YO1-CP9-0500	Application of Epstein-Barr Virus Markers to Diagnosis and Prognosis of NPC and Occult Tumors of Nasopharynx Area in USA
163.	LANIER, Anne P. Indian Health Service YO1-CP9-0501	Application of Epstein-Barr Virus Markers to Diagnosis and Prognosis of NPC and Occult Tumors of Nasopharynx Area in USA
164.	PEARSON, Gary Mayo Foundation NO1-CP9-1006	Application of Epstein-Barr Virus Markers to Diagnosis and Prognosis of NPC and Occult Tumors of Nasopharynx Area in USA

SUMMARY REPORT

RNA VIRUS STUDIES I

In the RNA Virus Studies I component research was supported by two funding mechanisms also. There were 99 grants with a funding level of 14.98 million dollars. The 99 grants utilize the murine (81), feline (11), primate (4), bovine (1), rat (1), and hamster (1) model systems. Studies of gene organization, control and expression comprise 39%, with 64% of these dealing with oncogenes; 36% are devoted to studies of virus-cell interaction; 14% are involved in the studies on the characterization and biological activity of retroviruses; 6% support research on cocarcinogenesis; 3% involve studies on the inhibition of viral replication and cell transformation; and 1% support conference grants. Two contracts were active during FY 83 although funds for their support were not provided this fiscal year. The studies supported by the contract mechanism dealt with various aspects of passive immunotherapy in the AKR-lymphoma system.

Studies in the RNA Virus Studies I area are concerned with experiments to elucidate molecular events associated with the conversion of a normal cell to the malignant phenotype utilizing retroviruses in animal model systems. The guiding principle in the efforts is that the malignant phenotype is a stably inherited trait: tumor cells give rise to offspring which are tumor cells. This suggests that oncogenic transformation may be the consequence of genetic alterations. This is clearly the case for cells transformed by oncogenic viruses where specific viral genes are responsible for the maintenance of the neoplastic state. The question naturally arises as to the nature of the genes responsible for naturally occurring tumors, and the genetic rearrangements thought to result in the aberrant activation of these genes. Progress has been enhanced by the important observation that cellular genes, homologous to viral oncogenes, in many instances appear to be responsible for the transfer of the tumor phenotype to fibroblasts in culture. These important observations have spurred a search for mechanisms by which these endogenous cellular genes may become altered to produce products with a potential for causing malignant transformation. The mechanisms responsible for the activation of cellular oncogenes may involve: 1) local changes or mutations in genes involving base changes or small deletions which alter the functional properties of the gene product; 2) gross changes in the relative position of genes which may either involve translocations of structural gene information from one chromosomal location to another or the introduction of activators such as viral LTRs adjacent to genes, such that the frequency of gene expression is enhanced; 3) changes in the numbers of genes involving gene amplification mechanisms which may obviously increase the abundance of specific gene products; and 4) changes in the activity of oncogene promotors either by changing the base sequence itself or by altering structure in the vicinity of regulatory information, as may be envisaged through changes in the pattern of methylation, the degree of supercoiling or other aspects of chromatin structure.

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; the more information gathered concerning this relationship seems likely to elucidate our understanding of the transformation process.

As a group, retroviruses often infect lymphoid tissues and many cause leukemias. Some, such as the feline leukemia virus (FeLV), cause many more deaths by predisposing the host to acute infections by other pathogens than by inducing leukemia. Cats infected with FeLV were previously shown to have prolonged homograft rejection responses, but, in the same study, no evidence was found that the humoral immune response was impaired. In a recent study (24, 25), the humoral response to the synthetic multichain polypeptide (L-tyrosine-L-glutamic acid)-poly-DL-alanine-poly-L-lysine, denoted (T,G)AL, was found to be significantly depressed in healthy cats that were naturally infected with FeLV compared to uninfected controls. In cats with persistent FeLV viremia the major antibody response to (T,G)AL, normally seen at days 9 to 14 after immunization, was both delayed and greatly reduced. Little is known about the way in which FeLV causes immunosuppression. Results by several investigators implicated a virion structural protein of approximately 15,000 daltons. In earlier studies, infection with FeLV was associated with prolongation of allograft rejection, thymic atrophy, depletion of paracortical lymphoid tissues, decreased response to T-cell mitogens, depressed peripheral blood lymphocyte counts, and diminished mobility of lymphocyte membrane capping. Despite these results, which appear to reflect both general impairment of lymphoid tissues and specific impairment of T-cell responses, earlier attempts to show that humoral immunity was depressed by FeLV infection were unsuccessful. Many of these studies were, however, conducted with cats that were inoculated in the laboratory with a particular strain of FeLV. The results of this study indicate that the humoral antibody response is diminished in cats that are naturally infected with FeLV.

Utilizing their experience in the FeLV system, investigators at Harvard University (25) initiated research to detect antibodies to cell membrane antigens associated with human T-cell leukemia-lymphoma virus (HTLV) in patients with acquired immune deficiency syndrome (AIDS). AIDS is a newly described disease that has recently been observed in several U.S. cities and in Haiti. The incidence of AIDS has been increasing dramatically. Although the disease was initially seen only in sexually active homosexual men, it has now been recognized in intravenous drug users, patients with hemophilia, Haitian immigrants, and heterosexual contacts of members of other high-risk groups. The syndrome, suspected to be of viral origin, is characterized by the development of Kaposi's sarcoma (KS), pneumonia caused by Pneumocystis carinii (PCP), and infections with various other opportunistic microorganisms. Such infections apparently develop because of an immune dysfunction that is characterized by lymphopenia with an imbalance of the normal ratio of T-helper

cells to T-suppressor cells. Patients with AIDS have increased titers of antibodies to cytomegalovirus and to the Epstein-Barr virus, and a higher prevalence of antibodies to hepatitis A virus and Treponema pallidum. The cytomegalovirus, which was previously considered as a potential cause of the African form of KS, has also been viewed as a candidate agent for a causative role in the form of KS seen in AIDS patients. Since individuals with AIDS are probably at greater risk than the normal population for infection with many agents, including those transmitted by blood or by close contact, the decision was made to determine if patients with AIDS had increased rates of exposure to HTLV. Serum samples examined for antibodies were obtained from 75 patients with AIDS including 72 men (60 homosexual) and 3 women. These included 34 cases of KS, 30 cases of PCP, 11 cases that had both PCP and KS, and 23 homosexual men with lymphadenopathy (LAS), a syndrome that sometimes progresses to AIDS. These serum samples along with matched and unmatched control subjects were examined for the presence of antibodies to cell membrane antigens associated with HTLV. Nineteen of 75 of the AIDS patients had antibodies directed to surface antigens of Hut 102, a reference T lymphoid cell line infected with the leukemia virus, as did two of the 336 control subjects. Six of 23 patients with LAS were also positive on Hut 102. Twenty-nine samples were tested from patients with chronic active hepatitis and 21 samples were tested from kidney dialysis patients. None of these 50 were positive. These results indicated that homosexual patients with AIDS and LAS have increased risk for infection with HTLV or a related agent. Such individuals should be monitored to determine rates for development of lymphoma, especially those of T-cell origin. The results also suggest that HTLV should, along with cytomegalovirus and other agents, be studied to determine what role, if any, it might play in the development of AIDS.

Retroviruses can be classified into two groups: those that contain oncogenes, and those that do not. Members of the first group (acute transforming retroviruses, or rapidly transforming retroviruses) induce neoplastic disease in infected animals within a few weeks after infection, and cause rapid transformation of target cells in tissue culture. These viruses contain oncogenes ("v-onc" genes) that were derived from normal cellular genes ("proto-onc" or "c-onc" genes) by recombination. Viruses of the second group (slowly transforming retroviruses), which lack oncogenes, induce neoplastic disease in animals only after a long latent period (4-12 months), and do not cause transformation of tissue culture cells at detectable frequency. A third class of retroviruses, which does not fit clearly into either of these groups, consists of viruses such as spleen focus forming virus (SFFV) and mink cell focus forming virus (MCF), which appear to be env gene recombinants. Although these viruses, in some cases, induce rapidly appearing lesions in infected animals, they do not appear to carry an oncogene of the classic type (i.e., a cell-derived oncogene). Rather, sequences located within the env region appear to be responsible for their pathogenic properties, by some unknown mechanism. Examples of all three types of viruses can be found in the RNA Virus Studies I area.

Acute Transforming Retroviruses

The genomes of highly oncogenic retroviruses, the sarcoma and acute leukemia viruses, contain specific genes responsible for oncogenicity as well as other

sequences required for virus replication. For example, the genome of Moloney sarcoma virus includes a single gene (mos) which is responsible for cellular transformation but is not involved in virus replication. Other sarcoma and acute leukemia viruses contain different transforming genes.

The transforming genes of sarcoma and acute leukemia viruses are homologous to DNA sequences present in normal uninfected cells. These normal cell homologs of viral transforming genes are highly conserved in vertebrate evolution and appear to represent normal cell genes which are not linked to viral DNA. Transcription of several of these genes has been detected in normal and neoplastic cells and, in some cases, normal cell proteins have been identified which are closely related to proteins encoded by the homologous viral transforming genes.

The highly oncogenic sarcoma and acute leukemia viruses thus appear to represent recombinants in which a transforming gene, derived from a homologous gene of normal cells, has been inserted into a retrovirus genome. The transforming genes of these viruses are expressed at high levels in virus-infected cells as a consequence of their association with viral transcriptional regulatory sequences. It is thus possible that transformation by these viruses is a consequence of abnormal expression of normal cell genes. Alternatively, transformation might result from structural differences betweeen the viral and cellular proteins.

The transforming genes detected by transfection of DNAs of human bladder carcinomas and a human mammary carcinoma have been recently isolated as biologically active molecular clones (14). These transforming sequences are homologous to sequences present in normal cell DNA and southern blot analysis indicates that activation of the transforming activity of these sequences in neoplasm DNAs is not a consequence of DNA amplification or gross DNA rearrangements detected by this analysis. In addition, a protein associated with expression of the human mammary carcinoma transforming gene has been identified by immunoprecipitation with sera from tumor-bearing mice. Further analysis of these neoplasm transforming genes should elucidate the molecular events involved in transforming gene activation and may contribute to understanding the molecular mechanisms of transforming gene function. The relationship between viral transforming genes and the transforming genes detected by transfection of neoplastic cell DNAs is being investigated by using molecular clones of viral transforming NIH cell lines. The results of this analysis to date indicate that the transforming genes of human bladder and lung carcinoma cell lines are homologous, respectively, to the ras genes of Harvey and Kirsten sarcoma viruses (4). Since the ras gene product, p21, is expressed in both transformed NIH cells and in the original human tumor cell lines, these results strongly suggest a causal role for the cellular ras genes in human bladder and lung carcinomas. Since common transforming sequences are activated in human lung and colon carcinomas, involvement of the cellular homolog of the Kirsten ras gene in human colon carcinomas is also implied.

These findings provide the first direct link between the cellular homologs of retroviral transforming genes and human neoplastic disease. In addition, they have at least three important implications for continued work in this

area. First, they provide additional stimulus for further investigation of relationships between transforming genes of retroviruses and naturally occurring neoplasms. Second, analysis of the bladder and lung carcinoma transforming genes will be greatly facilitated by previous work on the homologous viral transforming genes. Finally, since the ras^H and ras^K genes are members of a family of related cellular genes, the involvement of two different members of this gene family in human bladder, lung and colon carcinomas suggests the possibility that these genes may also be involved in other human carcinomas. If this is the case, further studies of these genes and their gene products may contribute to understanding the etiology of a significant class of human tumors.

In a recent series of experiments (4,93) investigators have located the genetic changes leading to the activation of the T24 oncogene within a 0.9-kb SacI-KpnI DNA fragment of the human c-has/bas-1 gene. Comparative sequence analysis of these 0.9 kb DNA fragments derived from the T24 oncogene and its normal counterpart revealed a single base pair difference: the guanosine residue present in the normal c-has/bas-1 gene has been changed into a thymidine residue in the T24 oncogene. This point mutation, within the exon sequences, led to the substitution of a glycine residue in the normal c-has/bas-1 gene-encoded p21 protein with a valine residue in the corresponding translational product of the T24 oncogene. These results, taken together, indicate that a single point mutation was responsible for the activation of this oncogene in T24 human bladder carcinoma cells.

Demonstration that a single genetic change can activate a human transforming gene poses the dilemma as to how these observations can be related with the widely accepted multi-stage model for the development of human neoplasias. It is possible that activation of the T24 oncogenes may represent a late, irreversible step in oncogenesis. Support for this hypothesis comes from the fact that NIH 3T3 mouse cells, the cells used to identify this oncogene, are thought to be preneoplastic rather than normal cells. However, the possibility that this oncogene may have a role in the onset of certain human cancers cannot be excluded at present. Development of biochemical and immunological reagents capable of specifically detecting the presence of the T24 bladder carcinoma oncogene (or its gene product) in both naturally and experimentally induced neoplasms should help to define the role of this dominant transforming gene in carcinogenesis.

Studies with Abelson murine leukemia virus (AbMuLV), Moloney sarcoma virus (Mo-MSV), rat sarcoma virus and feline sarcoma viruses have provided insight into the mechanism of action of the oncogene. AbMuLV is an acutely transforming retrovirus which induces lymphoid neoplasms in mice with latent periods of 3-5 weeks. The oncogenicity of AbMuLV is attributed to expression of a viral transforming gene (v-abl) which encodes a protein with tyrosine-specific kinase activity. Two recent findings (97), however, suggest that oncogenesis by AbMuLV may not be a single-step consequence of v-abl expression. First, AbMuLV infection of bone marrow cells in vitro stimulated proliferation of immature blast cells which did not exhibit the in vitro growth properties of tumorigenicity characteristic of cells from AbMuLV-induced neoplasms. These AbMuLV-infected cell populations evolved to fully neoplastic cells only after several weeks in culture, suggesting that

secondary events were required for expression of the neoplastic phenotype. Second, the loss of the AbMuLV genome was after prolonged in vivo passage of some AbMuLV-induced neoplasms, suggesting that expression of v-abl may be necessary for initiation, but not maintenance, of transformation; and that AbMuLV-induced neoplasms contain cellular transforming genes detectable by transfection which are distinct from AbMuLV sequences. Taken together, these results suggest that v-abl expression may induce early events in the neoplastic process and that secondary activation of a distinct cellular transforming gene may be involved in progression to neoplasia.

Mo-MSV is a replication-defective transforming retrovirus which arose by recombination between Moloney murine leukemia virus (Mo-MLV) and the proto-oncogene c-mos, present in the BALB/c mouse genome. Mo-MSV was initially isolated from a rhabdomyosarcoma of an Mo-MLV-infected mouse. From the initial Mo-MLV/Mo-MSV tumor, many variants of Mo-MSV were isolated. Given the detailed knowledge of the v-mos nucleotide sequence and the unique structure of its chimeric env-mos gene product, it was of interest to reexamine the apparent recombination points of these related variants with the precision of nucleotide sequencing. The purpose was to understand first, the recombinational events which led to diversity at the junctions between the env gene and v-mos previously observed by electron microscopy, and second, whether the predicted gene products of other strains would be similar to the v-mos gene product. In order to accomplish these objectives different variants of Mo-MSV were examined by nucleotide sequencing to compare the junctions between the acquired cellular sequence, v-mos, and the adjacent virus-derived sequences (46). These variants included 124-MSV, m1-MSV, and HT1-MSV and also the purportedly independent isolate Gazdar MSV. These four strains have an identical 5' junction between the murine leukemia virus env gene and the v-mos gene. This junction lies within the sixth codon of the chimeric env-mos coding region that encodes the transforming gene product. In contrast, at the 3' junction between the v-mos gene and the murine leukemia virus env gene, the three variations examined here were all different. Every transforming retrovirus that bears a cell-derived sequence has two junctions between viral information and cell-derived information. In Mo-MSV, these are referred to as the 5'-env:mos and the 3'-mos:env junctions. Unfortunately, knowledge of the nucleotide sequence at the junctions is not sufficient to define the recombination events that resulted in the original capture of the cellular sequence by the retrovirus. A small deletion was found in the COOH-terminal portion of the m1-MSV env-mos coding region, indicating that the COOH terminus of this transforming gene product must be different from that of 124-MSV or HT1-MSV. The data are consistent with the thesis that a virus closely related to HT1-MSV was the primordial Mo-MSV, and that all other related strains evolved from it by deletion or rearrangement. For several other transforming oncogenes besides mos, multiple variants or isolates have been described. For example, AbMuLV arose by recombination between Mo-MLV and the cellular proto-oncogene c-abl. The primordial strain is probably P160, which encodes a gag-abl fusion protein of MW 160,000. Another commonly studied strain, P120, is related to the P160 strain by a single internal deletion.

Many acutely transforming retroviruses that have acquired cellular inserts in the manner of Mo-MuSV express a viral phosphoprotein that has an associated

protein kinase activity. This protein kinase activity, usually detected as autophosphorylation, has been found to be associated with transforming proteins of Rous sarcoma virus, AbMuLV, Snyder-Theilen strain of feline sarcoma virus, Y73 avian sarcoma virus, and Fujinami avian sarcoma virus. First identified in the Rous sarcoma virus, the protein kinase activity of phosphorylated $p60^{src}$ has been shown to be an intrinsic property of the transforming protein.

Researchers have characterized a variant of Mo-MuSV-124, known as ts110 MuSV, that codes for a gag-mos polyprotein of 85,000 molecular weight termed $P85^{gag-mos}$. Two findings provided some insight into the possible activity of $P85^{gag-mos}$. The first is that $P85^{gag-mos}$ is phosphorylated in vivo at serine and threonine residues. Second, virions harvested from ts110-infected cells contain a unique protein kinase not found in either MuLV particles or wild-type MuSV (MuSV-349) particles. With this information available, a study was begun (1) to determine whether $P85^{gag-mos}$ is indeed a protein kinase. An immune complex kinase assay was used to show the in vitro phosphorylation of $P85^{gag-mos}$ by a manganese-preferring protein kinase activity. The results indicated that $P85^{gag-mos}$ is phosphorylated in anti-p30 immune complex kinase reactions. Phosphoamino acid analyses indicated that the in vitro phosphorylated $P85^{gag-mos}$ contained phosphoserine and phosphothreonine. Also that incubation of anti-p30 immunoprecipitates at 39°C drastically reduced, in a specific way, the kinase activity associated with $P85^{gag-mos}$. This result and other data suggest that the kinase is virus-encoded. Because $P85^{gag-mos}$, but not $Pr65^{gag}$, is phosphorylated in anti-p30 immunoprecipitates from MuLV-MuSV ts110 producer cells, the kinase enzyme is associated with $P85^{gag-mos}$ and not gag gene products.

Similarities have been found between two new mutations in the rat sarcoma virus oncogene and those present in the human bladder carcinoma gene (23). The viral oncogene $v\text{-}ras^{R}$ of the Rasheed strain of the rat sarcoma virus (RaSV) encodes a gag-fusion transforming protein (p29) which is immunologically related to p21 encoded by the transforming gene $v\text{-}ras^{H}$ of the Harvey murine sarcoma virus (Ha-MSV). Various investigators have reported that the $v\text{-}ras^{R}$ and the normal rat cellular counterpart of this gene are identical except for the replacement of glycine with arginine at residue-12 of p21. Comparison of the nucleotide sequence of the $v\text{-}ras^{R}$ with those of the $v\text{-}ras^{H}$ indicated that although $v\text{-}ras^{R}$ also encodes for arginine at the position corresponding to residue-12 of p21, the amino-terminus of p29 shows homologies with highly conserved gag-p15 related sequences not present in the Ha-MSV.

The $v\text{-}ras^{R}$ also contains several new amino acid replacements and point mutations within the p29 encoding region that are not present in p21 of Ha-MSV. Two of these changes at residues-118 and -181 of p29 are exactly the same as reported in the p21 of the human bladder cancer gene at residues-59 and -122 respectively. The critical autophosphorylation site at the threonine residue-59 of p21 has been replaced by alanine in both the human p21 and at the corresponding position in the rat p29. This confirms earlier observations that p29 of RaSV is not autophosphorylated.

The human p21 of both normal and oncogenic T24 genes differ from $v\text{-}ras^{H}$ encoded p21 in three amino acid changes. Two of these three changes are present in the $v\text{-}ras^{R}$. The results suggest therefore that the p21 encoding region of

the human bladder carcinoma gene may be more closely related to the v-ras$^{R}_{H}$ gene of RaSV than it is to the v-ras^{H} of Ha-MSV.

FeLV is horizontally transmitted between domestic cats and has been shown to be the etiological agent for lymphosarcoma and a variety of other feline diseases. Five feline sarcoma viruses (FeSVs) have been isolated from naturally occurring FeLV-associated feline fibrosarcomas. Three of them, the GA-, the ST-, and the SM-FeSV, have been characterized extensively. The ST- and the GA-FeSV contain the oncogene fes, which is related to the avian oncogene fps. The SM-FeSV contains the oncogene fms. The Parodi-Irgens FeSV (PI-FeSV) was isolated in 1973 from an 8-month-old FeLV-infected pet cat with a naturally occurring multicentric fibrosarcoma. Simian sarcoma virus (SSV) was isolated from a naturally occurring sarcoma of a pet woolly monkey and is the only acute transforming retrovirus which derived from a primate. In recent studies (31) the oncogene and the putative transforming protein of the PI-FeSV have been identified. The PI-FeSV is defective and needs a helper virus for its replication. The v-onc sequences in the PI-FeSV were found to be related to the v-sis sequences of the SSV. PI-FeSV nonproducer cells express two viral RNAs, a 6.8- and a 3.3-kilobase RNA. The 6.8-kilobase RNA contains gag, sis, and env sequences but lacks the pol gene. The 3.3-kilobase RNA, on the other hand, contains only env sequences. One feline leukemia virus-related protein product was detected in these cells, namely, a 76-kilodalton protein which contains determinants of the feline leukemia virus gag proteins p15 and p30. The v-sis sequences in the PI-FeSV have been located near the 5' end of the viral genome. Taken together, these results imply that the p76 protein contains both FeLV gag and sis sequences and probably is the transforming protein of this virus. In contrast, in SSV the sis sequences are located towards the 3' end of the viral genome, and the sis protein is thought to be expressed via a subgenomic RNA. PI-FeSV and SSV therefore use different schemes to express their onc-related sequences. The v-sis sequences in the PI-FeSV contain restriction sites which reflect the different origin of the v-sis sequences in the PI-FeSV and SSV. The homologous oncogenes of the PI-FeSV and SSV thus were transduced by two different retroviruses, FeLV and the simian sarcoma-associated virus, apparently from the genomes of different species.

In other studies (24, 25) utilizing the feline system, polyproteins (gag-fes) encoded by the ST-FeSV and the GA-FeSV were previously shown to be associated with mink or rat cells that were nonproductively transformed in vitro. Recent studies have demonstrated that the same gag-fes proteins were found in cat cells transformed in vitro. Of greater importance, these transformation-related proteins were also in cells taken from fresh biopsies of FeSV-induced tumors. Cells from fibrosarcomas induced with ST-FeSV had gag-fes proteins that were characteristic of this strain. Fibrosarcomas and melanomas were induced with GA-FeSV and both types of tumors contained the protein that is characteristic of cells transformed in vitro with this virus. Expression of these proteins in cultured tumor cells appeared to be independent of the passage level. Based on two-dimensional tryptic peptide analysis, the gag-fes proteins of cat tumor cells appeared to be indistinguishable from those found in cells transformed in vitro. The polyproteins of the cat tumor cells have a closely associated protein kinase activity, as demonstrated in the in vitro assay, and phosphorylated tyrosine residues. Gag-fes proteins of either the ST or GA

class were not present in cell cultures initiated from five spontaneous cat tumors.

In still other studies involving the feline system researchers have expressed concern about the true identity of the feline oncornavirus-associated cell membrane antigen (FOCMA). FOCMA was thought to be a transformation-specific antigen present in cells transformed by FeLV and by FeSV. The presence of FOCMA in FeLV-induced leukemias and FeSV-induced fibrosarcomas of cats suggested that it is the product of the FeSV-onc gene and might be the common transforming factor in both types of tumors. However, this thesis is inconsistent with the data of other investigators. FOCMA was also reported to be distinct from FeLV structural proteins on the basis of (a) absence of FOCMA expression in nontransformed, FeLV-infected fibroblasts or lymphocytes (b) failure of FeLV proteins to absorb FOCMA activity from naturally occurring viremic cat sera, (c) lack of correlation between virus neutralizing activity and anti-FOCMA activity in cat sera, and (d) FOCMA expression on FeLV-negative cat leukemia cells.

Recent studies (31) have shown that a common denominator in the activity of naturally occurring viremic cat antisera to FOCMA is, in fact, their reactivity to FeLV-C antigenic determinants. The cat antisera, monoclonal antibodies to FOCMA, and monoclonal antibodies to FeLV-C all reacted in immunofluorescence assays with FeLV-C-infected cells and immunoprecipitated a molecule electrophoretically indistinguishable from envelope glycoprotein of FeLV. Viremic cat antisera to FOCMA bound to budding virus particles of FeLV-C-infected cells, even though some of them could not be absorbed by mature virion proteins. Thus, the unusual feature of cat antibodies to FOCMA is their binding to nascent but not to mature virus particles. FOCMA-positive cat lymphomas expressed antigenic determinants of FeLV-C gp70, with or without productive infection. FeLV-negative tumors not expressing FeLV-C gp70 were also FOCMA negative. Furthermore, most of the viremic cat sera and the monoclonal antibodies to FOCMA did not react with FeSV-transformed nonproducer cells. The absence of FOCMA from these cells and from FeLV-negative lymphoid tumors and its presence in FeLV-C infected fibroblasts indicated that this antigen is virus encoded and not a cellular tumor-specific antigen.

Slowly Transforming Retroviruses

Previous studies have identified transforming genes in neoplasms induced by the weakly oncogenic retroviruses such as mouse mammary tumor virus (MMTV) and murine leukemia virus (MuLV). In contrast to acute transforming retroviruses, these weakly oncogenic retroviruses do not contain specific viral transforming genes and require long latent periods (4-12 months) for induction of neoplasia. The transforming genes detected by transfection of DNAs of MMTV-induced mouse mammary carcinomas were not linked to viral DNA sequences, indicating that oncogenesis by these viruses involves indirect activation of cellular transforming genes. In addition, restriction endonuclease analysis indicated that the transforming genes activated in MMTV-induced mammary carcinomas, or in MuLV-induced lymphoid neoplasms, were also activated in chemically induced or spontaneously occurring neoplasms of the same cell types.

The structure of the Mo-MuLV genome is known in great detail; indeed, the complete nucleotide sequence has been determined. The detailed functions carried out by the products of the gag, pol, and env genes, however, are largely unknown. For example, although it is known that the gag proteins are structural elements in the virion particle, the actual role of each mature protein (termed P15, P12, P30, and P10) is uncertain. A genetic analysis of the genome of Mo-MuLV was begun in an attempt to determine the various functions of the proteins and regulatory sequences required in the viral life cycle. A series of deletion mutations localized near the 5' end of the Mo-MuLV genome was generated by site-specific mutagenesis of cloned viral DNA (36). The mutants recovered from such deleted DNAs failed to synthesize the normal glycosylated gag protein $gPr80^{gag}$. Two of the mutants made no detectable protein, and a third mutant, containing a 66-base pair deletion, synthesized an altered gag protein which was not glycosylated. All the mutants made normal amounts of the internal $Pr65^{gag}$ protein. The viruses were XC positive and replicated normally in NIH/3T3 cells as well as in lymphoid cell lines. These results indicate that the additional peptides of the glycosylated gag protein are encoded near the 5' end, that the glycosylated and internal gag proteins are synthesized independently, and that the glycosylated gag protein is not required during the normal replication cycle. In addition, the region deleted in these mutants apparently encodes no cis-acting function needed for replication. Thus, all essential sequences, including those for packaging viral RNA, must lie outside this area.

MuLVs are endogenous, genetically transmitted viruses carried by a variety of inbred strains. Three major classes of MuLV have been defined on the basis of host range: ecotropic MuLVs, which infect and replicate preferentially on mouse cells; xenotropic MuLVs, which infect and replicate on cells of several heterologous species; and amphotropic MuLVs, which replicate on both mouse cells and cells of heterologous species. Depending on the genetic background of the host, different states of virus expression can occur, ranging from complete repression to partial expression of some viral polypeptides to production of complete infectious virus. In addition, infectious virus can be induced from cells of many inbred strains in vitro. For example, halogenated pyrimidines, such as 5-iodo-2-deoxyuridine (IdUrd), have been shown to induce both ecotropic and xenotropic MuLVs. Cycloheximide, an inhibitor of protein synthesis, only induces expression of xenotropic virus. These results suggest that ecotropic and xenotropic proviruses are subject to independent cellular regulatory mechanisms. The low infectivity of endogenous murine leukemia virus DNA in transfection assays suggests that virus transcription is regulated by linkage to cis-acting regulatory DNA sequences. Considerable evidence has also been presented linking various patterns of viral gene expression to differences in the extent of DNA methylation.

The endogenous ecotropic MuLVs of AKR mice have been extensively studied. A recent study (5, 15) has identified a noninducible endogenous ecotropic provirus of AKR/J mice. All AKR/J mice carry at least three endogenous ecotropic viral loci which have been designated Emv-11 (Akv-1), Emv-13 (Akv-3), and Emv-14 (Akv-4). Using two independent AKR/J-derived sets of recombinant inbred mouse strains, AKXL (AKR/J x C57L/J) and AKXD (AKR/J x DBA/2J), as well as the HP/EiTy strain (an Emv-13-carrying inbred strain

partially related to AKR/J mice), the association of these endogenous viral loci with virus expression have been examined. Strains which transmit Emv-11 or Emv-14 or both were found to produce virus spontaneously, whereas strains that transmit Emv-13 alone were negative for virus expression. Restriction endonuclease digestion and hybridization with an ecotropic virus-specific hybridization probe of DNAs from strains which transmit only Emv-13 yielded enzyme cleavage patterns identical to those observed with DNAs from strains transmitting Emv-11 or Emv-14 or both. These findings indicate the absence of any gross rearrangement of Emv-13 proviral sequences. Cell cultures derived from recombinant inbred strains that carry only Emv-13 failed to express detectable infectious virus, viral proteins, or cytoplasmic ecotropic virus-specific RNA even after treatment with 5-iodo-2-deoxyuridine or 5-azacytidine, an inhibitor of DNA methylation. These results indicate that a mechanism(s) other than methylation of Emv-13 proviral DNA is responsible for inhibition of Emv-13 expression.

To understand the molecular mechanisms by which the endogenous MMTV proviruses are expressed and produce late-occurring mammary tumors in C3Hf mice, genomic DNA from normal organs of mammary tumor-bearing and tumor-free mice and from 12 late-occurring C3Hf mammary tumors were analyzed by the use of restriction enzymes and the Southern transfer procedure (31,78). By using the restriction enzymes EcoRI and HindIII, it was found that in addition to the preexisting endogenous MMTV proviruses, new MMTV-specific proviral DNA was integrated into new sites in the host genome in all 12 of the tumors examined. PstI digests of C3Hf tumor DNA revealed that the new proviral DNA found in C3Hf tumors was of endogenous origin. Moreover, the respective sizes of at least one of the new DNA fragments generated by EcoRI or HindIII digestion were the same in at least 50% of the C3Hf tumors analyzed, suggesting that the integration site of this new proviral DNA could be at the same location in the host genome of these tumors. Results may imply that mammary tumorigenesis in C3Hf mice results from activation of cellular oncogenes by an MMTV proviral DNA promoter. Specific hypomethylation of MMTV proviral DNA was detected in the mammary tumors and spleens of C3Hf tumor-bearing mice. These results indicated that most, if not all, of the hypomethylated MMTV proviral DNA sequences were derived from the endogenous MMTV provirus located at the MTV-1 locus, a locus responsible for the production of MMTV antigens and increased incidence of mammary carcinoma in C3Hf mice. In spleens of non-tumor-bearing mice of ages 3, 6, 9, and 12 months, there was progressive hypomethylation of proviral DNA with increasing age, suggesting a possible correlation between demethylation of MMTV proviral DNA in the spleens of C3Hf mice and the expression of endogenous MMTV.

Recombinant Viruses

High-leukemic inbred strains of mice inherit DNA copies of ecotropic type C viruses, and expression of these loci results in a lifelong viremia. Despite compelling evidence that this viremia ultimately results in leukemia, a number of findings have suggested that the inherited ecotropic viruses themselves are not leukemogenic but rather give rise, by genetic recombination, to novel type C viruses which are the proximal leukemogenic agents of inbred mice. One group of recombinant viruses which may serve as inducers of leukemia are the mink cell focus-forming (MCF) viruses. MCF viruses apparently arise during the lifetime

of high-yield-ecotropic-virus mice by recombination between endogenous ecotropic and nonecotropic viruses and invariably appear in leukemias or at the sites of their imminent appearance. They possess novel gp70s and, as a result, a novel host range, being able to infect mouse cells like their ecotropic progenitors and mink cells like their putative xenotropic progenitors.

To test the hypothesis that MCF viruses are the proximal leukemogenic agents of inbred mice, the oncogenicity of a set of cloned MCF viruses obtained from preleukemic or leukemic mice of high leukemic strains was determined. Surprisingly, these viruses fell into two biological groups depending on their mouse strain of origin and the correlated property of whether that strain develops thymomas or leukemias localized grossly in the spleen, these being chiefly tumors of cell lineages other than T cells. MCFs isolated from thymus (class I MCFs) accelerated leukemia when injected into AKR mice, whereas isolates from nonthymic tumors (class II MCFs) did not. One MCF virus whose tissue of origin was uncertain had properties intermediate between those of the two classes. A preliminary analysis indicated that the genomes of the two biologically defined classes of MCF viruses could be distinguished by the methods of RNase T_1 fingerprinting and oligonucleotide mapping. Consistent oligonucleotide differences near the 3' end of the viral genomes were predictive of the origin from spleen versus thymus of an MCF virus. It was of interest to extend these studies by analyzing MCF viruses isolated from additional inbred strains (4, 45). Furthermore, an important question unanswered by the previous studies was, precisely which of the genetic elements known to reside in the 3' third of the viral genome consistently differs between biologically distinct MCF viruses and between MCF viruses and their nonleukemogenic ecotropic progenitors? In order to determine this, T_1 oligonucleotide maps were used, in conjunction with available nucleotide sequences of appropriate type-C viruses, to identify regions of the viral genome that distinguish two biological classes of mink cell focus-forming (MCF) viruses. It was found that leukemogenic MCF viruses from thymus differed from nonleukemogenic MCFs isolated from nonthymic neoplasms in nucleotide sequences encoding Prp15E and the U3 portion of the long terminal repeat (LTR). The thymic isolates possessed recombinant Prp15E genes, with the 5' to mid portion derived from their ecotropic parents and the extreme 3' portion invariably derived from their nonecotropic parents. These viruses probably derived the entire U3 portion of their LTRs from their nonecotropic parents. The nonthymic MCFs appeared to inherit their entire Prp15E coding region from their nonecotropic parents. Investigators failed to detect consistent differences in gp70-coding sequences between the two groups of MCFs, but this may simply reflect limitations of the data. The studies presented here, in conjunction with studies from a number of laboratories indicating a role for MCF gp70 in leukemogenesis, indicate that three genetic elements, gp70, p15E, and the U3 portion of the LTR, may all play a role in determining the leukemogenic phenotype of type C viruses of high-leukemic inbred mice.

Other investigators (48) have found a loss of leukemogenicity caused by mutations in the membrane glycoprotein structural gene of Friend spleen focus forming virus (F-SFFV). Friend virus infection of mice causes progressive leukemogenesis, a rapid splenic erythroblastosis, which develops weeks later into a disseminating erythroleukemia. Furthermore, the replication defective F-SFFV encodes a membrane glycoprotein with an apparent molecular weight of

55,000 (gp55) which is structurally and immunologically related to the membrane envelope glycoproteins of dual tropic murine leukemia viruses. Three spontaneous F-SFFV mutants which encode abnormally-sized, gp55-related glycoproteins with apparent molecular weights of 40,000, 54,000, and 58,000, respectively, have now been isolated. Northern and Southern blot analyses indicate that the mutant nucleic acids do not have substantial deletions or insertions in their glycoprotein gene regions. Protein fragmentation patterns indicate that the mutations affect nonoverlapping domains of the glycoprotein. Furthermore, these mutant glycoproteins seem to be defective in their processing to the plasma membranes. Although transmitted efficiently between cultured cells, the mutants have dramatically reduced leukemogenicities compared with the same titers of wild-type F-SFFV. It was concluded that the gp55 structural gene is necessary for initiating the erythroblast proliferative phase of Friend disease, and that changes in membranes can be primary causes rather than only secondary consequences of tumor progression.

Workshops, conferences and symposia supported by grants in FY 83 included the following: Molecular Neurobiology, June 1-8, 1983 under the auspices of Cold Spring Harbor Symposium on Quantitative Biology (R13 CA 02809). A Workshop supported by the Division of Cancer Cause and Prevention on Bovine Leukemia Virus (BLV) was held on May 4, 1983, for the purpose of defining the state of the art for the interdisciplinary research being conducted with BLV. Additionally, we hope to determine the areas of research needing further NCI support by the grant mechanism, to identify problem areas that need further stimulation and to determine the need for resources and services useful in BLV research. Because of the distant relationship of the HTLV with BLV and their possible common evolutionary origin, interest in BLV has increased during the past year. New initiatives for BLV research will be based upon input from the workshop.

GRANTS ACTIVE DURING FY 83

	Investigator/Institution/Number	Title
1.	ARLINGHAUS, Ralph B. Univ of Texas System Cancer Ctr 5 RO1 CA 25465-04	Biosynthesis and Characterization of Murine Oncornavirus
2.	AXEL, Richard Columbia University 5 PO1 CA 23767-05	Molecular Virology
3.	BACHELER, Lee T. Temple University 5 RO1 CA 29519-04	Organization and Expression of Leukemia Virus Genomes
4.	BALTIMORE, David Massachusetts Institute of Technology 5 PO1 CA 26717-04	Molecular Analysis of Oncogenic Viruses
5.	BEDIGIAN, Hendrick G. Jackson Laboratory 5 RO1 CA 31102-02	A New Murine Model for the Study of Nonthymic Leukemia
6.	BESMER, Peter Sloan-Kettering Inst for Cancer Res 1 RO1 CA 32926-01A1	Oncogenes of New Feline Sarcoma Virus Strains
7.	BURNS, William H. Johns Hopkins University 5 RO1 CA 30090-02	Role of Thymic Epithelium in Viral Leukemogenesis
8.	CARDIFF, Robert D. University of California (Davis) 5 RO1 CA 21454-06	MTV Gene Amplification and Expression
9.	CASEY, James W. Louisiana State University 5 RO1 CA 31702-02	Molecular Mechanisms of Retroviral Induced Leukemia
10.	CERNY, Jan Univ of Texas Medical Br (Galveston) 5 RO1 CA 32591-02	Regulatory Mechanisms of Neoplasia
11.	COGGIN, Joseph H., Jr. University of South Alabama 5 RO1 CA 23491-06	Etiology of a Lymphoma Epizootic in Hamsters

12.	COHEN, J. Craig Louisiana State Medical Center 7 RO1 CA 34823-01	Role of Endogenous Viruses in Mammary Carcinogenesis
13.	COMPANS, Richard W. University of Alabama (Birmingham) 5 RO1 CA 18611-09	Molecular Studies of Oncorna and Arenaviruses
14.	COOPER, Geoffrey M. Sidney Farber Cancer Institute 2 RO1 CA 18689-08	Infectious DNA for Endogenous RNA Tumor Virus Genes
15.	COPELAND, Neal G. Jackson Laboratory 5 RO1 CA 32324-02	Ecotropic MuLVs of Normal and Mutant Strains
16.	DARNELL, James E., Jr. Rockefeller University 5 PO1 CA 18213-08	Correlated Program in Viral Oncology
17.	DATTA, Syamal K. New England Med Ctr Hosp 5 RO1 CA 31789-04	Genetic-Viral-Immunologic Studies in New Zealand Mice
18.	DAVIDSON, Norman R. California Institute of Technology 5 RO1 CA 25991-03	Sequence Organization of Integrated Tumor Virus Genomes
19.	DINA, Dino Yeshiva University 2 RO1 CA 24223-04A1	Regulation of Murine RNA Tumor Virus Gene Expression
20.	DONOGHUE, Daniel University of California (San Diego) 1 RO1 CA 34456-01	Expression of Retroviral Envelope Gene Fusion Proteins
21.	DURAN-REYNALS, Maria L. Yeshiva University 5 RO1 CA 07160-18	Possible Neoplastic Effects of Non-Neoplastic Viruses
22.	ECKNER, Robert J. Boston University 5 RO1 CA 19562-08	Biological and Physical Properties of Friend Virus
23.	ELDER, John H. Scripps Clinic and Research Fdn 5 RO1 CA 25533-05	Sequence Studies of the gp70's of Recombinant Retrovirus
24.	ESSEX, Myron E. Harvard University 5 RO1 CA 13885-09	Oncornavirus-Associated Cell Membrane Antigens

25. ESSEX, Myron E.
Harvard University
5 RO1 CA 18216-07
Study of Feline Leukemia Virus

26. FAMULARI, Nancy G.
Sloan-Kettering Inst for Cancer Res
5 RO1 CA 27950-03
Influence of MuLV env and gag Genes in Leukemogenesis

27. FAN, Hung Y.
University of California (Irvine)
5 RO1 CA 32454-02
Studies of Murine Leukemia Virus Integration

28. FAN, Hung Y.
University of California (Irvine)
5 RO1 CA 32455-03
Expression and Localization of C-Type Virus Genes

29. FERRER, Jorge F.
University of Pennsylvania
1 RO1 CA 34231-01
Studies on a High Incidence Leukemia Herd of Cattle

30. FLEISSNER, Erwin J.
Sloan-Kettering Inst for Cancer Res
5 RO1 CA 15297-10
Viral and Mouse Genes in Leukemia Virus Infection

31. FLEISSNER, Erwin J.
Sloan-Kettering Inst for Cancer Res
5 PO1 CA 16599-09
Oncogenic Viruses Program Project

32. FRIEND, Charlotte
Mount Sinai School of Medicine
5 RO1 CA 10000-18
Filterable Agents and Tumor Induction in Mice

33. GARDNER, Murray B.
University of California (Davis)
5 RO1 CA 30912-02
Mammary Tumorigenesis in Hosts Lacking MuMTV DNA

34. GARDNER, Murray B.
University of California (Davis)
5 RO1 CA 31619-02
Genetic Control of Ecotropic Retrovirus in Wild Mice

35. GIRARDI, Anthony J.
Institute for Medical Research
5 RO1 CA 24940-05
Immunologic Studies in Mouse and Human Breast Cancer

36. GOFF, Stephen P.
Columbia University
5 RO1 CA 30488-03
Construction and Analysis of Retrovirus Mutants

37. GREENBERGER, Joel S.
Sidney Farber Cancer Institute
5 RO1 CA 26785-03
Requirements for Spontaneous Leukemogenesis In Vitro

38. HAAS, Martin
Salk Institute for Biological Studies
5 RO1 CA 30146-02
Viral Malignant Lymphomagenesis in X-Irradiated Mice

39. HANKINS, William D.
Vanderbilt University
5 RO1 CA 26306-03
A Friend Virus Transformation System In Vitro

40. HARFORD, Esther C.
Uniformed Serv Univ of Health Sci
1 RO1 CA 34582-01
Oncogenes (C-RAS) in Human Cancer Induction

41. HASELTINE, William A.
Sidney Farber Cancer Institute
5 RO1 CA 19341-07
The Molecular Biology of Replication RNA Tumor Viruses

42. HASELTINE, William A.
Sidney Farber Cancer Institute
5 RO1 CA 29294-03
Molecular Biology of Leukemia and Sarcoma Retroviruses

43. HAYS, Esther F.
Univ of California (Los Angeles)
5 RO1 CA 12386-10
Development of Lymphoma in the Thymus

44. HOOVER, Edward A.
Colorado State University
5 RO1 CA 32552-02
Pathogenesis of Animal Leukemia

45. HOPKINS, Nancy H.
Massachusetts Institute of Technology
5 RO1 CA 19308-08
Endogenous Viruses of BALB/c Mice

46. HUNTER, Anthony R.
Salk Institute for Biological Studies
2 RO1 CA 17096-09
Macromolecular Synthesis and Growth Control

47. HUNTER, Eric
University of Alabama (Birmingham)
5 RO1 CA 27834-03
Genetics of Primate "D" Type Retroviruses

48. KABAT, David
Univ of Oregon Hlth Sciences Ctr
5 RO1 CA 25810-05
Leukemogenesis by Friend Spleen Focus Forming Virus

49. KAPLAN, Henry S.
Stanford University
5 RO1 CA 03352-27
Biological Aspects of Carcinogenesis by Radiation

50. KAPLAN, Henry S.
Stanford University
5 RO1 CA 29079-03
Studies of Retroviruses from Human Lymphoma Cells

51.	KINGSBURY, David T. University of California (Berkeley) 7 RO1 CA 33275-01	Cloning and Analysis of Murine Endogenous Virogenes
52.	LERNER, Richard A. Scripps Clinic and Research Fdn 5 PO1 CA 27489-04	Immunological and Pathological Consequences of Endogenous Retroviral Expression
53.	LEVY, Jay A. Univ of California (San Francisco) 5 RO1 CA/AM 33137-02	Role of Endogenous Xenotropic Viruses
54.	LILLY, Frank Yeshiva University 5 RO1 CA 19873-06	Genetic Control of Resistance to Friend Virus Disease
55.	LILLY, Frank Yeshiva University 5 RO1 CA 19931-06	Mechanism of the H-2 Effect on Viral Leukemogenesis
56.	LILLY, Frank Yeshiva University 5 RO1 CA 26010-03	Genetic Resistance to Spontaneous Leukemia in Mice
57.	LUFTIG, Ronald B. University of South Carolina 5 RO1 CA 28077-05	Assembly of Murine Leukemia Viruses
58.	MAYER, Allen J. New York University 5 RO1 CA 23834-03	Endogenous Virus Expression and Leukemogenesis
59.	MERUELO, Daniel New York University Medical Center 5 RO1 CA 22247-06	Genetic Control of Virus-Induced Leukemia
60.	MERUELO, Daniel New York University Medical Center 5 RO1 CA 31346-02	Loci Affecting Radiation/RadLV Induced Leukemogenesis
61.	MODAK, Mukund J. Sloan-Kettering Inst for Cancer Res 5 RO1 CA 21404-06	Molecular Effects of Enzymatic DNA Synthesis
62.	MURPHY, Edwin C. Univ of Texas System Cancer Ctr 1 RO1 CA 34734-01	MUSV TS110: A Potential TS Transcriptional Mutant of MUSV
63.	O'DONNELL, Paul V. Sloan-Kettering Inst for Cancer Res 5 RO1 CA 31491-02	Kinetic Study of Virus-Accelerated Leukemia in AKR Mice

64. OLSEN, Richard G.
Ohio State University
5 RO1 CA 30338-03
FeLV Leukemogenesis and Preneoplastic Lesions

65. OZANNE, Bradford W.
Univ of Texas Hlth Sci Ctr (Dallas)
5 RO1 CA 23043-06
Peptide Transforming Factors from Transformed Cells

66. PARKS, Wade
University of Miami
5 RO1 CA 27890-03
MMTV in Spleen Lymphoid Cells: Immunologic Effect

67. PETERSON, David O.
Texas A & M Research Foundation
5 RO1 CA 32695-02
Genetic and Molecular Analysis of Steroid Responsiveness

68. PIKO Lajos
Veterans Administration Hospital
5 RO1 CA 24989-05
Gene Expression in Early Mouse Development

69. PINCUS, Theodore P.
Vanderbilt University
5 RO1 CA 30145-02
Genetic Restriction of Endogenous Retroviruses

70. PINCUS, Theodore P.
Vanderbilt University
7 RO1 CA 31920-01
Natural Host Control Mechanisms in Mouse Leukemia

71. PROFFITT, Max R.
Cleveland Clinic Hospital
5 RO1 CA 20242-07
Autoimmunity and Virus-Induced Leukemia

72. RASCHKE, William C.
La Jolla Cancer Research Foundation
5 RO1 CA 30903-03
Characterization of the Abelson Leukemia Virus

73. RASHEED, Suraiya
University of Southern California
5 RO1 CA 27246-03
Leukemia and Sarcoma Genes in Cellular Transformation

74. ROSENBERG, Naomi E.
Tufts University
5 RO1 CA 24220-05
Abelson Leukemia Virus Transformation of Lymphocytes

75. ROSENBERG, Naomi E.
Tufts University
1 RO1 CA 33771-01
RNA Tumor Virus-Hematopoietic Cell Interaction

76. ROSNER, Marsha A.
Massachusetts Institute of Technology
5 RO1 CA 32267-02
Isolation and Characterization of Retrovirus Receptors

77. ROY-BURMAN, Pradip
University of Southern California
5 RO1 CA 26809-03
Oncodevelopmental Gene Expression in Feline Leukemia

78. SARKAR, Nurul H.
Sloan-Kettering Inst for Cancer Res
5 RO1 CA 17129-09
Components of the Mouse Mammary Tumor Virus

79. SCHWARTZ, Robert S.
Tufts University
2 PO1 CA 24530-04A1
Experimental Leukemogenesis

80. SCHWARTZ, Robert S.
Tufts University
1 RO1 CA 31849-01
Experimental Model of Malignant Lymphoma

81. SOMERS, Kenneth D.
Eastern Virginia Medical School
5 RO1 CA 28474-03
Cellular Transformation by MSV

82. STAAL, Stephen P.
Johns Hopkins University
5 RO1 CA 26089-03
Leukemogenesis in AKR Mice

83. STEFFEN, David L.
Worcester Fdn for Experimental Biology
5 RO1 CA 30674-03
Mechanisms of Viral and Nonviral Leukemogenesis

84. THACH, Robert E.
Washington University
5 RO1 CA 13008-11
Replication of Virulent and Oncogenic Viruses

85. THEILEN, Gordon H.
University of California (Davis)
5 RO1 CA 16869-06
Feline Sarcoma Virus Antigens and Immunity

86. THOMAS, Christopher Y.
University of Virginia
5 RO1 CA 32995-02
Molecular Genetics of Leukemia Viruses of Inbred Mice

87. VAIDYA, Akhail
Hahnemann Med Coll and Hosp of Phil
5 RO1 CA 22413-06
Etiological Studies of Mammary Carcinoma

88. VARMUS, Harold E.
Univ of California (San Francisco)
5 RO1 CA 19287-08
Molecular Biology of Mouse Mammary Tumor Virus

89. VERMA, Inder M.
Salk Institute for Biological Studies
2 RO1 CA 16561-09
Reverse Transcriptase from RNA Tumor Viruses

90. VERMA, Inder M.
Salk Institute for Biological Studies
5 RO1 CA 21408-07
Genetic Organization of RNA Tumor Viruses

91. VOGT, Marguerite M.
Salk Institute for Biological Studies
5 RO1 CA 13608-11
Viral Gene Functions Involved in Transformation

92. WATSON, James D.
Cold Spring Harbor Laboratory
5 R13 CA 02809-28
Support for Symposia on Quantitative Biology

93. WEINBERG, Robert A.
Massachusetts Institute of Technology
5 RO1 CA 17537-09
Interaction of Sarcoma and Leukemia Genomes

94. WEISSMAN, Irving L.
Stanford University
5 RO1 CA 32031-02
The Receptor-Mediated Leukemogenesis Hypothesis

95. WHEELOCK, E. Frederick
Hahnemann Med Coll and Hosp of Phil
5 RO1 CA 32575-02
Role of Endogenous Viruses in Tumor Dormant States

96. WILSON, Michael C.
Scripps Clinic and Research Fdn
1 RO1 CA 33730-01A1
Regulation and Expression of the Gp70 Multigene Family

97. WITTE, Owen N.
University of California
5 RO1 CA 27507-04
Transformation by Abelson Murine Leukemia Virus

98. YAMAMOTO, Keith
Univ of California (San Francisco)
5 RO1 CA 20535-07
Mechanisms of Gene Regulation by Steroid Receptor Proteins

99. YANG, Wen K.
Oak Ridge National Laboratory
5 RO1 CA 30308-03
Mechanism of Fv-1 Restriction of Murine Leukemia Viruses

CONTRACTS ACTIVE DURING FY 83

100. BOLOGNESI, Dani
Duke University
NO1-CP3-3308
Studies Related to Passive Serum Therapy of AKR Leukemia

101. Kaplan, Henry
Stanford University
NO1-CP4-3228
Characterization of Hodgkin's Disease

SUMMARY REPORT

RNA VIRUS STUDIES II

In the RNA Virus Studies II program, there are 93 research grants with an estimated total cost of 12.4 million dollars. Of these, approximately 85% are predominantly involved with studies of avian tumor viruses. Five percent concern hepatitis B virus (HBV) and its relationship to primary hepatocellular carcinoma (PHC). 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 (genes responsible for the transformation of cells from normal to malignant) and possible explanation(s) of how viruses without definitive oncogenes are involved in oncogenesis. A few examples of the types of studies accomplished in the last year will follow.

The first area described will involve the isolation and characterization of new viral and cellular oncogenes. A variety of different types of neoplastic cells contain activated transforming genes which can be detected by their ability to efficiently transform NIH 3T3 mouse cells upon transfection. Examples of such neoplasms are chicken B cell lymphomas. These lymphomas are induced by infection with avian lymphoid leukosis virus (LLV), a retrovirus which induces neoplasms with long latent periods and does not contain a specific viral transforming gene. High molecular weight DNAs of these lymphomas transform NIH 3T3 cells with efficiencies of approximately 0.1 transformant per microgram DNA. In contrast, DNAs of LLV-infected non-neoplastic tissues lack transforming activity, indicating that a transforming gene has been activated during the neoplastic process. Analysis of NIH cells transformed by LLV-induced B-cell lymphoma DNAs indicates that the activated transforming gene detected by transfection is not linked to viral DNA sequences.

Recently the molecular cloning and characterization of the transforming gene detected by transfection of chicken B-cell lymphoma DNA has been accomplished. The cloned isolated transforming gene (known as λChBlym-1) is homologous to a family of sequences present in DNA of normal chicken cells. Homologous sequences are detected in human DNA, indicating that this gene family is well conserved in evolution. The nucleotide sequence of the transforming gene suggests that it encodes a small protein which shares homology with proteins of the transferrin family.

To examine the evolutionary conservation of the transforming sequence, M13R9, a probe derived from λChBlym-1 was hybridized to Southern blots of human DNA. Multiple homologous fragments were detected in BamHI-, EcoRI- and HindIII-digested human DNAs. Using hybridization and washing conditions of moderate stringency, similar intensities of hybridization to human and chicken DNAs were observed but the hybridization to human DNA was less intense using conditions of greater stringency. These results indicate that the gene family homologous to the λChBlym-1 transforming gene is well conserved in vertebrate evolution.

The M13R9 probe was also hybridized to molecular clones of the retroviral transforming genes abl, erb, fes, fms, mos, myb, myc, ras^H, ras^K, rel, sis and src. No hybridization was detected indicating that the transforming gene of λChBlym-1 is not homologous to the transforming genes of these sarcoma and acute leukemia viruses.

Thus a biologically active transforming gene detected by transfection of chicken B cell lymphoma DNA has been isolated and characterized. This transforming gene is homologous to a small family of normal cell genes which are highly conserved in vertebrate evolution. The high degree of evolutionary conservation observed for the transforming gene of λChBlym-1 is also characteristic of cellular homologues of retrovirus transforming genes. However, with the exception of the ras gene family, the cellular homologues of retroviral transforming genes are present as single copies in normal cell DNAs. In addition, no homology was detected between the transforming gene of λChBlym and the transforming genes of 12 distinct retroviruses, including ras^H and ras^K. The transforming gene of λChBlym-1 thus seems to represent a new transforming gene which is unrelated to viral transforming genes or to the transforming genes detected by transfection of human bladder and lung carcinoma DNAs, which are cellular homologues of viral ras genes. Since the cellular gene family homologous to the λChBlym-1 transforming gene is conserved in human DNA, it will be of interest to investigate the possible relationships between the members of this gene family and the different transforming genes detected by transfection of DNAs of human and mouse neoplasms representative of discrete states of B- and T-lymphocyte differentiation.

The nucleotide sequence of the λChBlym-1 transforming gene indicates that it encodes a small protein (molecular weight 7,800) which is homologous to the amino-terminal region of proteins of the transferrin family. Transferrin, an iron-binding protein of molecular weight approximately 80,000 daltons, is present in serum of all vertebrates and is an essential growth factor for cells in serum-free medium. Expression of a transferrin-related surface antigen and of transferrin receptors is closely correlated with proliferation of both normal and neoplastic cells, suggesting a possible involvement of these receptors in control of cell proliferation. In addition, transferrin has been reported to stimulate growth of lymphoctyes independently of its iron transport activity. If the homology of the small protein encoded by the λChBlym-1 transforming gene to transferrin reflects a functional relationship, it is thus attractive to speculate that the λChBlym-1 protein may function via interaction with a cellular regulatory system related to transferrin (57, 58).

SKV is a replication defective, transformation inducing retrovirus which was isolated in vitro from an endpoint diluted stock of the nontransforming, replication competent retrovirus tdB77. SKV causes morphological transformation and growth in soft agar of chicken embryo (CE) cells. Restriction mapping shows that SKV is a recombinant between tdB77 and chicken genomic sequences. In accordance with convention, the cell-derived transforming region has been given a three letter designation: ski. Ski does not cross hybridize with any of the previously described viral oncogenes. Ski is

represented in the cytoplasmic poly A plus RNA of uninfected CE cells by species of 7.5 and 5.7 Kb.

A fragment of a viral genome containing the viral ski gene has recently been cloned and its biological activity is now being tested after in vitro recombination with appropriate cloned viral vectors of avian and mammalian origin. The cloned probe is being used to study tissue specific distribution of the c-ski message. The influence of v-ski on both cell transformation and differentiation, and the conservation of c-ski in (at least) avian and mammalian taxa suggest that c-ski may have an important role in regulating processes of cellular growth and development (76).

To prove that ski is the SKV transforming gene a cloned ski-containing Xho fragment derived from the 5.7 kb SKV provirus was employed. This fragment was used to construct a 5.7 kb SKV genome by in vitro recombination at homologous Xho sites in a cloned RAV-1 provirus. Cotransfection of chicken embryo cells (CECs) with the reconstructed SKV (RAV-SKV) and the RAV-1 provirus yielded typical SKV foci. Northern blots of viral RNA produced by the transfected cells detected a single ski-containing genome of 5.7 kb. The virus transformed both CECs and quail embryo cells and showed the same stimulation of quail cell myogenesis previously observed upon SKV infection.

To determine whether ski was an oncogene, SKVs or SKV producing CECs (either SKV770 or RAV-SKV) were injected subcutaneously and intramuscularly into two-day-old chicks. Within two weeks of injection of SKV infected- or RAV-SKV infected CECs, half of the birds developed cutaneous squamous cell carcinomas at the site of subcutaneous injection. No such tumors developed in birds injected with RAV-SKV (approximately 10^3 ffu), SKV770 (less than 10^4 ffu), RAV-1 or RAV-1 infected CECs (76).

General comparisons of viral and cellular oncogenes have revealed the following exceptional differences: divergence between the amino acid sequences at the carboxy-termini of v-src and c-src (12 residues in c-src); and a large difference in size between the proteins encoded by v-myb (45kd) and c-myb (75kd) - as if only a portion of the coding domain of c-myb has been transduced into virus to generate v-myb.

Other features of the transduction of v-myb have been uncovered by comparison of the nucleotide sequences of v-myb and c-myb: the leftward recombination occurred within an intron of c-myb; the rightward recombination within an exon of the cellular gene; and the exons of c-myb have been spliced precisely during transduction to generate v-myb. These results represent the first detailed explication of the topography of a cellular oncogene with introns and sustain current views of how retroviruses might transduce cellular genes.

The expression of c-myb in various developmental lineages has been explored by searching for the protein encoded by the gene. Expression is prominent in immature cells in the erythroid and lymphoid series of hemopoiesis, but undetectable in myelomonocytic cells. In some established lines of leukemia cell, aberrantly modified forms of the c-myb protein are produced.

Retroviruses without oncogenes apparently initiate tumorigenesis by insertional mutagenesis: integration of viral DNA activates a previously quiescent cellular gene. The cellular gene is a c-myc in lymphomas induced by RAVs. Activation of c-myc requires only a single long term repeat (LTR); no other component of the viral genome is necessary. Preliminary evidence for a similar mechanism, affecting another cellular gene, has been obtained for the genesis of renal carcinomas by MAV-2. The activated cellular gene is presently unidentified, but appears not to be among the known cellular oncogenes.

The potential role of cellular oncogenes in the genesis of spontaneous human tumors has been explored by searching for expression of the genes in cultured cells derived from tumors. Over 100 lines of human tumor cells of numerous descriptions have been examined. Little evidence was found for enhanced expression of the known cellular oncogenes except in cells bearing evidence of gene amplification (double minute chromosomes or homogeneously staining regions on marker chromosomes). The amplification has affected c-myc in a human neuroendocrine tumor (APUDOMA COLO 320), and Kirsten c-ras in a mouse adrenocortical tumor (Y-1). Expression of the cellular amplified genes is enhanced approximately 50-fold in each instance, in accord with the 50-fold amplification of the genes.

The cellular response to Platelet-Derived Growth Factor (PDGF) was explored and a novel modification of $pp60^{c\text{-}src}$ that occurs within minutes of binding of PDGF to the cell surface and in concert with enhanced phosphorylation of tyrosine on other cellular proteins was found. The modification affects approximately 15% of the detectable $pp60^{c\text{-}src}$ and represents a new phosphorylation in the amino-terminal domain of the protein. Modification of $pp60^{c\text{-}src}$ is accompanied by 3- to 5-fold increases in its protein kinase activity. This is the first direct evidence that implicates a product of a cellular oncogene in the immediate response to regulators of normal cellular growth (7).

Fine structural analysis of the transforming proteins of PRCII, PRCIV, and Esh sarcoma virus has resulted in the mapping of the tyrosine phosphorylation sites, the identification the onc-gag junction fragments and has demonstrated sequence similarities among the various transforming proteins around the tyrosine phosphoacceptor site.

A detailed comparison of of the genome organization and structure of class II avian sarcoma viruses PRCII, PRCIV, PRCII-p and Fujinami sarcoma virus has been carried out. The transformation specific onc sequences were produced, residual gag and residual env information was identified and variable and constant regions within the onc domain were defined.

Work has also begun on the avian sarcoma and erythroleukosis virus S13. This virus is primarily sarcomagenic in white leghorn chickens. It induces transformation in chicken and Japanese quail fibroblasts; the transformed cells have a fusiform morphology. S13 also transforms cells of chicken bone marrow cultures but does not induce transformation in chicken yolk sac macrophages. S13 is a replication-defective virus; nonproducing transformed cells can be obtained from chicken fibroblasts and bone marrow cultures.

Preliminary experiments indicate that S13 does not contain nucleic acid sequences that hybridize with molecular probes of src, myc, erb A and erb B. It may contain an entirely new onc gene (85, 86).

UR2 is a newly characterized avian sarcoma virus whose genome contains a unique sequence that is not related to the sequences of other avian sarcoma virus transforming genes thus far identified. This unique sequence, termed ros, is fused to part of the viral gag gene. The product of the fused gag-ros gene of UR2 is a protein of 68,000 daltons (P68) immunoprecipitable by antiserum against viral gag proteins. In vitro translation of viral RNA and in vivo pulse-chase experiments showed that P68 is not synthesized as a larger precursor and that it is the only protein product encoded in the UR2 genome, suggesting that it is involved in cell transformation by UR2. In vivo, P68 was phosphorylated at both serine and tyrosine residues. Immunoprecipitates of P68 with anti-gag antisera had a cyclic nucleotide-independent protein kinase activity that phosphorylated P68, rabbit immunoglobulin G in the immune complex, and alpha-casein. The phosphorylation by P68 was specific to tyrosine of the substrate proteins. P68 was phosphorylated in vitro at only one tyrosine site, and the tryptic phosphopeptide of in vitro-labeled P68 was different from those of Fujinami sarcoma virus P140 and avian sarcoma virus Y73-P90. A comparison of the protein kinases encoded by UR2, Rous sarcoma virus, Fujinami sarcoma virus, and avian sarcoma virus Y73 revealed that UR2-P68 protein kinase is distinct from the protein kinases encoded by those viruses by several criteria. These results suggest that several different protein kinases encoded by viral transforming genes have the same functional specificity and cause essentially the same cellular alterations (3, 4, 88, 33).

The genetic structure and transforming sequence of UR2 has been analyzed by oligonucleotide fingerprinting. The sizes of the genomic RNAs of UR2 and its associated helper virus, UR2AV, were determined to be 24S and 35S, respectively, by sucrose gradient sedimentation. The molecular weight of the 24S UR2 genomic RNA was estimated to be 1.1×10^6, corresponding to 3,300 nucleotides, by gel electrophoresis under native and denatured conditions.

Partial sequence analysis of the UR2-specific oligonucleotides by RNase A digestion revealed that there are no homologous counterparts to these oligonucleotides in the RNAs of other avian sarcoma and acute leukemia viruses studied to date. UR2-transformed non-virus-producing cells contain a single 24S viral RNA which is most likely the message coding for the transforming protein of UR2. On the basis of the uniqueness of the transforming sequence, it was concluded that UR2 is a new member of the defective avian sarcoma viruses (3, 4, 88, 33).

A second major area of research concerns the detailed dissection of the molecular sequence of these oncogenes, and attempts to relate structural findings to functional activities. The specificity with which src (and other viral oncogenes) transform cells has been explored by examining the behavior of Rous sarcoma virus in chicken macrophages, cells that the virus can infect but does not transform. RSV-infected macrophages produce standard quantities of the src product, $pp60^{v\text{-}src}$, and the protein displays

tyrosine-specific protein kinase activity - just as it does in fibroblasts transformed by v-src. It appears likely that the failure of v-src to transform macrophages must be laid to presently unidentified properties of the host cell, not to failure of viral gene expression. $pp60^{v\text{-}src}$ is phosphorylated on both serine and tyrosine. The principle tyrosine phosphorylation (residue 416 in the protein) is not required for several of the prominent properties of $pp60^{v\text{-}src}$, including transformation of cultured fibroblasts, protein kinase activity both in vivo and in vitro, and localization of the protein to adhesion plaques within the plasma membrane.

Spontaneous mutations in v-src have been used to uncover translation of two separate proteins from two different reading frames within the coding domain of v-src. The results raised the possibility that a small (ca 7kd) protein is produced from the wild type v-src, in addition to $pp60^{v\text{-}src}$

Site-specific mutagenesis has been used to demonstrate that transformation of both fibroblasts and erythroid cells by avian erythroblastosis virus (AEV) can be attributed largely or entirely to the activity of v-erb-B. It appears that v-erb-A has either a minor role or no role at all in neoplastic transformation induced by AEV.

The topography of the v-erb locus has been explored further by mapping the splice-acceptor site that is used to generate the mRNA for v-erb-B. The nucleotide sequence of v-erb-B reveals substantial homology with the sequences of viral oncogenes that are known to be tyrosine-specific protein kinases. The functional significance of this homology is not yet clear (7).

The amino-terminal 8 kd of $pp60^{src}$ may serve as a structural hydrophobic domain through which $pp60^{src}$ attaches to plasma membranes. Two isolates of recovered avian sarcoma viruses (rASVs), 1702 and 157, encode $pp60^{src}$ proteins that have alterations in this amino-terminal region. The rASV 1702 src protein (56 kd) and the 157 src protein (62.5 kd) show altered membrane associations, and fractionate largely as soluble, cytoplasmic proteins in aqueous buffers, in contrast with the membrane association of more than 80% of the src protein of standard avian sarcoma virus under the identical fractionation procedure. Plasma membranes contain less than 10% of the amount of $pp60^{src}$ found in membranes purified from cells transformed by Rous sarcoma virus or control rASVs. The altered membrane association of these src proteins had little or no effect on the properties of chick embryo fibroblasts transformed in monolayer culture. In contrast, rASV 1702 showed reduced in vivo tumorigenicity compared with Rous sarcoma virus or with other rASVs that encode membrane-associated src proteins. Rous sarcoma virus-induced tumors are malignant, poorly differentiated sarcomas that are lethal to their hosts. rASV 1702 induces a benign, differentiated sarcoma that regresses and is not lethal to its hosts. These data support the role of amino-terminal sequences in the membrane association of $pp60^{src}$, and suggest that the amino terminus of $pp60^{src}$ may have a critical role in the promotion of in vivo tumorigenicity (26, 33).

The entire nucleotide sequence of the molecularly cloned DNA of Fujinami sarcoma virus (FSV) has been determined. The sequence of 1182 amino acids was deduced for the FSV transforming protein P130, the product of the FSV

gag-fps fused gene. The p130 sequence was highly homologous to the amino acid sequence obtained for the gag-fes protein of feline sarcoma virus, supporting the view that fps and fes were derived from a cognate cellular gene in avian and mammalian species. In addition, FSV P130 and $p60^{src}$ of Rous sarcoma virus were 40% homologous in the region of the carboxy-terminal 280 amino acids, which includes the phosphoacceptor tyrosine residue. These results strongly suggest that the 3' region of fps/fes and src originated from a common progenitor sequence. A portion (the U3 region) of the long terminal repeat of FSV DNA appears to be unusual among avian retroviruses in its close similarity in sequence and overall organization to the same region of the endogenous viral env DNA (33).

Using cDNAs specific to the inserts of avian sarcoma virus genomes, the existence and the transcription of cellular nucleotide sequences related to three onc genes of avian sarcoma virus (fps, yes and ros) in various cells were examined. The progenitor cellular sequences for these onc genes (c-onc) were present in uninfected chicken DNA in one or a few copies per haploid genome. These c-onc sequences were detectable in cellular DNA of a wide variety of vertebrates, and the homology between viral and cellular onc was inversely related to the phylogenetic distance of animal species. The pattern of expression of these c-onc genes in different tissues of chickens was found to be unique to each gene. The expression of c-fps and c-ros genes was generally repressed in many tissues, but c-fps was expressed at higher levels in bone marrow (2.5 copies per cell) and lung (1.1 copies per cell), whereas c-ros was mainly transcribed in kidney (2.5 copies per cell). On the other hand, c-yes transcripts were easily detectable in all tissues analyzed and were found at high levels in kidney (26 copies per cell). These c-onc expressions were unaffected by infection with avian sarcoma viruses that contained other onc genes (3, 33).

E26, a replication-defective, avian acute leukemia virus isolated in Bulgaria in 1962 from a field case of erythroblastosis in a chicken, was initially classified as an erythroblastosis (E) virus (1). Subsequently, it was proposed on the basis of in vitro transformation assays and in vivo studies to reclassify E26 as a myeloblastosis virus. However, recent analyses of differentiation parameters of leukemic cells from chicken and quail clearly indicated that in vivo the primary hematopoietic target cell for E26 belongs to the erythroid lineage, but also includes some myeloid cells. This directly confirmed earlier reports that E26 induces the proliferation of predominantly erythroid cells in various avian species, including chicken, turkey, guinea fowl and Japanese quail. Acutely transforming retroviruses of the avian leukosis/sarcoma group can be classified into seven subgroups on the basis of helper virus-unrelated, transformation-specific sequences present in the genomic viral RNA. Four subgroups of avian sarcoma viruses and three subgroups of acute leukemia viruses have been distinguished to date. On this basis, E26 and avian myeloblastosis virus (AMV) were classified as members of the same subgroup of acute leukemia viruses. Different members of a given oncogenic subgroup regularly have similar genetic structures and induce similar forms of neoplasia in the animal. However, the oncogenic spectra of E26 and AMV are distinct; cloned stocks of AMV exclusively induced myeloblastic leukemia, while E26 primarily causes erythroid or mixed erythroid and myeloid leukemia. Both viruses have

in common that they do not appear to transform fibroblasts. Additional oncogenic properties reported for the original isolate of AMV may reflect activities of the associated helper viruses. Despite the homology between their specific RNA sequences, the genetic structure and gene products of E26 RNA differ significantly from the structure and protein products of AMV RNA. These differences are consistent with the distinct oncogenic properties of these two viruses (55).

When cells become transformed by Rous sarcoma virus (RSV) they display a wide variety of biochemical, morphological, and regulatory alterations, including increases in the rate of glucose transport across the cell membrane and production of a plasminogen-specific protease (plasminogen activator), a decreased adhesiveness, a rounded morphology, and acquisition of the ability to grow in suspension (anchorage-independent growth). This collection of alterations is termed the transformed phenotype. All of the various manifestations of the transformed phenotype are dependent on the continuous expression of the viral transforming protein, $pp60^{src}$. This protein has been shown to be a protein kinase that phosphorylates on tyrosine residues.

It is now clear that many proteins become phosphorylated on tyrosine during transformation by RSV. Seven proteins containing phosphotyrosine were identified by two-dimensional gel electrophoresis. In addition, the cytoskeletal protein, vinculin, becomes phosphorylated on tyrosine; $pp60^{src}$ is itself phosphorylated on tyrosine, as is a 50,000-dalton protein which associates with $pp60^{src}$. The major phosphotyrosine-containing protein of transformed cells is the 36,000-dalton transformation specific protein which constitutes only about 10% of the total phosphotyrosine in the transformed cells. Genetic evidence indicates that $pp60^{src}$ has more than one primary cellular target which plays a biologically significant role in generating the transformed phenotype. The evidence is based on the existence of partial transformation mutants of RSV. These mutants cause the complete appearance of some transformation parameters, whereas others are induced poorly.

It is believed that the $pp60^{src}$ proteins coded for these partially transforming mutants are able to phosphorylate some cellular targets well, but phosphorylate others more poorly, resulting in the partially transformed phenotype. This differential phosphorylation could occur either because the mutant $pp60^{src}$ fails to recognize certain targets, or because its intracellular localization is altered so that only certain cellular targets are accessible. The association of biological parameters cannot be explained merely by quantitative variation in $pp60^{src}$ activity, since the mutants cannot be ordered into the same hierarchy for every parameter. A present hypothesis is that when certain transformation parameters are not induced, it is because particular cellular target cells proteins fail to be phosphorylated. It has previously been determined that phosphorylation of the 36,000-dalton protein (protein p) was neither necessary nor sufficient for loss of surface fibronectin and density-dependent growth inhibition; was not sufficient for loss of adhesiveness and increased glucose transport; and correlated best with the production of plasminogen activator, growth in soft agar, and tumor formation. The phosphorylation of five phosphotyrosine-containing proteins designated k, l, m, p and q in cells infected with a

wide variety of partially transforming mutant viruses were examined. The phosphorylation of protein q could be partially dissociated from the phosphorylation of protein p, indicating that the mutations in the src gene affected the specificity as well as the activity of $pp60^{src}$. Phosphorylation of these proteins is not sufficient for transformation-related changes in adhesiveness and glucose transport or for the appearance of a round morphology. The best correlation noted was between phosphorylation of proteins p or 1 or total phosphotyrosine content and plasminogen activator production and between phosphorylation of proteins q or 1 and increased hexose transport. However, even when good correlations were observed, significant exceptions were sometimes noted. It thus remains possible that some phosphorylations on tyrosine observed in Rous sarcoma virus transformed cells are not causally related to the expression of the measurable parameters of transformation (39, 90).

Thus, despite a great deal of work, and continuing identification of new oncogenes, the basic mechanism through which oncogenes initiate and maintain the transformed state are still unknown. In many cases, the activity of tyrosine phosphorylating kinases is involved, but the precise cellular targets for such enzymes which result in transformation have yet to be determined.

In studies of the relationships of HBV to PHC, the chimpanzee, which is the only animal other than man that becomes infected with HBV, was shown not to have integrated viral DNA in its liver cells. This is true despite the fact that 4 of the 5 animals studied had chronic persistant hepatitis. One chimpanzee subsequently developed chronic active hepatitis but still did not show evidence of integration. This indicates that HBV DNA integration is certainly not a frequent event in these animals, and may explain the fact that they do not develop PHC. In contrast, humans with PHC have HBV DNA integrated into unique sites in their liver cell DNA (71).

A patent application has been filed for a serologic test for the diagnosis of non-A non-B hepatitis in humans. This may be important since non-A non-B hepatitis resembles the disease caused by HBV in terms of its tendency to establish chronic infections and perhaps its potential for causing liver tumors as well (71).

On May 9-10, 1983, the RNA Virus Studies II Program held a workshop entitled "Chronic Carriage of Hepatitis B Virus: Neoplastic Sequelae and Possibilities for Intervention-Prevention." The meeting was co-chaired by Dr. Myron Essex of the DCCP Board of Scientific Counselors and Dr. W.S. Rutter, of the University of California at San Francisco. A number of cogent suggestions for future activities were made at the meeting which are now being reviewed and evaluated by staff for possible new initiatives.

GRANTS ACTIVE DURING FY 83

Investigator/Institution/Grant Number	Title
1. ASTRIN, Susan M. Inst for Cancer Research, Philadelphia 5 RO1 CA 27797-03	Control of Expression of Endogenous Viral Genes
2. AXEL, Richard Columbia University 5 RO1 CA 16346-08	Molecular Control of Chromatin Transcription
3. BALDUZZI, Piero C. University of Rochester 1 RO1 CA 32310-01A1	The Transforming Genes of Avian Sarcoma Viruses
4. BALUDA, Marcel A. University of California (Los Angeles) 2 RO1 CA 10197-16	Tumor Induction by Avian Myeloblastosis Virus
5. BEEMON, Karen L. Johns Hopkins University 5 RO1 CA 33199-02	Location and Function of M6A in Retrovirus RNAs
6. BEEMON, Karen L. Johns Hopkins University 7 RO1 CA 32557-01	Expression of RNA Tumor Virus Genes
7. BISHOP, John M. University of California (San Francisco) 5 RO1 CA 12705-12	Rous Sarcoma Virus: Replication and Cell Transformation
8. BOETTINGER, David E. University of Pennsylavnia 2 RO1 CA 16502-09	Genetic Analysis of RNA Tumor Viruses
9. BOETTINGER, David E. University of Pennsylvania 5 RO1 CA 30383-02	Virus Induced Myeloid Leukemia
10. BOSE, Henry B. University of Texas 5 RO1 CA 27003-03	Virus Sequences In REV-Transformed Cells
11. BREWER, John I. Northwestern Univ 5 RO1 CA 29461-03	Trophoblastic Tumors: New Organism/Immunology/Therapy
12. BRUGGE, Joan S. State Univ of New York (Stony Brook) 2 RO1 CA 27951-04	The Association of Two Cellular Proteins with $pp60^{src}$

13.	CARBON, John A. University of California (Santa Barbara) 5 RO1 CA 11034-15	Studies on Gene Organization and Expression
14.	CASPAR, Donald L. Brandeis University 5 RO1 CA 15468-10	Assembly of Viruses, Membranes and Tissues
15.	CHIRIKJIAN, Jack G. Georgetown Univeristy 5 RO1 CA 16914-07	RNA Modifying Enzymes in Normal and Neoplastic Cells
16.	COFFIN, John M. Tufts University 5 RO1 CA 17659-07	Relationship of Avian Tumor Virus RNA and Host Genome
17.	COFFIN, John M. Tufts University 5 RO1 CA 27108-03	Mechanism of Variability of Tumor Virus RNA
18.	COLLETT, Marc S. University of Minnesota 5 RO1 CA 29041-03	Structure and Function of the ASV Transforming Protein
19.	COOPER, Geoffrey M. Sidney Farber Cancer Institute 5 RO1 CA 18689-07	Infectious DNA for Endogenous RNA Tumor Virus Genes
20.	CRITTENDEN, Lymen B. U S Dept of Agriculture 5 RO1 CA 29656-02	Avian Endogenous Retroviral Gene Expression
21.	DUESBERG, Peter University of California (Berkeley) 2 RO1 CA 11426-14	Molecular and Genetic Analyses of RNA Tumor Viruses
22.	EHRLICH, Melanie Tulane Medical School 5 RO1 CA 19942-06	5-Methylcytosine in DNA: Cancer and Development
23.	EISENMAN, Robert N. Fred Hutchinson Cancer Research Center 5 RO1 CA 20525-07	Control Mechanisms in Avian Oncornavirus Replication
24.	ERIKSON, Raymond L. University of Colorado Medical Center 7 RO1 CA 34943-01	Biosynthesis of Viral RNA
25.	FARAS, Anthony J. University of Minnesota (Minneapolis) 5 RO1 CA 18303-08	RNA-Directed DNA Polymerase and 70S RNA of Oncornaviruses

26.	GOLDBERG, Allan R. Rockefeller University 5 RO1 CA 13362-10	RSV Functions Involved In Transformation
27.	GOULIAN, Mehan University of California (San Diego) 5 RO1 CA 11705-13	DNA Synthesis Studies
28.	GRANDGENETT, Duane P. St. Louis University 2 RO1 CA 16312-09	Avian Retrovirus DNA Synthesis and its Regulation
29.	GRANOFF, Allan St Jude's Children's Research Hospital 5 RO1 CA 07055-20	Studies of Lucke Tumor Associated Virus
30.	GRAY, Horace B. University of Houston 5 RO1 CA 11761-12	Hydrodynamics of Circular DNA Forms
31.	GUNTAKA, R. V. Columbia University 5 RO1 CA 28990-03	Synthesis Structure and Function--Avian Tumor Virus DNA
32.	HALPERN, Michael S. The Wistar Institute 5 RO1 CA 31514-02	Oncornavirus-Induced Immuno-suppression
33.	HANAFUSA, Hidesaburo Rockefeller University 5 RO1 CA 14935-10	Cellular Alteration Induced by Rous Sarcoma Virus
34.	HARRISON, Stephen C. Harvard University 5 RO1 CA 13202-11	Virus Structure and Assembly
35.	HAYWARD, William S. Rockefeller University 7 RO1 CA 34502-01	RNA Tumor Virus Gene Expression
36.	HOLOWCZAK, John A. Rutgers Medical School 5 RO1 CA 11027-15	Transcription and Translation in Pox Virus Infected Cells
37.	HOLTZER, Howard University of Pennsylvania 5 RO1 CA 18194-08	Conversion of Embryonic Cells into Transformed Cells
38.	HUMPHRIES, Eric H. University of Texas (Dallas) 5 RO1 CA 32295-01A1	Endogenous Avian Retrovirus in Non-Permissive Cells

39	HUNTER, Anthony B. Salk Institute Biological Studies 1 RO1 CA 17096-08	Macromolecular Synthesis and Cell Growth Control
40.	HUNTER, Eric University of Alabama 5 RO1 CA 29884-02	Site Specific Mutagenesis in the ENV Gene of RSV
41.	KAJI, Akira University of Pennsylvania 5 RO1 CA 19497-06	Replication of RNA Tumor Virus
42.	KOPROWSKI, Hilary Wistar Institute 2 PO1 CA 21124-06A1	Genetics and Virology of Cancer
43.	KUNG, Hsing-Jien Michigan State University 1 RO1 CA 33158-01	Erythroleukemia: Oncogene Activation by Retrovirus
44.	LAI, Michael M. University of Southern California 5 RO1 CA 16113-08	Structure and Replication of RNA Tumor Viruses
45.	LINIAL, Maxine L. Fred Hutchinson Cancer Research Center 5 RO1 CA 18282-08	Viral Coded Functions in Rous Sarcoma Virus
46.	LUSE, Donal University of Cincinnati 5 RO1 CA 31797	Viral Probe of Tumor-Specific DNA Replication Factor
47.	LUTWICK, Bruce E. Maimonides Medical Center 7 RO1 CA 32566-01	Hepatitis B Virus and Hepatocellular Carcinoma
48.	MARCUS, Philip I. University of Connecticut 5 PO1 CA 14733-10	Gene Expression, Virus Replication and Cell Growth
49.	MARTIN, G. Steven University of California (Berkeley) 5 RO1 CA 25464-03	Transformation of Differentiating Cells
50.	MARTIN, G. Steven University of California (Berkeley) 5 RO1 CA 17542-08	Genetics of RNA Tumor Viruses
51.	MASON, William S. Institute for Cancer Research 5 RO1 CA 26012	Replication of Rous Sarcoma Virus

52.	MAYOR, Heather D. Baylor College of Medicine 5 RO1 CA 14618-08	Growth and Maturation of Adeno-associated Satellite Viruses
53.	MENKO, Sue University of Minnesota 1 R23 CA 29289-02	The Effect of SRC on Cytoskeletal Functions
54.	MILMAN, Gregory Johns Hopkins University 5 RO1 CA 21650-07	Biochemistry of Mutation in Human Cells
55.	MOSCOVICI, Carlo University of Florida 2 RO1 CA 10697-17A1	Avian Leukemia Viruses and Cell Differentiation
56.	NADAL-GINARD, Bernardo Yeshiva University 5 RO1 CA 26860-02	Transforming Gene of Rous Sarcoma Virus
57.	NEIMAN, Paul E. Fred Hutchinson Cancer Research Center 5 RO1 CA 20068-08	Molecular Mechanisms in Neoplasia
58.	NEIMAN, Paul E. Fred Hutchinson Cancer Research Center 5 PO1 CA 28151-04	Retroviruses and Cancer
59.	PARSONS, J. Thomas University of Virginia 2 RO1 CA 27578-04	Expression of Avian Retrovirus Transforming Genes
60.	PARSONS, J. Thomas University of Virginia 5 RO1 CA 29243-03	Sarcoma Virus Specific Tumor Antigens
61.	POGO, Beatriz G. Mt Sinai School of Medicine 5 RO1 CA 29262-03	The Expression of Oncogenicity of Shope Fibroma Virus
62.	RHODE, Solon L., III Inst of Medical Res of Bennington 5 RO1 CA 26801-05	Replicon Control in Normal and Transforming Cells
63.	ROBBINS, Phillips W. Mass Institute of Technology 5 RO1 CA 14142-20	Cell and Virus Glycoproteins: Synthesis and Function
64.	ROBINSON, Harriet L. Worcester Fdn for Exper Biology 5 RO1 CA 23086-06	Inheritance and Expression of C-Type Viruses

65.	ROBINSON, Harriet L. Worcester Fdn For Exper Biology 5 RO1 CA 27223-04	Avian Leukosis Viruses and Cancer
66.	ROBINSON, William S. Stanford University School of Medicine 1 RO1 CA 34514-01	Duck Hepatitis B Virus: Infection and Disease
67.	ROHRSCHNEIDER, Larry R. Fred Hutchinson Cancer Research Center 5 RO1 CA 20551-07	Mechanisms of Oncornavirus Induced Transformation
68.	RUECKERT, Roland R. University of Wisconsin (Madison) 5 RO1 CA 08662-17	Structure and Synthesis of Retro- and Nodaviruses
69.	SCOTT, June R. Emory University 5 RO1 CA 11673-14	Lysogeny and Bacteriophage P1
70.	SEFTON, Bartholomew Salk Institute 5 RO1 CA 17289-08	Viral Membranes and Viral Transformation
71.	SHAFRITZ, David Albert Einstein College of Medicine 5 RO1 CA 32605-02	Hepatitis B Virus - Chronic Hepatitis - Liver Cancer
72.	SHALLOWAY, David I. Pennsylvania State University 5 RO1 CA 32317-02	Role of PP60c-SRC, Homolog of the RSV Oncogenic Protein
73.	SHANK, Peter R. Brown University 1 RO1 CA 32980-01	Stability and Disease Trophisms of Avian Proviral DNAs
74.	SIDDIQUI, Aleem University of Colorado 5 RO1 CA 33135-02	Expression of Hepatitis B Virus Genes and Hepatoma
75.	SMITH, Ralph E. Duke University 7 RO1 CA 35984-01	Biochemistry of RNA Tumor Virus Replication
76.	STAVNEZER, Edward Sloan-Kettering Inst for Cancer Res 5 RO1 CA 32817-02	The Origin, Structure, and Biological Activity of SKVS
77.	STOLTZFUS, Conrad M. University of Iowa 5 RO1 CA 28051-05	Retrovirus RNA Metabolism

78. SWANSTROM, Ronald
University of North Carolina
1 RO1 CA 33147-01A1
Retrovirus Replication; Interaction with Host Genome

79. TATTERSALL, Peter J.
Yale University
5 RO1 CA 29303-03
Molecular Basis of Parvovirus Target Cell Specificity

80. TAYLOR, John M.
Institute for Cancer Research
2 RO1 CA 22651-05
Early Events in Avian Retrovirus Replication

81. TEMIN, Howard M.
University of Wisconsin (Madison)
5 PO1 CA 22443-05
Molecular Biology and Genetics of Tumor Viruses

82. TEREBA, Allan M.
St Jude Children's Research Hospital
5 RO1 CA 28221-03
Localization and Mechanism of Retrovirus Integration

83. VANAMAN, Thomas C.
Duke University Medical Center
5 PO1 CA 30246-02
Regulatory Functions of Protein-Nucleic Acid Interactions

84. VIOLA, Michael V.
University of Connecticut
5 RO1 CA 27792-03
Pathogenesis of Paget's Disease of Bone

85. VOGT, Peter K.
University of Southern California
5 RO1 CA 13213-12
Interactions Between Avian Tumor Viruses and Their Hosts

86. VOGT, Peter K.
University of Southern California
5 RO1 CA 29777-02
Avian Oncovirus Transforming Genes and Proteins

87. VOGT, Volker M.
Cornell University
5 RO1 CA 20081-06
Avian Retrovirus Structure and Assembly

88. WANG, Lu-Hai
Rockefeller University
5 RO1 CA 29339-03
Transforming Genes of Avian Sarcoma Viruses

89. WATSON, Kenneth F.
University of Montana
5 RO1 CA 19729-07
Mechanism of Viral RNA-Directed DNA Polymerization

90. WEBER, Michael J.
University of Illinois (Urbana)
5 RO2 CA 12467-11
Early Cellular Changes in Viral Oncogenesis

91. WEINTRAUB, Harold M.
Fred Hutchinson Cancer Research Center
5 R01 CA 26663-05
Cell Transformation by RSV

92. WEISS, Gary B.
University of Texas
5 R01 CA 31800-02
Infidelity of Human RNA Directed DNA Polymerase

93. WELLS, Robert D.
University of Wisconsin (Madison)
7 R01 CA 34086-01
DNA Structure and Gene Regulation

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 Acting Branch 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 adequate purity, viability and potency, some of which are not available from the commercial sector. The BCB resources component provides some research materials and other supporting activities through contract operations in four general areas. These include: activities directed toward production, characterization and distribution of purified viruses and viral reagents; activities concerned with animal resources, including production of pathogen-free species of animals, breeding of cotton-topped marmosets, and maintenance of animal colonies; 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 human specimens.

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 international agreements with these countries covering the exchange of cancer research materials.

Two viruses were produced during this period to meet program needs: avian myeloblastosis virus (AMV) and Epstein-Barr virus (EBV). A large and consistently active supply of AMV reverse transcriptase is vital to biological carcinogenesis studies involving the interactions of cDNA copies of retrovirus genomes with cellular protein synthesis. To meet these needs, more than 5,600,000 units of AMV reverse transcriptase were produced and distributed to research laboratories. While the amount of transcriptase shipped has decreased somewhat since institutions are purchasing smaller amounts, the number of shipments has increased (850 vs 588 last year). Over 600 shipments were made to laboratories in the United States, and the remainder to foreign laboratories. EBV was one of the first viruses implicated in human cancer. High quality transforming virus and viral DNA is needed to continue the studies on the possible role of this agent in human cancer. Consumer demand has dropped considerably for the characterized EBV DNA during this period. At the same time, because of research interest in the EBV-determined nuclear antigen (EBNA), requests for large amounts of Raji cells have increased. This has necessitated modification of the production schedules, at no added cost to the Government,

to meet the changing interest of the research community. Production of both B95-8 and P3HR1 strains of virus continues in accord with the originally projected needs (5, 7).

Animals have an important role in the biological carcinogenesis research program. Experimentation for the biological activity of candidate human viruses must not be carried out on humans; therefore, 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 the utilization of these animals for these purposes. Since the marmoset appears to be especially suitable for use as a comparative model system, a colony of 60 cotton-topped marmosets consisting of 49 adults, nine juveniles, and two experimental animals is being maintained. The two experimental animals are assigned to Dr. Boris Lapin as part of the scientific bilateral agreement between the United States and Russia. 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. As a result of decreased need, the facility for maintaining large nonhuman primates has been eliminated from the program. Only administrative tasks relative to terminating the primate holding contract were performed during this period (3, 10).

Several research laboratories use eggs to study viral effects on cell differentiation. More than 8,000 fertile or embryonated eggs produced by an outbred specific pathogen-free, leukosis-free flock of Japanese quail were distributed to 12 laboratories for these and other cancer research projects. The quail are maintained under environmentally controlled conditions which preclude intercurrent infection by pathogenic microorganisms or infestation by parasites (4).

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. Therefore, a breeding program was 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. At present, there are 14 strains of congenic mice in various stages of development and two Gix-gp 70 mutant strains. The gene substitutions involved include Akvp, Fv-1, Gv-1, H-2, Pca-1 and Tla. In several cases, reciprocal substitutions of alleles have been effected between inbred strains that differ categorically in one or more characteristics pertaining to leukemia providing a quartet of inbred strains, two standard and two congenic with switched alleles, for each gene system. A total of 678 congenic mice were shipped to 23 recipients during the past year (1).

A transfer of funds in the amount of $241,511 was made to the National Institute for Allergy and Infectious Diseases to support production of captive-born woodchucks and evaluation of their suitability as models for studies of human hepatitis and hepatocellular carcinoma. It has been determined that the animals can be infected with woodchuck hepatitis virus during a limited period in their life, and that an on-site facility for research as well as breeding

is required. An experimental vaccine appears to be both immunogenic and protective against antigenemia. Also, it appears that chronic carriers can be established in the woodchuck. This animal may furnish a valuable model for studies on the development of hepatocellular carcinoma in humans and it is apparently superior to the duck in that histopathologic characteristics of hepatoma have been demonstrated in the woodchuck. The breeding effort to produce seronegative woodchucks will continue and the animals will be used in experimental studies (9).

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 cross-species tumor transplantations, the 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. To meet this need, procedures were carried out for interspecies and intraspecies cell identification for approximately 250 cultures. Three assays were utilized in these procedures: immunofluorescence staining for species specific surface antigens, isoenzyme analysis, and cytological analysis by means of chromosome banding (8).

In addition, during this period, research laboratories received over 200 shipments comprised of more than 5,000 human specimens and more than 1,800 viral materials from the inventory of frozen biological reagents. Materials received at the repository for storage included 185,000 milliliters of plasma containing AMV and 1,200 grams of AMV pellets. Data relevant to storage and distribution of research resource materials were added to the data base. Computerization of inventory data makes it possible to rapidly obtain information on availability, location and quantity of resources, permitting rapid response to requests by investigators. Inventory and accountability reports are prepared for program administrators as needed (2, 6).

In May 1981, the Branch began implementation of the resources "payback" system approved by the DCCP Board of Scientific Counselors. 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 included in a peer-review system; and the perception that free distribution of resources did not always result in the most effective utilization of available 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, the payback system is

being phased in over a period of time in such a way that investigators will 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 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 payback implementation.

Six of the ten resource contracts active during this period are now payback projects. These six include two for the production and distribution of viruses and viral reagents; one for specialized testing services and three for animal resources. The first contract implemented under the payback system was for the production and distribution of avian myeloblastosis virus (AMV) and AMV reverse transcriptase. Charges imposed this year for AMV materials have been 10 cents per unit of transcriptase and $1,000 per gram of virus. This fully implemented contract will receive about $560,000 for materials shipped during this period. In addition, approximately 150,000 units of AMV reverse transcriptase will be supplied gratis to participants in various bilateral scientific agreements. The recovered funds and the remaining inventory of frozen virus have made it unnecessary to add new funds to this contract for either FY82 or FY83 (5).

Consumer demand has dropped for both quail eggs and EBV since the implementation of the payback system. The contract for testing services, initiated as a phase-in payback contract, seems to be functioning well. Recipients are contributing toward operational costs and the number of tests performed are remaining at the projected level. Evaluation of the animal resource activities is not yet possible, due to insufficient data (1, 3, 4, 7, 8).

The payback system overall seems to be performing as expected. The demand level for high quality biological reagents not readily available from commercial sources has remained fairly constant. Reimbursement for full or partial costs of services has led to more careful use of costly resource reagents. This has resulted in a reduced level of effort or termination of several resource support contracts.

CONTRACTS ACTIVE DURING FY83

Investigator/Institution/Contract Number	Title
1. BOYSE, Edward A. Sloan-Kettering Institute for Cancer Research NO1 CP 71003	Influence of Virus-Related Genes on Susceptibility to Cancer
2. BURTON, Robert Information Management Services, Inc NO1 CP 11014	Computer Support for Resources Management
3. CLAPP, Neal K. Oak Ridge Associated Universities NO1 CP 21004	Operation of a Marmoset Colony for Cancer Research
4. FARROW, Wendall M. Life Sciences, Inc NO1 CP 61005	Germfree and SPF Quail Production
5. HOUTS, Gerald E. Life Sciences, Inc NO1 CP 11013	Production and Distribution of Avian Myeloblastosis Virus and AMV Reverse Transcriptase
6. McKINNEY, Cynthia E. Microbiological Associates NO1 CP 11000	Repository for Storage and Distribution of Viruses, Sera, Reagents and Tissue Specimens
7. NONOYAMA, Meihan Showa University Res Institute for Biomedicine in Florida NO1 CP 11012	Production, Purification and Concentration of Potentially Oncogenic DNA Viruses
8. PETERSON, Ward D. Children's Hospital of Michigan NO1 CP 21017	Inter and Intraspecies Identification of Cell Cultures
9. TENNANT, B. C. Cornell University NO1 AI 02651	Breeding Facility for Woodchucks (Marmota Munax)
10. VALERIO, Marian Litton Bionetics, Inc NO1 CP 91022	Operation of Facility to Provide and Maintain Nonhuman Primates for Cancer Research

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 combination 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.

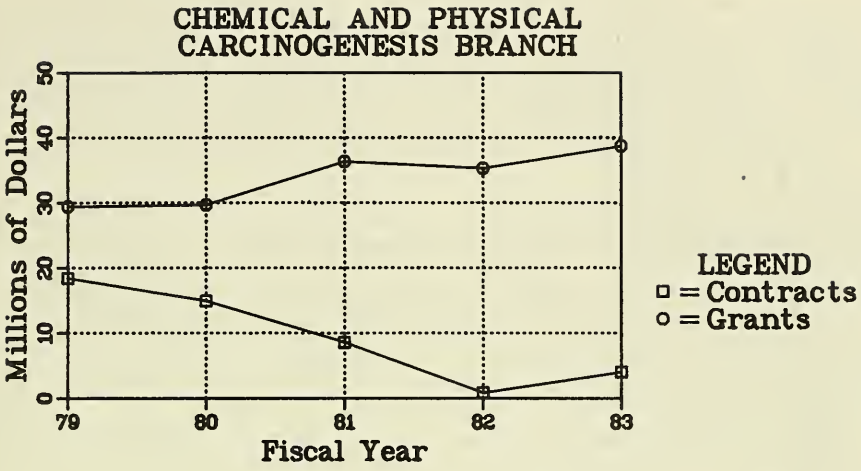
Grants and contracts administered by the staff of this Branch support five complementary categories of chemical and physical carcinogenesis research and associated resources: Molecular Carcinogenesis, Carcinogenesis Mechanisms, Biological and Chemical Prevention, Special Projects, and Research Resources. 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

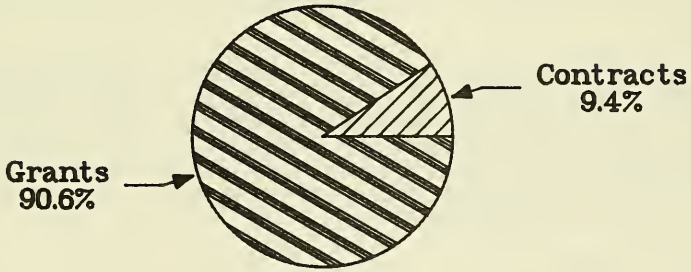
include mechanisms of action of candidate preventive agents, binding proteins and receptors, structure-function relationships, and the experimental use of combinations of preventive agents. The Special Projects category is characteristically concerned with a scrutiny of the broad domain of chemical and physical carcinogenesis research, with the object of identifying relevant research activities which would appear to merit an 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; the use of human cells and tissues in carcinogenesis research; and the occurrence and properties of mutagens in human foods. Until the third quarter of the current fiscal year, grants in the foregoing subject-matter areas and all Program Projects in this Branch had been assigned to the Special Projects category. More recently the cancer endocrinology grants were transferred to the Carcinogenesis Mechanisms category while grants dealing with tumor promoters and interspecies comparisons were moved to the Molecular Carcinogenesis category. Also, the Program Projects assigned to this Branch have been or will be reassigned to either of the latter two categories. 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 analogous heterosubstituted compounds, as well as the synthesis and distribution of retinoids including radiolabeled forms.

A summary of the number of grants, contracts, and associated funding relative to each of the above categories and to the Chemical and Physical Carcinogenesis Branch, as a whole, follows:

CHEMICAL AND PHYSICAL CARCINOGENESIS BRANCH

	FY 1983			
	CONTRACTS		GRANTS	
	No. of Contracts	$ (Millions)	No. of Grants	$ (Millions)
Carcinogenesis Mechanisms	0	0	66	5.81
Biological & Chemical Prevention	12	1.77	89	8.52
Molecular Carcinogenesis	0	0	93	9.29
Research Resources	12	2.26	0	0
Special Projects	0	0	83	15.10
TOTALS	24	4.03	331	38.72





FISCAL YEAR 1983 ESTIMATES	$ (Millions)	% of Program
Contracts	4.03	9.4
Grants	38.72	90.6
Total	$ 42.75	100.0

SUMMARY REPORT

BIOLOGICAL AND CHEMICAL PREVENTION

The Biological and Chemical Prevention program is responsible for research on agents that can inhibit, arrest, reverse, or delay the development of cancer in humans. Agents can derive from naturally occurring products such as foods consumed by man, from chemical synthesis, or from various biological sources. At the present time there are 56 grants in this program area with FY83 funding of approximately $8.52 million and 14 contracts with FY83 funding of approximately $1.77 million. Three additional support contracts in this program are discussed under Research Resources.

Research grants in the program support diverse types of studies including the experimental inhibition of carcinogenesis, the inhibition or suppression of malignant transformation in culture, mechanisms of action of preventive agents, synthesis of chemopreventive compounds, structure-function relationships, and pharmacologic disposition. Studies proceed on inhibition of carcinogenesis induced by chemical, physical and biological agents, against several stages of the tumorigenic process, and against the development of cancer at many organ sites. The modifying effects of anticarcinogens are investigated relative to a large number of biochemical and biological endpoints, which, in addition to tumorigenesis and transformation themselves, include the activity of the mixed function oxidase system, free radical generation and quenching, cell proliferation, differentiation, activation/detoxification of carcinogens, DNA repair, binding proteins or receptors for preventive agents, preneoplastic states and cytogenetic variables. The majority of these studies are in their first year, most of these resulting from a Request for Applications concerned with the mechanisms of biological and chemical prevention of carcinogenesis.

Contracts in the program support studies on antioxidant inhibition of tumorigenesis in liver, lung, digestive tract and mammary gland; on retinoid inhibition of tumorigenesis in urinary bladder and mammary gland; on synthesis and bioassay of new retinoids for potential future development; on synthesis of large amounts of selected retinoids and studies on their toxicity, and on synthesis of radiolabeled retinoids for metabolic and pharmacologic investigations. Research accomplishments follow on a number of these endeavors.

Grants Activity Summary

Antioxidants: Bovine adrenocortical cells can be prepared and cultured from two zones of the adrenal cortex, the zona fasciculata-reticularis and the zona glomerulosa. The zona fasciculata-reticularis is the cortisol (hydrocortisone) secreting-zone of the adrenal cortex while the zona glomerulosa secretes aldosterone. An interesting series of studies has employed these sources as model cell culture systems for investigations on steroid biosynthesis and its regulation, as well as for investigations on the fundamental mechanisms of antioxidant action. The adrenal cortex itself is known to contain an extraordinarily well developed complex of biological antioxidant systems. It has high contents of superoxide dismutase, glutathione peroxidase, glutathione reductase, glucose-6-phosphate dehydrogenase, and glutathione. It selectively concentrates sulfhydryl compounds, α-tocopherol and selenium. It has a high concentration of ascorbic acid and specific concentrating mechanisms for this substance, contains monodehydroascorbate

reductase and dehydroascorbate reductase, and nonenzymatically reduces dehydroascorbate to ascorbate. These antioxidant defenses are thought to be necessary to prevent or inhibit peroxidation resulting as a side effect of the operation of the adrenal cytochrome P-450s. Peroxidation is probably a general side effect resulting from the production of superoxide during electron transfer.

Previous studies have shown that bovine adrenocortical zona fasciculata cells in culture rapidly undergo changes in cytochrome P-450-dependent enzymatic activities. Specifically, these cells soon fail to synthesize cortisol, their major differentiated function, while continuing to synthesize deoxycortisol and other precursor steroids. These progressive alterations in the steroidogenesis pathway occur mainly as a result of accelerated loss of mitochondrial cytochrome $P\text{-}450_{11\beta}$, the hydroxylase activity at the steroid 11-position, upon interaction with 11β-hydroxylated enzyme products and substrates. Furthermore, adrenocorticotropin (ACTH) is not able to induce the 11β-hydroxylase under standard culture conditions. It was found that the hydroxyl radical scavenger, dimethyl sulfoxide (DMSO) and the antioxidant butylated hydroxyanisole (BHA) prevented the cortisol-induced loss of 11β-hydroxylase activity, partially at 19% O_2 and almost completely at 1% O_2. Further DMSO, BHA and ascorbic acid allowed some induction of cytochrome $P\text{-}450_{11\beta}$ by ACTH at 19% O_2 and much greater induction at 1% O_2. It was concluded that cytochrome $P\text{-}450_{11\beta}$ is subject to destruction or inactivation by interaction with 11β-hydroxylated steroids (the enzyme products) which act as pseudosubstrates. The inactivation was presumed to involve lipid peroxidation initiated by oxygen-derived free radicals released from the cytochrome P-450 • pseudosubstrate • oxygen complex due to the incapacity of the pseudosubstrate to be oxygenated. In this regard, release of oxygen-derived free radicals from mammalian microsomal cytochrome P-450 has been observed, and liver microsomes from cortisol-treated animals have been reported to show increased superoxide release.

The mechanism of loss of mitochondrial $P\text{-}450_{11\beta}$ hydroxylase has recently been examined in further detail in studies on the type of antioxidant compound that can protect the enzyme in the presence of 11β-hydroxylated steroids. It was found that not only was BHA effective, but also compounds similar in structure to BHA such as phenol, t-butylphenol, methoxyphenol and catechol. Other antioxidants were not effective such as ethoxyquin, nordihydroguiaretic acid, propyl gallate and α-tocopherol. It was concluded that the effective molecular structure is not that required for antioxidant activity, but rather that required for effective hydrogen donation. It is interesting that ascorbic acid is effective as a hydrogen donor in this system and is present in very high concentrations in the adrenal cortex. It is thought that hydrogen donation may be required to reduce radicals formed upon homolytic cleavage of the O-O bond of peroxides by cytochrome P-450 (22).

Similar studies have been carried out in culture using bovine adrenocortical cells from the outermost layer of cells of the adrenal cortex, the zona glomerulosa. Cytochrome $P\text{-}450_{CMO}$ in these cells is similar to cytochrome $P\text{-}450_{11\beta}$ in that loss of enzymatic activity occurs upon exposure to steroids of a variety of biological activities (glucocorticoids, mineralocorticoids, and androgens). Cytochrome $P\text{-}450_{CMO}$ is the enzyme that synthesizes aldosterone from corticosterone. Again, the molecular structure of the steroids required to cause loss of enzymatic activity is similar or identical to that found necessary for $P\text{-}450_{11\beta}$, being relatively insensitive to substitution at position 17 but requiring a β-hydroxy at position 11, with no activity for steroids having an α-hydroxy or a ketone at position 11. The loss of activity due to these steroid-P-450 interactions can be prevented by simultaneous exposure of the zona glomerulosa cells to an antioxidant (BHA) and a hydroxyl radical scavenger (dimethyl sulfoxide) (22).

In another project, the structure-activity relationship for a series of phenols had been investigated for their capacity to inhibit benzo(a)pyrene-induced neoplasia of the mouse forestomach. This polycyclic aromatic hydrocarbon, of course, requires activation to carcinogenic metabolites to induce neoplasia. Of 18 phenols tested, p-methoxyphenol was found to be the most potent. Further experiments with p-methoxyphenol have investigated its efficacy against the direct-acting carcinogen, β-propiolactone (βPL) either when fed in the diet prior to and during the 12-week period of carcinogen administration or when given by oral intubation one hour or four hours before each dose of βPL. Significant and comparable inhibition of forestomach tumorigenesis was obtained under each set of conditions; for example, p-methoxyphenol administered four hours before βPL reduces tumor incidence from 96% to 47% and tumors per animal from 5.4 to 0.5 while dietary administration reduces incidence from 100% to 41% and tumors per animal from 4.4 to 0.5. It is interesting that eleven other compounds of similar structures given four hours before this direct acting carcinogen did not significantly inhibit tumorigenesis. In both the B(a)P and βPL experiments where p-methoxyphenol has had high efficacy, it has come into direct contact with the target organ prior to carcinogenic insult. Further studies have shown that it is not effective against B(a)P-induced pulmonary adenoma induction, nor does it significantly inhibit forestomach tumorigenesis induced by B(a)P when fed subsequent to carcinogen administration. One possible mechanism for p-methoxyphenol inhibition of tumorigenesis against the direct-acting carcinogen βPL is a direct reaction between the inhibitor and the carcinogen. However, no reaction has been demonstrable in any of a number of solvent systems employed using thin layer chromatography or nuclear magnetic resonance as the analytical technique (52).

Anticarcinogenic activity of butylated hydroxyanisole (BHA) has been demonstrated against diverse types of chemical carcinogens at many organ sites, including breast, lung, skin, forestomach and large bowel. These studies in well-characterized animal model systems of carcinogenesis have extended up to 12 months in duration. This antioxidant is known to induce increased activities of detoxifying conjugating enzymes such as glutathione S-transferase and UDP-glucuronyl transferase, to increase cellular levels of nonprotein thiols, particularly glutathione, and to strengthen detoxifying pathways of xenobiotics in other ways. The exact mechanisms of these effects and of BHA's protective action are not known, but one suggestion made is that a BHA metabolite or metabolites may be involved, perhaps in conjunction with a binding protein for BHA or a BHA metabolite. Recently, two groups have identified BHA metabolites. In one case, mouse liver microsomal metabolites of radiolabeled BHA have been demonstrated on reverse phase high performance liquid chromatography. Four major peaks and three minor peaks eluted before the major isomer of BHA, which was used in these studies, and several additional peaks eluted after BHA. One of these metabolites has been isolated. It is a dimer of 3-BHA (major isomer of BHA). Its structure has been identified as 2,2'-dihydroxy-3,3'-di-t-butyl-dimethoxydiphenyl. Two additional metabolites have been purified with tentative identification as fatty acid esters of BHA (52).

A second study has demonstrated BHA metabolites as an outgrowth of fundamental studies on the effect of antioxidants on microsomal electron transport and the role of this process in the chemopreventive activity of these compounds. Since microsomal electron transport is dependent upon the oxidation of nicotinamide adenine dinucleotide phosphate (NADPH) and the activation of molecular oxygen, the effect of antioxidants on this process was studied. Experiments showed that butylated hydroxyanisole stimulates NADPH oxidation and O_2 consumption (NADPH oxidase activity) several fold in the presence of liver microsomes from phenobarbital-treated rats, with a concomitant inhibition of benzphetamine N-demethylase activity

and other monooxygenase activity on drug and carcinogen substrates. Half-maximum stimulation of oxygen consumption or inhibition of monooxygenase activity was achieved at BHA concentrations of approximately 20μM. The increase in NADPH oxidase activity could be accounted for, in part, by the recovery of reducing equivalents in the form of hydrogen peroxide. Since a lag in either stimulated NADPH oxidation or oxygen reduction was noted in the presence of BHA, a search for a BHA metabolite(s) was made which would be responsible for the observed uncoupling of cytochrome P-450-dependent monooxygenase and its flavoprotein reductase (NADPH-cytochrome P-450 reductase). High performance liquid chromatography and mass spectrometry have demonstrated that BHA is converted to t-butylhydroquinone and an unknown metabolite presumed to be t-butylquinone. Thus, in the presence of BHA, reducing equivalents flow from NADPH to the reductase to the BHA metabolites to molecular oxygen, with a concomitant inhibition of the monooxygenase activity. Monooxygenase activity is, of course, necessary for activation of many procarcinogens to their ultimate carcinogenic forms. Stimulation of NADPH oxidase activity with rat liver phenobarbital microsomes has also been seen with butylated hydroxytoluene, coumarin and 5,6-benzoflavone (43).

Recent studies have shown that chronic treatment with ciprofibrate and five other hypolipidemic drugs produces hepatocellular carcinomas in rats and mice. This group of structurally diverse compounds share unique biological properties, including the ability to induce marked hepatomegaly with an associated characteristic increase in liver peroxisomes. These ubiquitous cytoplasmic organelles contain catalase, several hydrogen peroxide-generating oxidases, carnitine acetyltransferase and other enzymes involved in the β-oxidation of long-chain fatty acids. The activities of these enzymes are elevated in association with peroxisome proliferation. Furthermore, certain industrial plasticizers also induce peroxisome proliferation, and at least one of these compounds also produces liver tumors in rats and mice upon prolonged dietary exposures. These compounds are intriguing since they do not appear to have mutagenic activity in either eukaryotic or the Salmonella/microsome assays, nor do they produce DNA damage in the lymphocyte ^{3}H-thymidine incorporation assay nor do they appear to bind to DNA or to cause chromosomal aberrations. One hypothesis for the carcinogenicity of these nonmutagenic compounds upon prolonged administration is that excessive levels of hydrogen peroxide are generated on a chronic basis as a result of increased peroxisomal β-oxidation of fatty acids. The excess peroxide generated or other activated oxygen species then mediate carcinogenesis, perhaps via lipid peroxidation chain reactions. Some support for this hypothesis may derive from the fact that accumulation of abundant quantities of lipofuscin, an indicator of oxidative polymerization reactions involving lipids and proteins, has been observed in liver during hepatocarcinogenesis by peroxisome proliferators. Since one of the actions of antioxidants is to inhibit lipid peroxidation, and since antioxidants can apparently decrease the rate of accumulation of lipofuscin occurring during the normal aging process, studies on the effects of several antioxidants on peroxisome proliferation carcinogenesis are underway. Results obtained so far from six-week acute administration studies indicate that peroxisome proliferation induced by the most potent of the peroxisome proliferators (ciprofibrate) cannot be inhibited by butylated hydroxyanisole, butylated hydroxytoluene, or ethoxyquin. Furthermore, these agents were not able to inhibit the induction of increased activities of the hydrogen peroxide-producing peroxisomal fatty acid β-oxidation system. This opens the possibility of exploring the hypothesis that these antioxidants can inhibit carcinogenesis induced by these agents, and in doing so, throw additional light on the mechanism of carcinogenesis by these ostensibly non-genotoxic agents (44).

Vitamins and Retinoids: A number of vitamins and retinoids both inhibit the development of cancer in a variety of experimental animal systems and suppress malignant and phenotypic transformation in cell culture. The basic mechanisms of these effects continue to be explored in hopes of elucidating the fundamental roles played by these agents in chemoprevention. Two recent series of studies, both in cell culture, have extended knowledge on the chemopreventive and biological actions of these compounds.

Previous work has shown that the retinoid, retinyl acetate, suppresses the development of morphological transformation of cultured mouse embryo fibroblasts by the carcinogenic polycyclic aromatic hydrocarbon, 3-methylcholanthrene (MCA). This culture system, the C3H 10T1/2 clone 8 mouse embryo fibroblast system, has been widely used in the recent past by many investigators for in vitro mechanistic studies on neoplastic transformation under defined conditions. It was found that not only was retinyl acetate (ROAc) highly active in suppressing morphological transformation, but also that a dose-response relationship for inhibition was clearly demonstrable. Furthermore, since ROAc was not added until seven days after removal of the carcinogen, its inhibitory effect could not have been against the production of reactive metabolites of MCA, nor against fixation of the chemical damage caused by the carcinogen, which appears to be essentially complete by four days post carcinogen. Since removal of ROAc from inhibited cultures allowed the appearance of transformed foci with the same latency and the same frequency as occurs in cultures receiving MCA only, it was concluded that the retinoid was maintaining "initiated" cells in a phenotypically normal state and that removal of the retinoid permits their progression towards neoplastic transformation. Recent experiments have extended these results to the actual isolation of a clone of MCA-treated 10T1/2 cells which appear to possess basic characteristics expected of "initiated" cells. In the presence of ROAc, this clone, (designated INIT/10T1/2) exhibits contact inhibited growth control and is morphologically indistinguishable from the parental 10T1/2 cell line. The "initiated" cells have essentially the same growth rate and saturation density as 10T1/2 cells. Furthermore, the potent phorbol diester tumor promoter, 12-O-tetradecanoylphorbol-13-acetate, accelerates the formation of transformed foci in ROAc-deprived INIT/10T1/2 cells by reducing the latency period, with, apparently, no increase in the total number of transformed foci. Stabilization of the initiated state of transformation seems to occur in vivo as well. In these experiments, INIT/10T1/2 cells injected into immunodeficient nude mice formed fibrosarcomas after latent periods of six to eight weeks. However, injection of INIT/10T1/2 cells into mice whose standard chow diet was supplemented by retinoid gavaged three times per week resulted in complete suppression of tumor formation. The retinoid employed in this case was the synthetic retinoid N-(4-hydroxyphenyl) retinamide which is a known inhibitor of epithelial carcinogenesis in animal systems. This inhibition of INIT/10T1/2 cell neoplastic transformation is apparently not due to selective retinoid toxicity since, upon cessation of retinoid administration, all surviving mice develop tumors approximately six weeks later. The INIT/10T1/2 cell line may provide an important system to study early biochemical changes in control mechanisms which result in neoplastic growth. It is, perhaps, significant to point out that the reversibility of the inhibitory effects of retinoids shown in this cell culture system has also been demonstrated in inhibition of chemically induced mammary carcinogenesis in animals; taken at face value, these results imply that continuous, perhaps long-term administration of retinoids may be necessary for chemoprevention in humans (8).

The second set of studies in cell culture has also employed C3H 10T1/2 clone 8 mouse embryonic fibroblastic cells. Previous work in this system has shown that ascorbic

acid also completely inhibits morphological transformation produced by the same polycyclic aromatic hydrocarbon, MCA. This 100% inhibition of transformation again occurs at non-toxic levels of the inhibitor in culture. This remarkable inhibition following a 24-hour exposure to MCA can be obtained either by immediate and continued addition of ascorbic acid to the cultures or by delaying as long as 23 days before inhibitor addition or by addition of ascorbic acid just one day after carcinogen and continuing for 21 days. Even 7 days exposure to the inhibitor 1 day after MCA inhibits morphological transformation by 75%. Expression of transformation in all these experiments was scored at 42 days after carcinogen. These results are significant since they indicate that ascorbic acid may not only have activity as an anti-initiating agent or as a blocking agent against the formation of N-nitroso carcinogens, but also that it can completely inhibit transformation when added as late as 3 weeks after a 24 hour exposure to MCA. Furthermore, it has been shown that certain morphologically transformed cells can be reverted back to a normal phenotype following subculture in the presence of ascorbic acid. These cells do not grow initially in semi-solid medium, although they later gain this capacity. They are also capable of producing tumors in immunodeficient nude mice after considerable additional subculture. In contrast, morphologically transformed cells which are not responsive to reversion by ascorbic acid, grow in agarose and are tumorigenic at an early passage. This suggests that ascorbate can block oncogenic progression in the early stages of the tumorigenic process.

These studies have recently been extended in an interesting manner employing x-ray irradiation as the carcinogen in place of MCA. It was found that ascorbic acid, again at low, non-cytotoxic concentrations, can completely suppress radiation-induced transformation in 10T1/2 cells when added 6 days after radiation exposure. Furthermore, under certain conditions, ascorbic acid has been found to enhance radiation-induced cytotoxicity; thus, appropriate use of ascorbic acid has the potential to increase the therapeutic effectiveness of radiation while at the same time decreasing potential hazards such as the induction of secondary malignancies. The induction of secondary malignancies subsequent to the use of certain chemotherapeutic agents is a known phenomenon. Preliminary data indicate that ascorbic acid may also be protective against certain of these agents as well (5).

Contracts Activity Summary

Inhibition of Carcinogenesis in Animal Systems: 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 underway investigating BHA and BHT inhibition of neoplasia at four organ sites: mammary gland, liver, colon, and lung. Recent results now indicate that BHA inhibits methylazoxymethanol (MAM) acetate-induced colon carcinogenesis in the CF_1 mouse in a dose-related manner. In these studies, BHA was provided at four levels in the diet (300, 1000, 3000, and 6000 ppm) from two weeks before through two weeks after carcinogen administration. This schedule was designed to determine the protective effect of BHA when present during the initiating phase of MAM acetate-induced colon carcinogenesis. It is significant not only that BHA appears to inhibit colon carcinogenesis in a dose-related manner, but also that a dietary level as low as 300 ppm appears to partially inhibit both tumor incidence and multiplicity (65).

Dose-response studies have also been completed on the inhibition of dimethylbenz-anthracene (DMBA)-induced mammary tumorigenesis by BHT. In the experimental design employed, the antioxidant was given in the diet at four levels (again 300 to 6000 ppm) beginning two weeks prior to carcinogen administration and continuing to the end of the study at 150 days post carcinogen. At both high and low doses of carcinogen, significant inhibition of mammary adenocarcinoma formation was found, as well as pronounced reductions in the accompanying adrenocortical lesions (57).

A most interesting result has been obtained in the BHT dose-response study on inhibition of N-2-fluorenylacetamide (FAA)-induced liver cancer. In this study, four levels (300 to 6000 ppm) of BHT were fed to F-344 rats concurrently with a high dose of FAA. BHT alone produced no neoplasms in any organ, but the higher doses of BHT enhanced liver activities of gamma-glutamyltranspeptidase (GGT) and glutathione S-transferase. The effect of BHT on FAA hepatocarcinogenesis was evaluated by measurement of preneoplastic and neoplastic liver lesions at 6, 12, 18, and 25 weeks of simultaneous feeding. Altered foci, identified by a positive GGT reaction and iron exclusion, were reduced by BHT at all time points and the final tumor incidence was decreased. Thus, the number of foci was predictive of tumor development. Conversely to the effect on liver, BHT enhanced the incidence of bladder tumors, also in a dose-dependent manner. The final tumor incidences (%) in liver and bladder respectively were as follows: FAA alone, 100 and 0; FAA + 300 BHT, 95 and 2; FAA + 1,000 BHT, 90 and 2; FAA + 3,000 BHT, 73 and 18; FAA + 6,000 BHT 56 and 44. These findings suggest that BHT altered the metabolism of FAA to inhibit hepatocarcinogenesis but to enhance bladder carcinogenesis. Additionally, BHT may be a promoter of bladder carcinogenesis. These novel findings require detailed mechanistic elucidation, but in any case, they clearly demonstrate the complexity of interactions that can be produced by agents that modify the carcinogenic process (71).

A fourth study on the dose-response relationship for phenolic antioxidants concentrates on the efficacy of BHA in inhibiting carcinogen-induced pulmonary adenoma. Experiments were performed to determine conditions under which low doses of 3-BHA, the major isomer of 2(3)-BHA, might enhance the activity of carcinogen detoxification systems. For this purpose the effects of feeding low doses of 3-BHA for long-time intervals on glutathione S-transferase activity and tissue levels of glutathione were determined. The concentrations of 3-BHA added to the diet were 100 ppm, 50 ppm, and 25 ppm. These diets were fed to female ICR/Ha mice for a period of either 4 weeks or 12 weeks. Induction of increased glutathione S-transferase activity and tissue levels of glutathione were found in the livers of mice fed 100 or 50 ppm 3-BHA in the diet. The magnitude of the induction was approximately the same in the mice fed 3-BHA for the two different time intervals studied. Thus, prolongation of feeding will not by itself result in a progressively greater

enhancement of glutathione S-transferase activity or tissue levels of glutathione. Further studies then were carried out to ascertain whether other dietary constituents might increase the inductive effects of these low doses of 3-BHA in the diet. Pure compounds and natural products were employed in these experiments. The compounds include indoles, terpenes, plant sterols, glutathione and powdered cocoa beans. A substantial enhancing effect was obtained with (+)-limonene. In liver and small bowel (+)-limonene increased 3-BHA inductive effects by approximately 50%. These data are of interest since (+)-limonene occurs in high concentration in citrus fruits. Lesser enhancing effects were obtained with indoles, a constituent of cruciferous vegetables, and also with cocoa beans. These studies suggest that other constituents of the diet might be of importance in the overall impact of 3-BHA as a protective compound against chemical carcinogenesis (69).

Several experiments have also been carried out to determine conditions under which 3-BHA inhibits lung tumor formation. In one experiment the effects of simultaneous administration of 3-BHA and indomethacin were ascertained when these substances were given prior to and during B(a)P administration. Both 3-BHA and indomethacin have been shown to inhibit components of the arachidonic acid metabolism cascade, an attribute which has been associated with inhibition of carcinogenesis in some experimental systems. The two compounds were given separately or together. Although each by itself inhibited pulmonary adenoma formation, indomethacin did not show an enhancing effect on 3-BHA inhibition. Furthermore, an experiment testing the efficacy of these compounds to inhibit B(a)P-induced lung adenomas when administered during the post-initiation period showed that neither BHA, indomethacin, nor the two given simultaneously were effective (69).

The ability of three retinoids to prevent the proliferation and the neoplastic response of the urothelium in the female B6D2F$_1$ mouse following exposure to the carcinogen N-butyl-N-(4-hydroxybutyl) nitrosamine (HOBBN) has been investigated. Following HOBBN exposure, this particular mouse strain develops carcinoma in situ which rapidly progresses to a very aggressive, flat, invasive, poorly differentiated transitional cell carcinoma of the bladder. Retinoids were administered in the diet after 10 weekly doses of the carcinogen. Two of the retinoids had either no effect or produced only a slight improvement in the overall carcinogenic response of the mice to this bladder carcinogen. By contrast, N-(4-hydroxyphenyl) retinamide produced a significant reduction in the incidence of bladder carcinomas (66% to 25%) and a consequent increase in hyperplasias (21% to 49%). Furthermore, the urothelia in these animals were strikingly better differentiated than those in the placebo-fed groups (59).

BIOLOGICAL AND CHEMICAL PREVENTION

GRANTS ACTIVE DURING FY83

Investigator/Institution/Grant Number	Title
1. AUERBACH, Arlene D Rockefeller University 1 RO1 CA 33948-01	Effects of Anticarcinogens on Fanconi Anemia Chromosomes
2. AWASTHI, Yogesh C Univ of Texas Medical Branch (Galveston) 2 RO1 CA 27967-04	Mechanism of Anti-Carcinogenic Effect of Antioxidants
3. BAILEY, George S Oregeon State University 1 RO1 CA 34732-01	Mechanisms of Inhibition of Chemical Carcinogenesis
4. BANERJEE, Mihir R Univ of Nebraska (Lincoln) 2 RO1 CA 25304-04	Chemical Carcinogenesis Mammary Gland Organ Culture
5. BENEDICT, William F Children's Hospital of Los Angeles 5 RO1 CA 31574-02	Ascorbic Acid Transformation and Oncogenic Progression
6. BENSON, Ann M Johns Hopkins University 1 RO1 CA 32479-01A1	Modulation of Enzyme Profiles by Anticarcinogenic Agents
7. BERNSTEIN, Isadore A Univ of Michigan (Ann Arbor) 1 RO1 CA 32470-01	Mechanism for Retinoid Neutralization of Tumor Promotion
8. BERTRAM, John S Roswell Park Memorial Institute 5 RO1 CA 25484-03	Inhibition of In Vitro Transformation by Retinoids
9. BRINCKERHOFF, Constance E Dartmouth College 5 RO1 CA 32476-02	Action of Retinoids on Synovial Cells
10. CHOPRA, Dharam P Southern Research Institute 5 RO1 CA 26696-03	Biology of Airway Epithelial Lesions
11. CHUNG, Fung-Lung American Health Foundation 1 R23 CA 32272-01	Screening for Inhibitors of N-Nitrosamine Carcinogenesis

12. CROCE, Carlo M
Wistar Institute of Anatomy and Biology
1 R01 CA 32495-01
Retinoic Acid Induced Differentiation

13. CURPHEY, Thomas J
Dartmouth College
1 R01 CA 32478-01
Pancreatic Cancer and Retinoids--Model and Mechanism

14. DAWSON, Marcia I
SRI International
1 R01 CA 30512-02
Novel Retinoids for Chemoprevention of Epithelial Cancer

15. DAWSON, Marcia I
SRI International
1 R01 CA 32428-01
Retinoid Tumor Inhibitory Activity-Toxicity Probe

16. GRUBBS, Clinton J
Southern Research Institute
1 R01 CA 30986-01A1
Chemoprevention of Cancer Caused by Anticancer Agents

17. HADDOX, Mari K
University of Texas Health Science Center (Houston)
1 R01 CA 32444-01
Mechanism of Retinoid Inhibition of Cell Proliferation

18. HECHT, Stephen S
American Health Foundation
1 R01 CA 32519-01
Chemoprevention of Nitrosamine Carcinogenesis by BHA

19. HILL, Donald L
Southern Research Institute
5 R01 CA 26389-03
Disposition and Chemopreventive Activity of Retinoids

20. HILL, Donald L
Southern Research Institute
5 R01 CA 26815-03
Biotransformation of Retinoids in Vitro

21. HILL, Donald L
Southern Research Institute
5 R01 CA 30604-02
Prevention of ENU-Induced Brain Cancer by Retinoids

22. HORNSBY, Peter J
Univ of California (San Diego)
5 R01 CA 32468-02
Antioxidant Action in a Model Cell Culture System

23. JACOBSON, Herbert I
Albany Medical College
1 R01 CA 32531-01
A Marker for Studying Inhibition of Colon Carcinogenesis

24. JOHNSON, Eric F
Scripps Clinic and Research Foundation
1 R01 CA 34910-01
Modulation of Carcinogen Activation/Detoxification

25.	KOEFFLER, H Phillip Univ of California (Los Angeles) 1 R01 CA 33936-01	Action of Retinoids on Myeloid Leukemia Cells
26.	KRINSKY, Norman I Tufts University 1 R01 CA 32524-01	Anticarcinogenic Mechanisms of Carotenoid Pigments
27.	LUDLUM, David B Albany Medical College 5 R01 CA 32446-02	Repair of Carcinogenic Lesions in DNA
28.	MARNETT, Lawrence J Wayne State University 1 R01 CA 32506-01	Cancer Chemoprevention and Arachidonate Metabolism
29.	MATHEWS-ROTH, Micheline M Brigham and Women's Hospital 5 R01 CA 23053-06	Carotenoids as Antitumor Agents for Skin Tumors
30.	MAYS, Charles W University of Utah 5 R01 CA 28314-03	Reducing Cancer Risk By Radionuclide Chelation
31.	MCCORMICK, Anna M University of Texas Health Science Center (Dallas) 1 R01 CA 31676-01	Metabolism of Chemopreventive Retinoids
32.	MCCORMICK, David L IIT Research Institute 5 R23 CA 30646-02	Interactions Among Modifiers of Mammary Carcinogenesis
33.	MCCORMICK, J Justin Michigan State University 1 R01 CA 32490-01	Inhibition of Carcinogen--Transformation of Human Cells
34.	MEDINA, Daniel Baylor College of Medicine 5 R01 CA 11944-12	Biology of Mammary Preneoplasias
35.	MEDINA, Daniel Baylor College of Medicine 5 R01 CA 32473-02	Selenium Inhibition of Mouse Mammary Tumorigenesis
36.	MEHTA, Rajendra G IIT Research Institute 1 R01 CA 34664-01	Hormone and Retinoid Interaction in Mammary Tissue
37.	MOORE, Malcolm A Sloan-Kettering Institute for Cancer Research 1 R01 CA 32516-01	Mechanisms of Biological Prevention of Leukemogenesis

38. MUFSON, Robert A
New York University
1 R01 CA 32485-01

Effects of Retinoids on Human Epidermal Keratinocytes

39. NAPOLI, Joseph L
University of Texas Health Science Center
1 R01 CA 32474-01A1

Determinants of Vitamin A Homeostasis

40. NILES, Richard M
Boston University
1 R01 CA 32543-01

The Effect of Retinoids on Growth and Differentiation

41. ONG, David E
Vanderbilt University
5 R01 CA 20850-07

Cancer and Vitamin A

42. PETERSON, Per A
University of Uppsala
9 R01 CA 32583-04

Retinol Metabolism with Special Regard to the Eye

43. PROUGH, Russell A
University of Texas Health Science Center (Dallas)
1 R01 CA 32511-01

Inhibitor Effects on Mono-oxygenase Function

44. REDDY, Janardan K
Northwestern University
5 R01 CA 32504-02

Antioxidants and Peroxisome Proliferator Carcinogenesis

45. REWERS, John J
University of Texas
1 R01 CA 34469-01

Inhibition of Chemical Carcinogenesis by Interferon

46. ROGERS, Adrianne E
Massachusetts Institute of Tech
1 R01 CA 32498-01

Azaserine Carcinogenesis--Effects of Methionine, Choline

47. RUDDLE, Nancy H
Yale University
1 R01 CA 32447-01

Lymphotoxin and Interferon Inhibition of Carcinogenesis

48. SLAGA, Thomas J
Univ of Texas System Can Ctr
1 R01 CA 34521-01

Inhibition of Tumor Promotion by Antioxidants

49. STRAUSS, Bernard S
University of Chicago
5 R01 CA 32436-02

Plasminogen Activator and Error-Prone DNA Synthesis

50. THOMPSON, Henry J
University of New Hampshire
1 R01 CA 32465-01

Breast Cancer Chemoprevention and Polyamine Biosynthesis

51.	WATANABE, Kyoichi A Sloan-Kettering Institute for Cancer Research 1 RO1 CA 32535-01	Chemical Mechanisms of Carcinogenesis Protection
52.	WATTENBERG, Lee W Univ of Minnesota (Mnpls-St Paul) 2 RO1 CA 14146-10	Inhibition of Carcinogenesis by Phenols and Thiols
53.	WIEBEL, Friedrich J GSF-Research Center Gesellschaft fur Strahlen 1 RO1 CA 32541-01	Carcinogen Inactivation by Conjugation with Glutathione
54.	WOLF, George D Massachusetts Institute of Tech 5 RO1 CA 13792-06	Vitamin A and Glycoproteins of Skin Tumors
55.	WOLF, George D Massachusetts Institute of Tech 1 RO1 CA 32014-01	Mechanism of Retinoid Action on Bladder Cancer
56.	YANG, Chung S University of Medicine and Dentistry of New Jersey 5 RO1 CA 28298-02	Effects of BHA on Carcinogen Metabolism

CONTRACTS ACTIVE DURING FY 83

Investigator/Institution/Contract Number		Title
57.	COHEN, Leonard A American Health Foundation NO1-CP-05722	Dose Response Studies on Phenolic Antioxidants (Mammary Gland)
58.	DAWSON, Marcia I SRI International NO1-CP-05600	Synthesis of New Retinoids for the Chemoprevention of Epithelial Cancer
59.	HICKS, Marion R Middlesex Hospital Medical School NO1-CP-05602	Chemoprevention of Epithelial Cancer by Retinoids (Bladder)
60.	KURTZ, Perry J Battelle Memorial Institute NO1-CP-85650	Studies on Toxicology of Retinoids
61.	MCMURRAY, John E Cornell University NO1-CP-05716	Synthesis of New Retinoids for the Chemoprevention of Epithelial Cancer

62. MEEKS, Robert G
Southern Research Institute
NO1-CP-85615
Studies on Toxicology of Retinoids

63. MOON, Richard C
IIT Research Institute
NO1-CP-05718
Chemoprevention of Epithelial Cancer by Retinoids (Mammary Gland)

64. OKAMURA, William H
Univ of California (Riverside)
NO1-CP-05715
Synthesis of New Retinoids for the Chemoprevention of Epithelial Cancer

65. REDDY, Bandaru S
American Health Foundation
NO1-CP-05721
Dose Response Studies on Phenolic Antioxidants (Intestinal Tract Model)

67. REDDY, Bandaru S
American Health Foundation
NO1-CP-85659
Studies of Natural Inhibitors of Chemical Carcinogens

68. SHELLABARGER, Claire J
Department of Energy/Brookhaven National Laboratories
NO1-CP-00202
Chemoprevention of Epithelial Cancer by Retinoids (Mammary Gland)

69. WATTENBURG, Lee W
University of Minnesota
NO1-CP-05605
Dose Response Studies on Phenolic Antioxidants

70. WELSCH, Clifford W
Michigan State University
NO1-CP-05717
Chemoprevention of Epithelial Cancer by Retinoids (Mammary Gland)

71. WILLIAMS, Gary M
American Health Foundation
NO1-CP-05723
Dose Response Studies on Phenolic Antioxidants (Liver)

SUMMARY REPORT

CARCINOGENESIS MECHANISMS

Included under this heading are studies relating to the metabolism and mechanisms of action of carcinogens and their metabolites. Also included are studies that involve isolation, identification, and synthesis of known and suspect carcinogens; molecular structure-activity relationships and carcinogen-mutagen relationships. The sole instrument of support for this area is the research grant. Currently there are 74 grants in this program area with FY83 funding of approximately $5.81 million.

Grants Activity Summary

The grants in the program are most easily classified by the agent under study. Approximately half are concerned with studies of polycyclic aromatic hydrocarbons, alkylating agents, and aromatic amines. The remainder consists partly of studies involving many carcinogens from more than one of the above-mentioned groups; plus investigations of the mechanisms of action of materials, like fibers or foreign bodies and a variety of chemicals that do not fall into the above-mentioned classifications.

Polycyclic Aromatic Hydrocarbons: This area remains very active. At the present time, 15 grants are devoted to various aspects of polycyclic aromatic hydrocarbon (PAH) carcinogenicity. Included are studies on the metabolism of specific PAHs, on mechanisms of action of selected series of PAHs, and of structure activity relationships.

One metabolic study is designed to evaluate the possible biological effects of 3-methylcholanthrene (3-MC) and its 1-hydroxy and 2-hydroxy derivatives in rat lung using microsome fractions and isolated perfused lung (64). Another project is studying metabolic pathways used by prokaryotic and eukaryotic microorganisms to oxidize benzo(a)pyrene (BaP), benzo(a)anthracene (BaA), and 3-MC. Remarkable similarities were found between the oxidation of PAHs by mammals and by the filamentous fungus Cunninghamella elegans. The metabolic products found indicated that the organisms may contain enzymes similar to those known to metabolize these compounds in mammalian systems. For example, a cytochrome P-450 monooxygenase had been implicated in the initial oxidation of naphthalene by C. elegans. The formation of other metabolites suggested that the organism may contain the enzymes epoxide hydrolase, sulfotransferase, and UDP-glucuronosyltransferase. Further studies indicated that the enzyme activities did exist, and efforts were made to locate their activities in cellular fractions. Glutathione-S-transferase was found almost exclusively in the cytosol fraction. However, a small amount of activity (1-2%) was always detected in the membrane fraction obtained by centrifugation at 100,000 g. which could not be removed by repeated washing. It could not be determined whether the microsomal fraction contained a distinct glutathione-S-transferase or whether the activity was due to soluble protein trapped in membrane vesicles. Preliminary observations also suggested that more than one glutathione-S-transferase might be present in the soluble fraction. Two substrates for the enzyme were studied; each gave different results. When 1-chloro-2,4-dinitrobenzene was used, storage of cell extracts at 4°C resulted in a steady decline in activity until about 10% of the original activity was lost. At this point the rate of inactivation decreased significantly. Only 2% of the activity was lost in a similar study using p-nitrobenzyl chloride as the substrate. Other studies also indicated different

rates of inactivation with these two substrates. Epoxide hydrolase activity was found in the microsomal fraction, and UDP-glucuronosyltransferase was found only in the soluble fraction. The role of these enzymes is unknown. Further studies will determine whether these enzymes participate in essential metabolic reactions or are present to protect the fungus from the effects of environmental chemicals (19).

Under the classification of mechanisms of action, an attempt is being made in one project to study the early critical events in PAH carcinogenesis in mouse skin as a specific target tissue. An initiation-promotion protocol for initiation of skin tumors in mice has been developed for these studies, using inbred mice that have been selectively bred for susceptibility and resistance to two-stage carcinogenesis (61). Another project using mouse skin and other systems is attempting to obtain a better understanding of the mechanisms that are involved in the induction of cancer by PAHs and, in particular, of the routes by which hydrocarbons of varying carcinogenic potencies are activated by metabolism. Among the significant observations made by this group is that bay-region diol-epoxides are not necessarily the only vicinal diol-epoxides that are involved in the metabolic activation of PAH and that the skin of animals not susceptible to tumor formation by PAH can metabolize and activate BaP by mechanisms similar to those that are involved in the skin of animals that are susceptible (59).

Several grants support development of new synthetic methods for PAHs and their analogs. In one project, the principal investigator is developing stereo- and regiochemically controlled synthetic methods for bay-region diol-epoxide metabolites of BaA, 7,12-dimethylbenz(a)anthracene (DMBA) and 3-MC plus the putative active metabolites of azuleno(1,2,3-cd)phenalene and cyclopenta(a)phenanthrene. The chemical properties of the biologically active compounds along with their in vitro and in vivo reactions with nucleic acids are being investigated in conjunction with the bioorganic mode of action of the parent hydrocarbons and their metabolites (30).

Two grants are supporting research where methylated derivatives of PAH are being synthesized for purposes of studying molecular structure-activity relationships. One is studying the mechanistic basis for the enhancing effect in carcinogenicity of a bay-region methyl group and the inhibiting effect of a substituted peri position. Specific methylated isomers of benzo(b)fluoranthene and benzo(j)fluoranthene are being synthesized and bioassayed in order to determine the structural requirements for carcinogenicity of these compounds (26). Another group is evaluating the molecular basis for the carcinogenic differences of the twelve methylbenzo(a)-anthracenes (MeBaA). This is being done by assessing the differences in metabolic pathways that may account for their different carcinogenicities and determining their mutagenicity and tumor initiating ability. In a recent publication, these investigators have shown that liver microsomes from 3-MC pretreated male Sprague Dawley rats metabolized 12-MeBaA predominately to (-)trans-5,6-dihydrodiol with S,S absolute stereochemistry as the predominant enantiomer. Under similar conditions, the major enantiomer formed from BaA is (+)BA-trans-5R,6R-dihydrodiol. According to the authors, this is the first example indicating that the methyl substituent of a PAH can drastically alter the stereo-selective preference of the microsomal drug-metabolizing enzyme systems toward a substrate in the formation of a dihydrodiol metabolite at an unsubstituted aromatic double bond. In another paper, the same group has reported that in the case of 8-MeBaA, the major enantiomeric trans-dihydrodiol formed at the 3,4- and 5,6-double bonds, respectively, have the same absolute configuration regardless of the rat liver microsomal enzyme system used in the in vitro incubation of 8-MeBaA. However, the results of the study indicate that cytochrome P-448 and cytochrome P-450 interact with different faces of the

8,9-double bond of 8-MeBaA. In these experiments, the rat liver enzyme systems were obtained from rats pretreated with either 3-MC (activates cytochrome P-448) or phenobarbital (activates cytochrome P-450). The 8-MeBaA-trans-8,9-dihydrodiol formed by liver microsomes from 3-MC treated rats or by a reconstituted rat liver enzyme system containing cytochrome P-448 and epoxide hydrolase was enriched with the (-)-enantiomer. The 8-MeBaA trans-8,9-dihydrodiol formed by liver microsomes from either untreated or phenobarbital-treated rats was enriched with the (+)-enantiomer. The authors state that this indicates that a methyl substitution can drastically alter the stereo-selective property of the drug-metabolizing enzyme systems towards a substrate molecule at the methyl-substituted aromatic double bond (74).

In another project, the investigators are synthesizing bay-region diol-epoxides of aza substituted PAHs, specifically of benz(a)acridine (BaACR) and benz(c)acridine (BcACR) and testing them for biological activity. The mutagenic activities of BaACR, BcACR and a number of their derivatives, including 12 epoxides and diol epoxides have been examined in Salmonella typhimurium strains TA98 and TA100 and in Chinese hamster V79 cells to determine the importance of bay-region activation of aza-PAH and results were reported in a recent paper. The bay-region diol-epoxides and tetrahydroepoxides of BcACR were found to be from 1 to 4 orders of magnitude more mutagenic to bacterial and mammalian cells than were their non-bay-region counterparts. These results suggest that the bay-region theory of PAH carcinogenicity can be extended to certain aza-PAHs. The authors note that perhaps the most striking structure-activity relationship deduced from the study is that the position of the nitrogen heteroatom in the aromatic ring system has a profound effect in the biological activity of the epoxides. In all mutagenic test systems studied, the bay-region diol-epoxides and tetrahydro-epoxides of BcACR are substantially more mutagenic than are the analogous derivatives of BaACR. Tumorigenicity studies of many of these compounds are in progress, and consistent with the mutagenicity results, the substituted BcACRs are significantly more tumorigenic than the substituted BaACRs. In this paper, the authors note that relatively little is known about the metabolism of the benzacridines (31). Another grant is supporting studies on the metabolic disposition of selected nitrogen containing PAHs in mammalian systems in vivo and in vitro. BcACR is one of the compounds under study (40).

It is known that certain hydrocarbons can potentiate the carcinogenicity of some carcinogenic hydrocarbons and can inhibit cancer induction by others. The effects of these hydrocarbons in the metabolic activation of carcinogenic PAHs and the induction of biological effects in hamster embryo cell cultures is the subject of another grant in this group. A recent report described the effects of benzo(e)-pyrene (BeP) on the metabolism and binding of BaP-7,8-diol in hamster embryo cell cultures. Earlier this group had shown that simultaneous treatment with BeP increased the diols, decreased the water soluble metabolites, and altered the DNA adducts formed from BaP in early passage Syrian hamster embryo cell cultures. In this study, it was found that as the concentration of BeP increased, the amount of BaP-7,8-diol metabolized decreased. Both oxidation of the BaP-7,8-diol to more polar derivatives and conjugation to glucuronic acid decreased as the concentration of BeP increased. The amount of BaP-7,8-diol bound to DNA decreased as the concentrations of BeP increased. The major DNA adducts formed in all cultures resulted from the binding of the anti- and syn-isomer of BaP-7,8-diol-9,10-epoxide. The ratio of the anti- to syn-diol-epoxide-DNA adducts decreased as the concentration of BeP increased. These results, in the authors' opinion, demonstrate that BeP induces concentration-dependent decreases in the metabolism and DNA binding of BaP-7,8-diol

and alters the proportion of the two isomeric diol-epoxides bound to DNA, suggesting that the major effects of BeP on BaP metabolism involve alterations in the secondary metabolism of BaP (4).

Alkylating Agents: Eleven grants fall under this heading, ten of which are concerned exclusively with either nitrosamines or nitrosoureas.

Chemical studies of nitrosoamine formation are being performed in depth in work supported by two grants. In one, competitive routes for production of nitrosamines from tertiary amines via nitrosoammonium ion formation are being investigated. The overall goal of this project is to investigate new routes for nitrosamine formation and to find ways of inhibiting these processes (35). In another grant, the same principal investigator had earlier elucidated the characteristics of a model chemical reaction which cleaves a β-hydroxynitrosamine to a smaller fragment nitrosamine. He has also shown that S-9000 fraction from rat liver is capable of producing an analogous biochemical fragmentation of the nitrosamine C_{α} and C_{β} bond. This biochemical observation is under further investigation (34).

Several grants support research on metabolism and mechanisms of action of N-nitroso-compounds. One project is investigating the metabolism of various N-nitroso drugs and related compounds in a variety of animals including the pig (36). The mechanism by which two nitrosoureas induce liver tumors in rats is being studied in another project. This observation is unusual since most compounds of this class do not induce liver tumors in the rat (43). Under the mechanisms of action designation, the ability of several N-nitroso compounds to alkylate cellular macromolecules, particularly DNA and RNA, is being studied. In one project, the principal investigator is investigating the mechanism by which certain dialkyl nitrosamines and their β-oxidized derivatives methylate nucleic acids in rat liver (2). In another project, the ability of a variety of nitrosamines and related hydrazo- compounds to alkylate cellular macromolecules is being studied in both in vivo and in vitro systems (36). The mechanism of action of N-nitrosobis(2-oxopropanol)amine, a potent pancreatic carcinogen in an in vitro system, is the subject of another grant (47).

A new project is investigating the ability of nitrogen dioxide, an important atmospheric pollutant, to form N-nitroso compounds in vivo. This study developed from experiments showing that N-nitrosomorpholine was produced in mice gavaged with morpholine and exposed to atmospheric nitrogen dioxide (44).

Possible correlations between N-nitroso compounds and colon cancer are under investigation in groups of patients drawn from populations known to be at high or at low risk for development of colon cancer. The experiment involves studying nitrate metabolism in these patients under carefully controlled dietary conditions (33).

Finally, one project in this group is attempting to determine whether different chemical classes of toxic methylating agents act through a common ultimate active species. Several methylating agents are being studied, such as methylnitrosamines, methylnitrosourea, dimethylhydrazine, N-methyl-N-nitro-N-nitrosoguanidine, and methyltriazines. The work is designed to identify any compounds which act by way of a novel species and to obtain quantitative chemical data to characterize each of the alternate intermediates (73).

Aromatic Amines: Various aspects of the chemistry, metabolism, and mechanisms of action of this class of carcinogens are subjects of twelve grants.

Two grants are concerned with pure chemistry. One is studying the synthetic utility of 1-aza-1'-oxa(3,3)sigmatropic rearrangements of N-aryl-O-vinyl- and N,O-divinyl-hydroxylamines to 1,4-iminoketones and related compounds. The development of a variety of procedures for synthesis of N-aryl- and N-vinylhydroxylamines and for effecting O-vinylation of hydroxylamines are important goals of this project (10). The other is investigating the chemical reactivity of aryl hydroxylamines in aqueous solutions in order that a better and more fundamental understanding of the behavior of such compounds in biological systems can be realized (63).

The majority of grants in this area are concerned with metabolism. Determining the capacity of rat mammary gland to metabolize N-arylacetamides and N-arylaceto-hydroxamic acids is the goal of a very interesting project. Identification of metabolites of these carcinogens excreted in the milk of lactating rats and the consequences of lactational transmittal of these compounds in the suckling young are an important component of this project (37). The role of metabolism in the biliary excretion of drugs is the subject of another grant. In this project, the metabolism of the azo dye, N,N-dimethyl-4-aminoazobenzene is being studied in depth. The function of hepatic glutathione in the metabolism and biliary excretion of this compound is under investigation. The roles of iron, lipid peroxidation, and heme oxygenase are also being considered (32). In studies directed towards determining the genetic susceptibility to xenobiotic toxicity, the capacity of primary cultures of hepatocytes to N-acetylate xenobiotics is being determined. The principal investigator is attempting in these in vitro systems to reflect the genetically determined acylator polymorphism. The ultimate goal of this project is to determine at a molecular level the toxicity of xenobiotics that are N-acetylated and to further define the relationship between the risk of toxicity and the acetylator phenotype of liver cells (41). 4,4'-Methylene-bis(2-chloroaniline) (MCA) is an industrially used aromatic amine which is carcinogenic for rats, mice, and dogs. It is not known whether MCA is carcinogenic in humans, but it bears a structural resemblance to other aromatic amines which are known to be potent human urinary bladder carcinogens. The objective of this study is to compare the metabolic activation of MCA in rats and humans. The studies on rats, both in vivo and in vitro, are designed to determine which urinary metabolites are related to activation or detoxification of MCA. The studies on humans consist of examining urine from workers occupationally exposed to MCA to determine whether metabolites are present which correspond to activation or detoxification pathways in rats (46).

The mechanism of action of single ring arylamine carcinogens is the subject of another grant. Most of the compounds under study have a common structural feature in that they have a methyl or a methoxy function ortho to the amine. The research includes studies of the in vivo metabolism of O-toluidine, carcinogenicity of O-toluidine and O-nitrosotoluene and mutagenicity of N-oxidized derivatives of aniline, O-toluidine, 2'-methyl-4-aminobiphenyl and 3,2'-dimethyl-4-aminobiphenyl in Salmonella typhimurium. In the last-mentioned experiment, it was observed that N-oxidized derivatives of the compounds possessing an O-methyl group were generally more active than the corresponding unsubstituted compounds (16).

Another grantee is attempting to apply semi-empirical molecular orbital theory to study the reactivity of strong electrophiles with free nucleic acid bases and nucleosides. Initially the principal investigator is working with the known chemical behavior of the nitrenium ion of the carcinogenic arylamines and amides with various bases. Using the information developed from these studies, he will attempt to predict the course of nucleic acid modification by untested electrophilic agents (28).

Other Agents: This grouping is the largest. It consists of 36 grants supporting research with a variety of agents not in the other categories or with mixtures of agents from more than one category.

Four grants in this group are concerned with physical organic chemistry of carcinogens and their analogs, and are supporting studies on mechanisms of action from a purely chemical point of view. In one of the most interesting projects, crystallographic techniques are being applied to determine the three-dimensional structures of biologically active molecules and to determine the possible stereochemistry of interaction of these molecules with other molecules during processes of biological interest. Two areas of study are being pursued. The first is an investigation of the stereochemistry of certain enzyme reactions. The second is an investigation of the structures of series of polycyclic mutagens, carcinogens and their metabolic products, and molecular complexes of such compounds. The latter studies were done in order to derive an accurate three-dimensional description of the interaction of an activated carcinogen with a protein or nucleic acid (20).

Metabolic studies or mechanisms of action of several carcinogens are the subjects of eight grants. Two groups are involved in a collaborative study on the pharmacology of carcinogen activation in intact cells. The major goal of these joint projects is to delineate mechanisms whereby nutritional factors and pharmacological agents influence the metabolic activation and conjugation of precarcinogenic and model drug substances in intact cells using isolated perfused organs as experimental models (29, 68). In another project, the principal investigator has shown that carcinogenic chemicals common in the environment can adversely affect the developing preimplantation embryo without killing it. The effect of exposure to such compounds on implantation rate, birth rate, and the development of birth defects or tumors in live offspring from treated blastocysts following transfer to surrogate mothers is now being studied (27). Another grantee is studying carcinogen activation by cultured mammary cells and has recently shown that intraorgan cell specificity exists. Cells from a 50-55 day old virgin Sprague Dawley female rat mammary gland was divided into parenchymal and stromal enriched populations. A mediated mutagenesis assay was used to quantitate the ability of these cells to activate carcinogens. They found that the potent mammary carcinogen, DMBA, was activated by both mammary parenchymal and stromal cells, while the non-mammary carcinogen aflatoxin B_1 (AFB_1) was not activated by either cell type. The weak mammary carcinogen BaP was activated by the stromal cells and not by the parenchymal cells from which mammary carcinomas arise. The authors state that their data suggest that the intraorgan relationship between cell types that activate a carcinogen, and cell types that undergo neoplastic transformation, may in part explain the organ specificity of a carcinogen (24).

Fibers are the subject of four grants, and foreign body carcinogenesis is the subject of the fifth in this grouping. The foreign body (FB) tumorigenesis project has been the most interesting. In a recent report on chromosomal aberrations in FB tumorigenesis of mice, the authors note that virtually all FB sarcomas of mice possess abnormal chromosome numbers. These observations proved to be remarkably stable in transplantation experiments and in in vitro cultures. Thus they suspected an etiological connection. Preneoplastic and neoplastic cells were studied at various stages of progression by means of chromosome banding in an attempt to identify specific chromosome changes relative to the tumorigenic process. A considerable variety of numerical chromosome derangements were observed leading the authors to note that it appears that many different patterns of genetic disorders and imbalance may lead to initiation. They claim that this hypothesis is consistent

with observations which indicated the non-uniformity of initiation events in FB tumorigenesis. The primary alteration of the chomosome number in a preneoplastic parent cell may be explained by faulty mitosis with unequal chromosome distribution to the daughter cells. This may be the result of an inherent karyotype lability which is characteristically seen in murine mesenchymal stem cells of the microvasculature, the cell type of FB sarcoma origin. Other murine cell types, such as fibroblasts, do not share that property to the same extent. In man, the vascular stem cells appear karyotypically more stable, and this conforms with the finding that human FB sarcomas are rare. Secondary chromosome aberrations were noted to be unstable and variable during early neoplasia. Only preneoplastic cells of advanced FB-reactive capsules and their homologous tumor cells had structural chromosome aberrations in common. Apparently such aberrations gained stability during advanced stages of preneoplastic progression, possibly by giving affected cells a competitive growth advantage. Thus, they may contribute to tumor promotion and modify the determinants of late tumor characteristics. In another report, the same group states that they have unequivocally shown that FB-induced sarcoma cells of mice produce fibronectin. The presence of fibronectin may be related to the general finding that these sarcomas fail to metastasize hematogenously. In every other way, the tumors must be considered to be malignant: cell doubling time increases abruptly when autonomous growth commences, growth control seems irreversibly lost; histopathologically many of the tumors are highly anaplastic; they are transplantable; local invasiveness is always apparent although initial growth is nodular; metastases per continuity into regional lymph nodes do occur. It follows that these properties of malignancy are not contingent on the absence of fibronectin (6).

The remaining 19 grants cover research on metabolism or mechanisms of action of agents not included in any of the above-mentioned categories. In this group, there are two projects studying the mutagenicity and carcinogenicity of malondialdehyde. They are both using purified malondialdehyde and are finding, contrary to earlier reports, that the compound is only weakly mutagenic. This carcinogenicity study is not yet completed (3, 39).

Transplacental carcinogenesis is the subject of another grant. The agent under study is diethylstilbestrol (DES). In a recent paper, investigations of the disposition of (^{14}C)DES were reported in the near term pregnant Wistar rat. Both constant maternal iv infusion and direct fetal ip injection of (^{14}C)DES were utilized as exposure routes. Maternal infusions resulted in stable maternal plasma levels of DES during a 3-hour period. At 3 hours, fetal plasma levels were 2 to 3 times higher than maternal plasma DES. The chorioallantoic placenta and visceral yolk sac concentrated DES to levels 5 to 7 times that in maternal plasma. DES was highly concentrated in the extra-embyonic membranes, and high fetal plasma levels of DES were maintained. Fetal reproductive tract had total ^{14}C activity levels 20 to 25 times higher than those found in maternal plasma. In the fetal reproductive tract, only 20% of the total ^{14}C activity was found to be DES whereas greater than 50% was found to be oxidative metabolites. Thus, the fetal reproductive tract highly concentrated DES and especially its oxidative metabolites. The authors state that this finding demonstrates that it is critical to examine the individual fetal organs because measurement of the entire fetus resulted in an inability to detect oxidative metabolites. In the mother, 2% of the DES was excreted unchanged, and 98% was in the form of water-soluble conjugates. Seventy percent of the radioactivity was associated with DES in the whole fetus. β-glucuronidase treatment released only another 10% of the DES. The remainder of the glucuronides were oxidative metabolites. The degree of covalent binding of ^{14}C originally associated with DES was significantly higher in the fetal reproductive tract when compared with fetal

liver. However, there was some binding in all tissues analyzed. Similar metabolites were found in maternal and fetal livers. Direct fetal injections of (^{14}C)DES resulted in similar metabolite profiles in fetal tissues. The degree of covalent binding of (^{14}C)DES to cellular constituents was tissue specific, especially the fetal reproductive tract.
It is proposed that both the estrogenic activity of DES and the potential reactivity of its metabolites may be responsible for the toxicity expressed in the fetus. In as much as direct fetal injection of (^{14}C)DES resulted in high tissue localization of both oxidative and conjugative metabolites within the fetus, it is suggested that fetal tissues may metabolize DES (42).

Determination of whether there is a relationship between surface charge and the ability of various potentially toxic and carcinogenic particulate metal compounds to be phagocytized by cultured Syrian hamster embryo cells and Chinese hamster ovary cells is the subject of another project in this section (15). Another group is evaluating the possible role of mutagens in rat urinary bladder carcinogenesis induced by the administration of sodium saccharin following freeze ulceration of the bladder. Preliminary results suggested that the carcinogenic process can occur without a mutational event since neither freeze ulceration nor sodium saccharin are mutagenic. A series of experiments have been designed to follow up this interesting observation (11).

Carcinogen metabolism by the adrenal cortex is the subject of another grant. The adrenal cortex is adversely affected by a number of foreign compounds including carbon tetrachloride (CCl_4) and polychlorinated biphenyls, but the role of adrenal metabolism in the toxicity of such compounds has not been determined. This group recently reported on studies that were carried out to evaluate the effects of CCl_4 administration on adrenal and hepatic corticosteroid metabolism in rats. Their results indicate that exposure to CCl_4 decreases plasma corticosterone concentration, probably the net result of effects on adrenal corticosterone production and on hepatic steroid metabolism. However, the toxicological significance of these observations are not presently known. This group also reported on a study of the effects of adrenocorticotropic hormone (ACTH) administration to guinea pigs on the activities of adrenal microsomal monooxygenases. ACTH treatment decreased the rates of adrenal benzphetamine demethylation and BaP hydroxylation but had no effect on the same reactions in hepatic microsomes. Adrenal microsomal steroid hydroxylation reactions were unaffected (21-hydroxylation) or stimulated (17α-hydroxylation) by ACTH. Although ACTH treatment decreased adrenal BaP hydroxylase activity, the relative quantity of the various BaP metabolites as determined by high performance liquid chromatography, did not change. Adrenal microsomal cytochrome P-450 concentrations were decreased by ACTH but proportionately less than the decreases in adrenal xenobiotic metabolism. The results of these experiments indicate that ACTH selectively decreases the rates of adrenal xenobiotic metabolism, perhaps by producing a selective decline in the concentrations of those cytochrome P-450s involved in the metabolism of foreign compounds (12).

CARCINOGENESIS MECHANISMS

GRANTS ACTIVE DURING FY83

Investigator/Institution/Grant Number	Title
1. ALWORTH, William L Tulane University of Louisiana 5 R01 CA 23014-03	Modification of Mammalian Epoxide Hydrase Activity
2. ARCHER, Michael C Ontario Cancer Institute 2 R01 CA 26651-04	Mechanism of Nitrosamine Alkylation of DNA and RNA
3. ASTLE, Lynn University of Utah 5 R01 CA 32628-02	Gastrointestinal Carcinogenicity of Malondialdehyde
4. BAIRD, William M Purdue University West Lafayette 5 R01 CA 28825-03	Modifiers of Chemical Carcinogenesis in Cell Culture
5. BAKER, Donald G Univ of Virginia (Charlottesville) 5 R01 CA 25890-02	Influence of Hyperthermia on X-Ray Carcinogenesis
6. BRAND, K Gerhard Univ of Minnesota (Mnpls-St Paul) 5 R01 CA 10712-15	Initiation and Promotion in Foreign Body Tumorigenesis
7. BRYAN, George T Univ of Wisconsin (Madison) 5 R01 CA 11946-11	Carcinogenicity and Metabolism of 5-Nitrofurans
8. BUHLER, Donald R Oregon State University 2 R01 CA 22524-05A2	Pyrrolizidine Alkaloid Toxicity, Metabolism and Binding
9. CAVALIERI, Ercole L Univ of Nebraska Medical Ctr 1 R01 CA 32376-01	Mechanisms of Mammary Carcinogenesis by Hydrocarbons
10. COATES, Robert M Univ of Illinois (Urbana-Champaign) 5 R01 CA 20436-06	Hydroxylamine Rearrangements and Carcinogenesis
11. COHEN, Samuel M Univ of Nebraska Medical Ctr 1 R01 CA 32313-01	Non-Mutational Multistage Urinary Bladder Carcinogenesis
12. COLBY, Howard D West Virginia University 2 R01 CA 22152-04	Adrenal Carcinogen Metabolism

13. CORBETT, Michael D
University of Florida
5 R01 CA 32385-02
Hydroxamic Acid Production in Microbial Ecosystems

14. CORBETT, Michael D
University of Florida
5 R01 CA 32395-02
Hydroxamic Acid Production by Marine Organisms

15. COSTA, Max
University of Texas Health Sciences Center (Houston)
1 R01 CA 29581-01A1
Surface Charge and Phagocytosis--Toxic Metal Particulates

16. FIALA, Emerich S
American Health Foundation
5 R01 CA 26395-03
Single Ring Arylamine Carcinogens: Mechanism of Action

17. FIELD, Lamar
Vanderbilt University
5 R01 CA 30321-02
Thiono-type Compounds and their Relation to Cancer

18. FORD, George P
Pacific Northwest Research Fdn
5 R01 CA 30475-02
The Prediction of Nucleoside-Carcinogen Reactivity

19. GIBSON, David T
University of Texas (Austin)
2 R01 CA 19078-07
Microbial Degradation of Carcinogenic Hydrocarbons

20. GLUSKER, Jenny P
Institute for Cancer Research
5 R01 CA 10925-34
Application of Crystallographic Techniques

21. GOLD, Avram
Univ of North Carolina Chapel Hill
5 R01 CA 28622-03
Activation of Polycyclic Environmental Mutagens

22. GOLD, Barry I
University of Nebraska Med Ctr
2 R01 CA 24554-04A1
Epoxidation in Chloro-Olefin Carcinogenesis

23. GOLDMAN, Peter
Beth Israel Hospital
5 R01 CA 15260-09
Carcinogen Metabolism by Host Intestinal Bacteria

24. GOULD, Michael N
Univ of Wisconsin (Madison)
5 R01 CA 28954-03
Carcinogen Activation by Cultured Mammary Cells

25. GURTOO, Hira L
Roswell Park Memorial Institute
2 R01 CA 25362-04
Genetics of Aflatoxin Metabolism--Role in Carcinogenesis

26.	HECHT, Stephen S American Health Foundation 1 R01 CA 32242-01	Carcinogenic Methylated PAH: Structural Requirements
27.	IANNACCONE, Philip M Northwestern University 5 R01 CA 29675-02	Effects of Exposure to Carcinogens on Blastocysts
28.	IRVING, Charles C University of Tennessee Center Health Sciences 5 R01 CA 26165-03	Conjugation Reactions in Arylamine Carcinogenesis
29.	KAUFFMAN, Frederick C Univ of Maryland (Baltimore) 5 R01 CA 20807-06	Pharmacology of Carcinogen Activation in Intact Cells
30.	KOREEDA, Masato Univ of Michigan (Ann Arbor) 5 R01 CA 25185-05	The Bioorganic Chemistry of Arene Oxides
31.	LEHR, Roland E Univ of Oklahoma (Norman) 2 R01 CA 22985-07	Diol Epoxide and Other Derivatives of PAH and AZA-PAH
32.	LEVINE, Walter G Yeshiva University 2 R01 CA 14231-10A2	Role of Metabolism in the Billiary Excretion of Drugs
33.	LIPKIN, Martin Sloan-Kettering Institute for Cancer Research 5 R01 CA 28805-02	Nitrate Metabolism in Gastroin- testinal Cancer
34.	LOEPPKY, Richard N Univ of Missouri (Columbia) 5 R01 CA 22289-06	Nitrosamine Fragmentation and Nitrosamine Carcinogenesis
35.	LOEPPKY, Richard N Univ of Missouri (Columbia) 2 R01 CA 26914-04	Carcinogenesis: Nitrosamine Formation and Inhibition
36.	MAGEE, Peter N Temple University 5 R01 CA 23451-05	Formation and Metabolism of N-Nitroso Compounds
37.	MALEJKA-GIGANTI, Danuta Univ of Minnesota (Mnpls-St Paul) 5 R01 CA 28000-04	Mammary Carcinogenesis by Arylhy- droxamic Acids
38.	MANDEL, Richard Boston University 2 R01 CA 27324-04	Additive and Synergistic Effects of Mutagens

39.	MARNETT, Lawrence J Wayne State University 5 RO1 CA 22206-05	Studies on Malondialdehyde
40.	MC MURTREY, Kenneth D Univ of Southern Mississippi 5 RO1 CA 29903-03	Toxicology of Polynuclear Heterocyclic Carcinogens
41.	MC QUEEN, Charlene A American Health Foundation 1 RO1 CA 33144-01	Genetic Susceptibility to Xenobiotic Toxicity
42.	MILLER, Richard K University of Rochester 5 RO1 CA 22335-06	Transplacental Carcinogenesis
43.	MIRVISH, Sidney S Univ of Nebraska Medical Ctr 5 RO1 CA 24776-03	Mechanism of Liver Carcinogenesis by Two Nitrosoureas
44.	MIRVISH, Sidney S Univ of Nebraska Medical Ctr 1 RO1 CA 32192-01A1	N-Nitroso Compounds Formed In Vivo from Nitrogen Dioxide
45.	MORRISON, Harry A Purdue University West Lafayette 5 RO1 CA 18267-05	Cutaneous Photobiology and Drug Phototoxicity
46.	MORTON, Kenneth C Michigan Cancer Foundation 1 RO1 CA 32303-01	Metabolism of Activation of MCA
47.	NAGEL, Donald L Univ of Nebraska Medical Ctr 5 RO1 CA 31016-02	An In Vitro Model for Alkylation by Pancreas Carcinogens
48.	O'FLAHERTY, Ellen J University of Cincinnati 5 RO1 CA 29917-03	Quantitative Considerations in Urethan Carcinogenesis
49.	PAQUETTE, Leo A Ohio State University 5 RO1 CA 12115-12	Unsaturated Polyolefins and Hydrocarbon Carcinogenesis
50.	PARTHASARATHY, Rengachary Roswell Park Memorial Institute 5 RO1 CA 23704-05	Stereochemistry of Thiol-Disulfide Interchanges
51.	REICH, Edward Rockefeller University 5 RO1 CA 08290-18	Chemotherapeutic Deoxynucleosides and Other Agents
52.	REINKE, Lester A Univ of Oklahoma Health Sci Ctr 5 RO1 CA 30137-03	Influence of Ethanol on Carcinogen Activation

53.	RIVERA, Evelyn M Michigan State University 5 RO1 CA 17862-06	The Biology of Rat Mammary Hyperplasias
54.	ROMAN-FRANCO, Angel A University of Puerto Rico Medical Sciences 5 RO1 CA 28894-03	Mechanism of Action of Carcinogenic Fibers
55.	SCHAAP, A Paul Wayne State University 5 RO1 CA 15874-09	Enzymatically Generated Singlet Oxygen in Carcinogenesis
56.	SCRIBNER, John D Pacific Northwest Research Fdn 5 RO1 CA 23712-05	Early and Critical Events in Chemical Carcinogenesis
57.	SHIMAMURA, Tetsuo Rutgers Medical School 1 RO1 CA 30106-01A2	Mechanisms of Development of Urinary Bladder Cancers
58.	SIMENHOFF, Michael L Thomas Jefferson University 5 RO1 CA 26571-03	In Vivo Nitrosamines and Cancer in Renal Failure
59.	SIMS, Peter University of London 5 RO1 CA 21959-06	Mechanisms of Activation of Polycyclic Hydrocarbons
60.	SINSHEIMER, Joseph E Univ of Michigan (Ann Arbor) 5 RO1 CA 25770-03	Epoxide Toxicity in Alkene Metabolism
61.	SLAGA, Thomas J Univ of Tennessee (Knoxville) 5 RO1 CA 20076-07	Polycyclic Hydrocarbon Metabolism and Binding in Skin
62.	SPECK, William T Case Western Reserve University 5 RO1 CA 23692-06	Potential Hazards of Phototherapy
63.	STERNSON, Larry A University of Kansas (Lawrence) 5 RO1 CA 28782-03	Chemical Characterization of Arylhydroxylamines
64.	STOMING, Terrance Medical College of Georgia 7 RO1 CA 33586-01	Metabolism of 3-Methylcholanthrene in Liver and Lung
65.	SUN, Albert Y Univ of Missouri (Columbia) 2 RO1 CA 26586-04	Chlorinated Water and Membrane Function and Neoplasia

66. SUZUKI, Yasunosuke
Mount Sinai School of Medicine
5 R01 CA 24311-03
Pathogenesis of Experimental Malignant Mesothelioma

67. SUZUKI, Yasunosuke
Mount Sinai School of Medicine
5 R01 CA 29432-03
Carcinogenic and Fibrogenic Effects of Zeolites

68. THURMAN, Ronald G
Univ of North Carolina Chapel Hill
5 R01 CA 23080-06
Pharmacology of Carcinogen Activation in Intact Cells

69. TOTH, Bela
Univ of Nebraska Medical Ctr
1 R01 CA 31611-01A1
Carcinogenesis and Chemistry of Cultivated Mushrooms

70. UNDERWOOD, Graham R
New York University
5 R01 CA 25073-03
Study of Ultimate Carcinogen from Aromatic Amines

71. VOLLHARDT, K Peter
Univ of California (Berkeley)
5 R01 CA 20713-06
Activated Mutagenic and Aromatic Hydrocarbons

72. WALSH, Christopher T
Massachusetts Institute of Tech
5 R01 CA 20574-06
Reactive Heterocycles--Cancer and Biomechanism

73. WEINKAM, Robert J
Purdue University West Lafayette
5 R01 CA 28631-03
Chemotherapeutic and Carcinogenic Methylating Agents

74. YANG, Shen K
US Uniformed Services University of Health Sciences
5 R01 CA 29133-02
Metabolic Activations of Monomethyl benz(a)anthracenes

SUMMARY REPORT

MOLECULAR CARCINOGENESIS

The Molecular Carcinogenesis program area includes 157 grants with FY83 funding of approximately $9.29 million and one contract with no FY83 funding. The grants consist of 153 R01 (Research Project) grants and four R23 (Young Investigator) grants. Research in the program area focuses on the characterization of carcinogen-macromolecule interactions (30 grants); changes in biological macromolecules, cell structure, ultrastructure, and functions as a result of carcinogen or cocarcinogen exposure (32 grants); the identification of biochemical and molecular markers and properties of cells transformed by carcinogens (22 grants); the genetics and mechanisms of cell transformation (12 grants); the development of carcinogenicity/-mutagenicity testing procedures (9 grants); the mechanisms of carcinogen-induced mutagenesis and genetic damage (10 grants); enzymes associated with carcinogenesis induced by chemical and physical carcinogens (16 grants); and the role of DNA repair in carcinogenesis (34 grants). Expanded descriptions of individual subject areas along with examples of research accomplishments are provided below.

Grants Activity Summary

Carcinogen-Macromolecule Interactions: The projects in this subject area focus on studies on the identification, quantitation and characterization of carcinogen-nucleic acid adducts. The interest in the identification and characterization of DNA adducts stems from the role alterations in DNA play in the initiation of carcinogenesis. Most of the carcinogens used in these studies are metabolized by cellular xenobiotic metabolizing enzymes to a variety of metabolites of which one or a few are reactive and bind to nucleic acids and/or protein. The identification and quantitation of the binding species are generally determined by chromatographic and radioisotope techniques. The levels and persistence of specific DNA adducts are often related to the organ specificity of the carcinogen and indicate which of the adducts are biologically relevant. For many carcinogens such as the polycyclic aromatic hydrocarbons, alkyl nitrosamines, N-2-acetylaminofluorene, and aflatoxin B_1, the reactive metabolites and the identity of the various nucleoside adducts are known. The chemical nature and physical conformation of the adducts is thought to determine the biological effect of the adduct. For this reason several investigators are focusing on the chemical and biophysical characterization of carcinogen-DNA adducts and on the resultant conformational changes the adducts may introduce into the DNA molecule. Several different techniques have been utilized for the characterization of carcinogen-nucleic acid adducts. These include high pressure liquid chromatography, absorption and fluorescence spectroscopy, nuclear magnetic resonance, optically detected magnetic resonance, linear and circular dichroism spectroscopy and x-ray crystallography. In addition, computer analysis of possible carcinogen-DNA adduct conformations has allowed the building of molecular models for the most likely conformations. The results of these studies give information as to the possible mechanisms by which a carcinogen may cause a mutation or other alteration in the DNA structure.

The relatively low carcinogenicity of the polycylic aromatic hydrocarbon benzo(e)-pyrene (B(e)P) as compared to the high biological activity of the isomeric benzo-(a)pyrene (B(a)P) has been an intriguing problem. It is known that B(a)P is metabolized to a diol epoxide (±)7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydro-benzo(a)pyrene (B(a)PDE) which is its ultimate carcinogenic form. The analogous

diol epoxide of B(e)P,(±)9β,10α-dihydroxy-11α,12α-epoxy-9,10,11,12-tetrahydrobenzo-(e)pyrene (B(e)PDE) is negligibly formed metabolically. This provides at least one possible reason for the low biological activity of B(e)P. According to the bay region theory, B(e)PDE should only be slightly less active than B(a)PDE. Both, in fact, have been shown to be mutagenic, but while B(a)PDE is highly tumorigenic, B(e)PDE is inactive or only weakly tumorigenic. The molecular basis for these differences is poorly understood, but may result from important conformational differences between the two molecules. Using the techniques of absorption and fluorescence spectroscopy and electric linear dichroism, the conformational properties of the covalent adducts of B(e)PDE to DNA have been described. These are compared to the non-covalent complexes obtained from the binding of 9,10,11,12-tetrahydroxy-tetrahydrobenzo(e)pyrene (B(e)PT) to DNA. Two types of binding sites were shown to occur for B(e)PDE-DNA adducts, an exterior binding site similar to the one observed with B(a)PDE-DNA adducts and a quasi-intercalative type of binding site which is not observed in covalent B(a)PDE-DNA adducts. The extent of binding of B(e)PDE to DNA was shown to be four to eight times less than the binding of B(a)PDE to DNA under the same experimental conditions. The data obtained suggest that the reduced reactivity of B(e)PDE can be attributed to steric hindrance due to quasi-diaxial conformations of the two hydroxyl groups in one of the two bay regions of B(e)PDE (44).

It has recently been shown that DNA can exist in a left-handed helical conformation as opposed to the right-handed conformation of the conventional B-form of DNA. The left-handed helical DNA, termed Z-DNA, was shown to occur at high salt concentrations in DNA containing regions of alternating deoxyguanine-deoxycytidine bases. It has also been shown that the methylation of poly(dG-dC)•poly(dG•dC) at the N-7 position of guanine or the C-5 position of cytidine facilitates the transition of B- to Z-DNA at physiological salt concentrations. The reaction of the carcinogen 2-acetylaminofluorene, which forms adducts at the C-8 position of guanine, was shown to readily facilitate the conversion of poly(dG-dC)•poly(dG-dC) from the B- to the Z-DNA form. The effect of aflatoxin B_1 adduct formation on the salt induced conversion of poly(dG-dC)•poly(dG-dC) from the B- to Z-DNA form was examined, since the major adduct of aflatoxin B_1 is at the N-7 position of guanine. It was found that reaction with aflatoxin B_1 strongly inhibited the salt-induced conversion of the polymer from the B- to the Z-DNA. This inhibition could be detected even at the relatively low binding level of one aflatoxin B_1 molecule to 42 nucleotides. The reason for this inhibition is not fully understood at this time. The change in DNA conformation from the B- to Z form is thought to be associated with the regulation of gene function. The binding of carcinogens to DNA interferes with the normal equilibrium between the right- and left-handed forms of DNA and thus could also interfere with normal transcriptional activity (124).

Changes in Cellular Macromolecules and in Cell Functions: The types of research activities in this subject area include studies on alterations 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. Biochemical and immunochemical methods have been used to isolate and identify nonhistone chromosomal proteins, phosphoproteins, membrane glycoproteins, and other cytosolic proteins which are either altered or specifically appear in chemically induced hepatocarcinogenesis models. Neoplastic cells have been shown to manifest a variety of morphological and biochemical phenotypes different from their normal cell counterparts which are presumed to result from a substantial reprogramming of the cellular genome during neoplastic transformation. The acquisition of new surface properties that influence cellular adhesiveness and cell-cell

communications is one property of cells transformed to the malignant phenotype. There is evidence that alterations in plasma membrane glycoproteins may be responsible, in part, for the altered functional properties of the tumor cell. Five different subsets of glycoproteins have been identified by Dr. Walborg and his group (146). These include bile canalicular and liver biomatrix glycoproteins, Con A and soybean agglutinin binding glycoproteins and rat leukemia virus associated glycoproteins. Antibodies toward these glycoproteins are being developed in order to elucidate their biological significance.

The identification and characterization of nonhistone chromosomal proteins specific for neoplasia are the focus of several studies because they have been implicated in various aspects of differential gene expression. Sequential changes in the immunological specificity of nonhistone protein antigens has been observed during the carcinogenic process. In the laboratory of Dr. Hnilica (67) three nonhistone chromosomal proteins from Novikoff hepatoma chromatin have been isolated. These proteins were shown to have molecular weights of 39,000 (p39), 49,000 (p49) and 56,000 (p56) and were identified as cytokeratins. The p56 protein was found to be present in normal rat liver, 24 hr regenerating rat liver, and fetal rat liver or kidney, although in much smaller amounts than in Novikoff hepatomas. The p49 antigen was present only in Novikoff ascites hepatoma and in a cell line, NAHTC, derived from these ascites cells. The p39 antigen has been found in every carcinoma line examined but not in a representative sarcoma, fibrosarcoma, or Walker 256 carcinosarcoma. These results suggest that the p39 antigen is protein specific for malignant cells of epithelial origin and could be a useful marker for the presence of these cells.

Enzymes involved in DNA synthesis or RNA transcription such as the DNA polymerases, DNA ligases, DNA methylases and RNA polymerases may also be modified by the action of carcinogens. In the laboratory of Dr. Becker (4) an error-prone DNA polymerase (DNA polymerase α) has been identified in N-2-acetylaminofluorene-induced hepatic nodules. This enzyme is being purified and characterized as to its role in other carcinogen regimens and tumors. An altered pattern of DNA ligases has also been observed in these nodules and work is in progress to determine the mechanism of their deficiency and to determine whether it participates in the accumulation of DNA breaks which has been observed in these nodules. This laboratory has also demonstrated that nuclear DNA isolated from spontaneous and chemically induced hepatocarcinomas is significantly undermethylated. The site of methylation in DNA is predominently at the 5' position of cytosine and is usually contained in the sequence CpG. DNA methylation is hypothesized to be involved in the control of gene expression, the repair of DNA, the maintenance of chromosome structure, the establishment of preferred sites for mutation, and in certain systems, protection of DNA against enzymatic degradation. There is much evidence arguing for an association between the hypomethylation of DNA and active gene expression. In general, cytosine residues are found to be methylated in the DNA of cells where specific genes are inactive and the cytosine sites lack methyl groups in cells where genes are expressed. In systems where cell differentiation can be induced, such as the Friend erythroleukemia system, it has been observed that as the cells differentiate, the level of unmodified methylation sites in their DNA increases. In addition, agents which can cause hypomethylation of DNA, ethionine and 5-azacytidine, can also trigger cell differentiation. The methylation of cytosine involves a postreplication DNA modification by the DNA methyltransferases or DNA methylases. Several groups are currently attempting to purify these DNA methyltransferases and to investigate the regulation of this enzyme in response to carcinogens and promoters.

When Friend erythroleukemia cells are treated with 5-azacytidine and 5-aza-2-deoxycytidine, a rapid, time-dependent and dose-dependent decrease in DNA methyltransferase activity and synthesis of markedly undermethylated DNA is observed. The evidence obtained shows that the hypomethylation of DNA appears to result from the loss of DNA methyltransferase. It was shown that the analogs must be incorporated into DNA to mediate their effect on the enzyme and that a minor substitution of 5-azacytosine for cytosine in DNA (about 0.3%) is sufficient to inactivate more than 95% of the enzyme in the cell. The cytosine analogs act as inducers of differentiation in the same concentration range where they act to inhibit DNA methylation. These results support the hypothesis that methylation of cytosine residues in DNA plays a role in determining gene activity (24 and 1).

Exposure of cells to carcinogens has been shown to affect DNA replication, RNA transcription and processing, and protein synthesis. In one study the injection of aflatoxin B_1 into rats was shown to result in a rapid inhibition of hepatic nucleolar RNA synthesis. This was shown to not be due to the direct inhibition of RNA polymerase I. The other possibilities for this effect could be due to the alteration by the carcinogen of the nucleolar DNA template or by the alteration of nucleolar chromosomal proteins which normally regulate the expression of nucleolar genes. In addition to aflatoxin B_1, the carcinogen methylazoxymethanol acetate (MAMA) was shown to be a potent inhibitor of both nuclear and nucleolar RNA synthesis. The data indicate that the mechanism of this inhibition is at multiple sites. It appears to impair chromatin template function and it also selectively inhibits RNA polymerase II activity. The enzyme is not totally inactivated but may have been transformed into a catalytically deficient state. Unexpectedly, it was observed that MAMA treatment in vivo causes a dramatic condensation of nucleoplasmic chromatin. Since the biochemical action of MAMA is believed to be similar to that of dimethylnitrosamine in that the methylation of guanine in DNA would result, the impaired chromatin template function could be a direct result of DNA methylation. The MAMA-induced chromatin condensation could also effect chromatin template function and may be related to DNA methylation (157).

The fidelity of DNA synthesis has been shown to be altered by chemical and metal carcinogens. Metal cations such as Be, Cd, Ni and Cr, which are suspected human carcinogens, and Ag, Co, Cu, Mn and Pb, which are known animal carcinogens or bacterial mutagens, were shown to increase the infidelity of DNA synthesis. Another effect of the metals on DNA is that they increase the rate of depurination. In the laboratory of Dr. Loeb (101) it has been established that an apurinic site is a mutagenic lesion. The results obtained from his studies indicates that mutagenesis resulting from apurinic sites is associated with bypass of these noncoding lesions during DNA synthesis. Another possible way that chemical carcinogens can affect the fidelity of DNA synthesis was demonstrated by Topal and Baker (145). A nontoxic dose of N-methyl-N-nitrosourea was given to synchronized C3H 10T1/2 clone 8 cells during their S phase. From the analysis of the deoxyribonucleotide DNA precursor pool, it was shown that a nucleic acid residue in the precursor pool is 190 to 13,000 times more susceptible to methylation than a residue in the DNA duplex, depending upon the site being modified. For example, it was shown that the N-1 position of adenine in dATP from the pool was 6.3 times more methylated than the same position in the DNA, even though the adenine content of the pool is a small fraction (0.0005) of the adenine content of the DNA helix. The group has demonstrated that the modified nucleotides can be incorporated into DNA and thus, some of the methyl adducts detected in the DNA may have come from incorporation of the modified precursors. The incorporation of these modified nucleotides is expected to contribute to mutation and neoplastic transformation.

Markers and Properties of Transformed Cells: Research included in this subject area involves studies on the documentation of 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 evidence obtained to date strengthens the supposition that the development of most cancers involves a multistep process in which cells progress from normal to initiated, preneoplastic, and premalignant stages to the end point of malignant neoplasia. In order to characterize cells at each stage, a detailed analysis and knowledge of the sequence of relevant biochemical and biological alterations associated with the development of chemically induced carcinogenesis is needed. To achieve this purpose, a variety of model systems, both animal and cell culture, are being used. Of the animal model systems, the predominant one currently in use is the rat chemically induced hepatocarcinogenesis model. Although this model was established some time ago, the treatment regimens being employed have undergone a variety of changes depending on the purpose of the experiment and on the endpoint desired. Chronic or intermittent exposure regimens have been used along with initiation-promotion type regimens in which various different initiating carcinogens and promoting stimuli are used. The sequential appearance of foci of altered hepatocytes, nodules, and hepatocellular carcinomas can be observed and analyzed. There are other interesting model systems which are being established and analyzed by one or more laboratories. For example, a model of renal carcinogenesis in the rat is being established in which adenocarcinomas or mesenchymal tumors are selectively induced following a single dose of dimethylnitrosamine. Cell cultures representative of the renal tumor types are being established in order to correlate in vivo phenomena in renal carcinogenesis with events occurring in vitro.

Another interesting experimental system involves the establishment and sequential analysis of stages of oral carcinogenesis using hamster buccal pouch epithelium. The buccal pouch consists of a flat epithelium which has no glandular elements and normally lacks histochemical evidence of gamma glutamyltranspeptidase (GGT) activity. Whole mounts of this epithelium can be prepared for analysis. Also, with this system it appears that it will be possible to relate the cells displaying altered growth in vitro to populations of presumptive initiation sites in vivo. This is not possible with other existing models.

Research relevant to respiratory carcinogenesis is being conducted using a rat tracheal implant system. The properties of carcinogen initiated cells can be studied in short-term organ culture where normal tissue interactions can be preserved. The cells can also be studied while growing in cell culture and also in vivo by allowing the cells to repopulate denuded trachea which are implanted into nude mice. Properties of normal and carcinogen-treated human respiratory epithelium can also be studied by using the denuded rat trachea implants in nude mice. These types of studies are being initiated and represent exciting new approaches to studying respiratory neoplasia and human respiratory neoplasia in particular. It should allow us to better extrapolate animal carcinogenesis results to their human counterpart. Research using other animal model systems, i.e. breast, colon, pancreas, bladder, and prostate is being handled by the Organ Systems Program of NCI.

In addition to the utilization of animal systems, the in vitro transformation of cells in culture occupies the focus of several other research groups. The use of cell cultures which are derived from in vivo carcinogenic lesions allows investigators to more easily analyze properties of the cells in question. The ability to transform cells in culture allows for the study of mechanistic questions regarding

chemically induced transformation. For some of this research, standard rodent fibroblast or epithelial cell lines have been used. With the increasing success in transforming human fibroblast and epithelial cells following the pioneering work of Kakunaga and Milo and DiPaolo, several groups of investigators are increasingly turning to the use of human cell cultures in their research. This focus has been and will continue to be vigorously supported by NCI.

Upon transformation by chemicals, most cells acquire altered growth properties which allow them to proliferate under selective growth conditions. This can involve the ability to grow in soft agar (anchorage independent growth), the loss of contact inhibition of growth, or the ability to grow in medium containing low calcium. Several biochemical and molecular markers have been used to identify transformed, preneoplastic and neoplastic cells. The histochemical expression of GGT activity and the loss of histochemically determined glucose-6-phosphatase and ATPase activity are common markers used to identify carcinogen-altered liver cells and other epithelial cells. Other enzyme markers such as the presence of epoxide hydrolase, alkaline phosphatase isozymes and aldehyde dehydrogenase isozymes are being evaluated. Functional markers for liver cells being utilized currently include the production of albumin, alpha fetoprotein, transferrin, and fibrinogen. An increasing need is being seen for the development of genetic markers of neoplasia. The development of chromosomal abnormalities and aneuploidy in transformed cells are now being evaluated.

The laboratory of Dr. Emmanuel Farber at the University of Toronto has provided results which have had a 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. In the hepatocarcinogenesis model, populations of altered hepatocytes are induced by an initiating dose of a carcinogen. The initiated hepatocytes acquire the property of resistance to the inhibitory effects of toxins on cell proliferation. These resistant hepatocytes can be selectively stimulated to proliferate by a mitogenic stimulus for liver in the presence of a concentration of 2-acetylaminofluorene in the diet sufficient to inhibit the proliferation of a majority of hepatocytes. Hyperplastic nodules then appear and metastasizing hepatocellular carcinoma is found within such nodules. Since there appears to be some continuity among the carcinogen-induced resistant hepatocyte, a small subpopulation of hyperplastic nodules and liver cancer, the resistant hepatocytes may play an important role as an early precursor population in hepatocarcinogenesis. The role of cell proliferation per se on the resistance of hepatocytes was studied by using primary cultures of hepatocytes prepared at various time intervals up to 2 weeks following partial hepatectomy. A progressive increase in resistance until 48 hr to the cytocidal effect of aflatoxin B_1, 2-acetylaminofluorene, N-hydroxy-2-acetylaminofluorene, methotrexate, or methyl methanesulfonate was demonstrated, which returned to the resting level of susceptibility by 2 weeks. The ability of S-9 liver fractions to generate mutations from 2-acetylaminofluorene and aflatoxin B_1 in the Ames mutagenesis assay decreased and returned to control values in a manner paralleling the resistance. The levels of total cellular cytochromes P-450 were measured and found to decrease following partial hepatectomy and remain at 28 to 36% less than controls for at least a week. The glutathione and total soluble sulfhydryl content were found to increase following partial hepatectomy in a pattern consistent with a partial role for glutathione in the resistance phenomenon as it relates to 2-acetylaminofluorene. From the data it appears unlikely that the levels of microsomal activation enzymes play the only role in the acquisition of resistance, since it is seen with compounds that do not require metabolic activation as well as with compounds that do. There is a growing realization that glutathione may play a major

controlling role in the removal or trapping of some reactive chemicals in the liver. In this study alterations in glutathione levels appear to relate to the resistance of only one hepatotoxin, 2-acetylaminofluorene, even though, on the basis of other results, it was anticipated that aflatoxin B_1 toxicity might be most closely related to changes in glutathione levels. The reasons for this discrepancy remain unknown. In a different study but using the same hepatocarcinogenesis protocol, the long term administration of glutathione had no observable protective influence on liver cancer development initiated by diethylnitrosamine. The answers to questions that remain concerning the phenomenon of resistance require a better understanding of the biochemical and metabolic basis of resistance (35).

In the laboratory of Dr. Ronald Lindahl, studies on the elucidation and characterization of 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 isozymes have differing substrate and coenzyme preference, substrate Km, immunochemical properties and sensitivity to inhibitors. Hepatomas induced in male Sprague-Dawley rats by 2-acetylaminofluorene have been shown to have a unique aldehyde dehydrogenase phenotype, which is characterized by an increase in total aldehyde dehydrogenase activity. This is 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. In addition, several aldehyde dehydrogenases are inducible in normal liver by various xenobiotics, among which are two effective promoters of hepatocarcinogenesis, phenobarbital and 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). A cytosolic aldehyde dehydrogenase is induced in certain genetically defined lines and certain strains of rats by phenobarbital. TCDD induces a second cytosolic isozyme in both phenobarbital-responsive and non-responsive lines. The TCDD-inducible isozyme is immunochemically identical to at least one of the tumor isozymes, although neither the tumor nor the TCDD-inducible isozymes are related to the phenobarbital-inducible isozyme. An experiment to determine when the tumor aldehyde phenotype is first detectable during the tumor induction process was conducted by inducing with a brief feeding of 2-acetylaminofluorene followed by tumor promotion using phenobarbital. A significant change in hepatic aldehyde dehydrogenase activity was observed, the phenotype being characterized by the appearance of a new cytosolic isozyme. This isozyme was kinetically, electrophoretically, and immunochemically distinct from the normal liver aldehyde dehydrogenase isozymes and from isozymes induced in hepatomas induced by 2-acetylaminofluorene. The new isozyme was shown to be NAD-dependent, sensitive to disulfiram inhibition, and to cross-react with antiserum to a normal liver aldehyde dehydrogenase inducible by phenobarbital in several lines of rats. The animals used in this study had been shown previously to be non-responsive to aldehyde dehydrogenase induction by phenobarbital. Since no animals receiving only phenobarbital expressed this new isozyme and the phenotype appears only in animals receiving the combined 2-acetylaminofluorene/phenobarbital treatment, it was concluded that the carcinogen must play a significant role in its induction. This may be the first example in hepatocarcinogenesis of a new enzyme phenotype induced by a combination carcinogen/promoter treatment under conditions where neither carcinogen nor promoter alone are capable of such an induction (99).

An apparently unique calcium binding protein has been found in several rat Morris hepatomas. This protein has been purified to homogeneity and a specific antiserum has been raised against it. It has a molecular weight of 11,500, is acidic (isoelectric point of 3.9), has an amino acid composition devoid of tryptophan and with a high phenylalanine to tyrosine ratio, and strongly binds two calcium ions per protein molecule. This protein, named oncomodulin, has properties similar to

members of the troponin C superfamily, which includes parvalbumin, calmodulin, and troponin C. Unlike other calcium-binding protein regulators, oncomodulin either does not exist or is present in undetectable amounts in normal fetal and adult tissues. Using specific radioimmunoassays, several types of mouse, rat and human tumors were tested and the seemingly de novo appearance of oncomodulin and increased levels of calmodulin were observed. Tryptic peptide mapping showed that oncomodulin in rat and mouse tumors was identical, and was not a fragment of either calmodulin or troponin C. A function for this protein is unknown but a possible role may be in the deregulated cell proliferation of cancer cells, which can proliferate in calcium-deficient media that does not allow the proliferation of non-neoplastic cells (105).

Rhodamine and cyanine dyes have been used by many investigators to localize mitochondria in living cells by epifluorescence microscopy. The specificity of these dyes appears to result from the high membrane potential (negative inside) across mitochondria and the net positive charge carried by the dyes at physiological pH. These mitochondrial supravital dyes have been used in a number of studies in which mitochondial function or destruction or cell viability are being monitored. In studies conducted by Dr. Lan Bo Chen it was observed that mitochondria in cardiac muscle cells and myoblast-fused myotubes displayed an unusually long (3-5 days) retention time of rhodamine 123. Fifty different keratin-positive carcinoma or transformed cell lines were tested. Mitochondria with prolonged retention of rhodamine 123 were detected in most of the transitional cell carcinoma, adenocarcinoma, and chemical carcinogen-transformed epithelial cell lines and in some squamous cell carcinoma lines but not in any oat cell carcinoma lines. Nontumorigenic or tumorigenic cells of fibroblastic, neural or hematopoietic origin released the dye within 1 to 16 hours after being transferred to dye-free medium. That there are exceptions indicates that this phenotype is not essential for neoplasia. However, the property of extended rhodamine 123 retention may be a selective marker diagnostic for transformed epithelial cells. The difference in rhodamine 123 retention between carcinoma cells and other cell types might also be exploited for cancer chemotherapy. The effects of rhodamine 123 on the clonal growth of human and mouse bladder cells and on normal mouse bladder epithelial cells was explored. It was observed that the dye markedly reduced the clonal growth of carcinoma cells but had little effect on nontumorigenic epithelial cells in vitro. Known anticancer drugs such as arabinosyl cytosine and methotrexate did not display such selective inhibition in vitro. Thus, the rhodamine dyes and their analogs may be useful selective drugs for carcinomas (22).

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 have an influence on cell transformation. There is a large body of data demonstrating a high correlation between the mutagenicity and carcinogenicity of various chemicals. This supports the hypothesis that somatic mutations are involved in the process leading to neoplasia. Somatic cell hybridization techniques have been used to study the types of mutations leading to the transformation of BHK cells by chemical carcinogens. The chemically induced transformants examined show the characteristics of a single-step, recessive mutation. Temperature sensitive transformants which result primarily from base change mutations rather than frameshift mutations have also been isolated. Similar types of studies are being conducted to determine the number of complementation groups into which the various transformants isolated fall. This will allow a determination of the number of functional alterations which are needed to lead to

the expression of transformation. In another study, the role of H-2 haplotypes in influencing the relative resistance (or relative susceptibility) to local tumorigenesis induced by 3-methylcholanthrene is being determined. It has been determined that the H-2^K haplotype confers a high degree of susceptibility to local tumorigenesis by 3-methylcholanthrene and that this effect is a recessive trait. Further studies to understand the mechanism of the observed effect are in progress.

Fish of the genus Xiphophorus are being used in order to evaluate the biochemical genetics of the carcinogenesis process. These fish have proven to be a good model system for the study of a wide range of genetic, evolutionary, physiological and ecological problems. Using interspecific backcross hybrids of Xiphophorus fishes, genotype-specific susceptibilities to the induction of neuroblastoma and fibrosarcoma/rhabdomyosarcoma by the direct acting carcinogen N-methyl-N-nitrosourea has been demonstrated. In experiments to identify genes involved in the regulation of melanomas, a significant association with the locus coding for esterase-1 was the only one found. A major goal of the current studies in this area is to use genetic mapping techniques to provide markers for each of the 24 pairs of acrocentric or teleocentric chromosomes in Xiphophorus. These can then be used to identify genes controlling tumor induction and growth in these fishes. Various strains of fish with selected genotypes can then be produced which will be maximally susceptible to carcinogens. It is envisioned that this will result in an aquatic vertebrate in vivo system for use in the evaluation of putative carcinogens.

Newer studies on the role of specific genes and gene products in chemically induced cell transformation have been initiated. In one study of this type the hypothesis to be tested is that the transcriptional activation of genes, which are progenitors of sarcoma virus genes, is required before chemical mutagens can initiate transformation in cultured rat cells. It is also proposed that initiation involves the production of mutations in at least one copy of the sarcoma virus genes. In these studies the techniques of somatic cell hybridization and two-dimensional gel electrophoresis are being used to provide information concerning the nature of the genetic lesion and altered gene expression resulting from the chemically induced transformation of cells. In support of the above hypothesis, there has been a veritable explosion of publications demonstrating the isolation and characterization of genes responsible for the transformation of cells to malignancy. Newly developed recombinant DNA, gene cloning and DNA sequencing techniques have been employed in this research. To date, several different transforming genes have been isolated from different human tumor cells and their homology to various viral oncogenes has been established. Recently, it was shown that there is increased transcription of the cellular homologue of the transforming gene of Harvey sarcoma virus during the early stages of liver regeneration. Increased transcription of the transforming gene was also observed during chemically induced hepatocarcinogenesis. Further studies are underway to extend these findings.

Another major focus of projects in this subject area are studies designed to test the cell cycle specificity of the induction of cytotoxicity, mutagenesis, and neoplastic transformation by chemical carcinogens. Also, the quantitative relationship between the level, persistence, and species of carcinogen-nucleotide adducts and the concomitant cell transformation frequency are being determined. There is a substantial amount of information supporting the hypothesis of cell cycle specificity of carcinogenesis. It has been shown that in mouse embryo C3H 10T1/2 cells, G_1 and S phase cells are susceptible to cytotoxicity and mutation, while only S phase cells (in synchronized cultures) are susceptible to neoplastic transformation by exposure to alkylating agents. In adult rat liver, the hepatocytes are

generally resistant to carcinogenesis by a single exposure to agents capable of inducing cancer in other tissues. Hepatocyte susceptibility to carcinogenesis is increased by certain treatments which stimulate the proliferation of carcinogen damaged cells. Additional work is in progress to determine more specifically in rat liver the phase of the cell cycle which is most susceptible to the initiation effect of carcinogenic chemicals.

The mechanism of radiation carcinogenesis induced by x-rays or UV light in cell culture systems and the interactive effect of various chemical agents is the focus of an important study. The role of DNA recombinational events, free radical intermediates, cell growth modification, patterns of cell differentiation, and the induction of specific proteases is being examined in the mouse embryo C3H 10T1/2 cell line and in a human diploid cell transformation system which is being developed.

Development of Carcinogenicity Test Systems and Mechanisms of Mutagenesis: The development of carcinogenicity test systems subject area includes projects in which epithelial and fibroblast cell culture systems, specially constructed bacterial strains, erythroid cells, and a ^{32}P-labeling test are being used to monitor the effects of exposure to known and potential carcinogens. The end points being measured include cell transformation, mutagenesis, or the generation of DNA damage. The differential sensitivity of cells to low or high calcium concentrations in the medium is a selective property in three of the test systems. Most tumor cells have the capability to proliferate in medium containing low calcium concentrations, while normal cells do not. The carcinogen-induced resistance to low calcium concentrations of rat liver cells is the basis of one such test system. Mouse epidermal cells, on the other hand, can be subcultured in the absence of feeder layers in low calcium medium. In the presence of high calcium medium these cells cease to grow and terminally differentiate. Epidermal cells altered by chemical carcinogens, however, continue to grow in high calcium medium and do not terminally differentiate. This difference in growth response to high calcium is being used to select cells transformed by carcinogens. Work is in progress to isolate and characterize cloned epidermal cell lines for use in a testor system, to identify and resolve the sources of variability in this system and to identify additional markers of transformation of epidermal cells.

In another laboratory epidermal cells from skin tumor sensitive (SENCAR) mice are being used to develop a reliable and quantitative in vitro transformation system. In this system the epidermal cells are grown on a mouse fibroblast feeder layer in medium containing standard calcium levels. Under these conditions the epidermal cells can grow, be subcultured and terminally differentiate (stratify, form keratin, and cornify) in a manner analogous to normal skin. It was felt that this should allow studies of epidermal carcinogenesis and differentiation to be conducted under more normal conditions. The SENCAR mouse skin tumorigenesis system has been used extensively for the testing of chemical compounds for their carcinogenic activity using an initiation-promotion protocol. Investigations on the critical biochemical events in initiation and promotion have been conducted using this animal model. The development of an in vitro transformation system should facilitate investigations into mechanistic questions involving initiation and promotion as well as allowing the detection of carcinogens and promoters.

There is considerable interest in developing methods that will allow investigators to determine whether people have been exposed to harmful levels of chemical carcinogens. Two laboratories are developing such methods and their work is

directed toward measuring the level of base substitution mutations produced in erythrocytes. Monoclonal antibodies are being produced which can recognize mutant hemoglobin and spectrin molecules. These will be labeled by conjugation with fluorescein. Erythrocytes are labeled with the fluorescently conjugated antibodies specific for the variant proteins. The mutant cells are then enumerated using high-speed fluorescence activated cell sorting and automated scanning microscopy. The method has the capability of detecting one abnormal cell in 10^7 cells and thus the frequency of background and mutagen/carcinogen-induced somatic mutations can be determined.

In another project a bacterial test system for carcinogens will be developed. The basis for this study comes from a theoretical paper published by Dr. Martin Pall (Proc. Natl. Acad. Sci. USA 78: 2465-2468, 1981) in which he describes a mechanism by which carcinogenesis might occur. The mechanism proposed is that initiation involves a mutation which produces a tandem duplication of certain genes (a proto-oncogene is suggested) and that promotion involves the further amplification of the same genes by unequal crossing over and sister chromatid exchange. When sufficient gene product is produced, the cell will then become transformed. In the proposed work certain predictions of the theory will be tested. The main prediction to be tested using derivatives of the bacterium, Salmonella typhimurium, will be that carcinogenic chemicals can cause tandem duplications more efficiently than noncarcinogenic chemicals can. If shown to be valid, the work can lead to the understanding of molecular events in carcinogenesis and also to improved carcinogen test systems.

Most of our present knowledge about interactions between carcinogens and biological macromolecules has been provided by the use of radioactive chemical carcinogens. A newly developed enzymatic ^{32}P-postlabeling method has been described which allows the analysis of DNA's modified by non-radioactive chemicals. In this study DNA was either reacted in vitro with N-hydroxy-2-acetylaminofluorene, N-acetoxy-2-acetylaminofluorene, and 7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene or isolated from the livers of rats treated with N-hydroxy-2-acetylaminofluorene and benzo(a)pyrene. The DNA preparations were then enzymatically digested to deoxyribonucleoside 3'-monophosphates and then converted to (5'-^{32}P) deoxyribonucleoside 3',5'- bisphosphates by the T4 polynucleotide kinase-catalyzed (^{32}P) phosphate transfer from (α-^{32}P) ATP. The ^{32}P-labeled nucleotide adducts are then separated by thin-layer chromatography and detected by autoradiography. This method allows for the detection of one adduct in 10^7-10^8 normal nucleotides and appears to be applicable to the ultrasensitive detection of a large number of carcinogen-DNA adducts of diverse structure without requiring radioactive carcinogens or specific antibodies (56 and 123).

In addition to the development of mutagenicity test systems are projects which seek to understand how mutations and DNA or chromosome damage are generated by carcinogenic chemicals. Specifically synthesized oligonucleotides of defined base sequence are being used to examine the molecular mechanism of frameshift mutagenesis. The base sequence specificities of the interactions of frameshift mutagens with oligonucleotides are being studied and correlated to their mutagenic activity in Ames testor strains having known base composition in the frameshift site. Studies are also being supported for the study of the mechanism and genetic control of frameshift mutagenesis in yeast. The recently sequenced His4 gene system with existing (+1 G/C) and new (-1)frameshifts are to be used to construct testor strains. DNA sequencing and recombinant DNA technology have been used in these studies. The types of studies to be undertaken include an assessment of the effect of transcription on mutation frequency, the effect of having an origin of replication in

close proximity, an analysis of gene duplication, and a study of the potential role of the nuclear envelope in mutagenesis.

Mutagenesis by aflatoxin B_1 is being investigated at the molecular level in a prokaryotic experimental system involving E. coli, phage ϕX174 and the plasmid pBR322. It has been demonstrated that the activated form of aflatoxin B_1 causes the covalent modification primarily of guanine residues, leading to alkali-labile sites in DNA. DNA sequence analysis was used to identify alkali-labile sites induced by aflatoxin B_1 and to determine the frequency of alkali-labile aflatoxin B_1 modifications at particular sites on a DNA fragment of known sequence. The influence of flanking nucleotide sequences on aflatoxin B_1 modification in a number of DNA fragments of known sequence was investigated. The results show that, in general, all guanine residues in single-strand DNA are equally subject to aflatoxin B_1 modification.

In the double-stranded form, however, guanine residues flanked by (A-T)-rich sequences appear to be poor targets for the induction of alkali-labile lesions by aflatoxin B_1, while guanine residues occurring as members of G-C clusters are highly favorable target sites for this type of modification. It thus appears that the DNA sequence plays a role in the site of binding of aflatoxin B_1 (70).

DNA damage also leads to aberrations at the higher organizational level of the chromosome. Projects are being supported whose goals are to understand the molecular mechanisms which lead to the formation of chromosome abberrations and to investigate the biological significance of the sister chromatid exchange assay. Standard cytogenetic techniques as well as cell fusion techniques to form prematurely condensed chromosomes, DNA elution techniques, and techniques required to get drugs and enzymes into cells are being utilized to study chromosome damage by various chemical agents. In experiments using bleomycin it was shown that both DNA damage and chromosome damage exhibit two-component dose-response curves. The results suggest that there is a differential sensitivity to bleomycin within the cellular chromatin. It was also demonstrated that the chromatid exchange rate was independent of dose rate, which suggests that rapidly repaired DNA lesions are not involved in the formation of exchanges (66).

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 monooxygenases, epoxide hydrolases, and glutathione-S-transferases which are involved in the activation and detoxification of polycyclic aromatic hydrocarbons and the N-acetyl-and acyl transferases which are involved in the metabolism of aromatic amines. Also included are studies on the role of the polyamine synthetic enzymes, primarily ornithine decarboxylase, in UV or chemically induced carcinogenesis.

In many of the studies involving polycyclic aromatic hydrocarbon metabolizing enzymes, the multiple forms of cytochromes P-450, epoxide hydrolases, and cytochrome P-450 reductases are purified before their use. In other cases the organization of the enzymes in the microsomal membrane is being studied to determine the role of protein-protein and protein-lipid interactions in the function of the monooxygenase system. In order to study the topography of proteins in the endoplasmic reticulum membrane of rat liver, a cleavable cross-linking reagent, dithiobis(succinimidyl propionate), was used. The cross-linking of a protein with a molecular weight of 52,000 to form an apparent dimer was observed in untreated, phenobarbital or 3-methylcholanthrene-induced microsomes. In phenobarbital and 3-methylcholanthrene-induced microsomes a 48,000 dalton protein was cross-linked to a protein of about

57,000 by the reagent. In all three types of microsomes, a 52,000 dalton protein was also cross-linked to a protein of about 79,000. An oligomeric protein of approximate molecular weight 180,000 which contained three cross-linked proteins, two of molecular weight 52,000 and one of 79,000, was found in phenobarbital and control microsomes. It was suggested that the 52,000 molecular weight protein may be cytochrome P-450, the 48,000 molecular weight protein epoxide hydrolase, and the 79,000 molecular weight protein NADPH- cytochrome P-450 reductase. The investigators speculate that these integral membrane proteins are closely located or may have some of their population existing in molecular complexes (155).

Other studies involving polycyclic aromatic hydrocarbon metabolizing enzymes are focused on the mechanism of induction of the aryl hydrocarbon hydroxylase enzyme system in liver and other cells. The cloning and characterization of the genes for the polycyclic aromatic hydrocarbon metabolizing enzyme system is being pursued in order to determine the number, function, and control of gene expression in this enzyme system. The cytochrome P-450 genome has been successfully cloned from rat liver DNA and studies using this probe are being conducted. In a different study somatic cell hybridization studies were conducted using mutants of the mouse hepatoma line, Hepa-1, with deficiencies in aryl hydrocarbon hydroxylase activity. The Ah locus which controls this activity is believed to be comprised of regulatory, structural, and probably temporal genes which may or may not be linked. Of the mutant clones isolated having either decreased or nondetectable amounts of aryl hydrocarbon hydroxylase activity, a small minority were shown to be dominant. Most of the mutants, however, were shown to be recessive. The recessive mutants were shown to fall into at least three different complementation groups indicating that the defects represent several distinctly different genes associated with the Ah locus. A cytosolic receptor which binds avidly to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is the major regulatory gene product of the Ah locus. The parent cell line and six mutant clones were examined for Ah receptor levels in the cytoplasm and for the accumulation of the inducer-receptor complex in the nucleus. Clones c1 and c5 which belong to one complementation group and c3, the dominant clone, were found to have essentially normal Ah receptor levels and to possess normal kinetics for the translocation of the inducer-receptor complex into the nucleus. However, these clones possess very low or nondetectable basal or inducible aryl hydrocarbon hydroxylase activity. A mutation in the P-450 structural gene or other genes responsible for the induced hydroxylase activity is suspected in these clones. Clones c2 and c6, which belong to a second complementation group, were found to be receptor-deficient mutants. These cells have no more than 10% of wild-type Ah receptor levels, normal kinetics of nuclear translocation of inducer-receptor complex, and no more than 20% of wild type inducibility of hydroxylase activity by either TCDD or benz(a)anthracene. Clone c4, which belongs to a third complementation group, was found to have normal cytosolic levels of Ah receptor, to be defective in the nuclear translocation of the inducer-receptor complex, and to lack any basal or inducible aryl hydrocarbon hydroxylase activity. The regulation of aryl hydrocarbon inducibility has been known to be complex. In the mouse, a minimum of six distinct alleles and two nonlinked genetic loci is required to explain the regulatory component of the Ah locus. The ease with which the benzo(a)pyrene-resistant clones were developed from the Hepa-1 cell line and the heterogeneity of the mutants thus far characterized demonstrate the potential usefulness of the established cell culture lines for studies on the mechanism of induction of aryl hydrocarbon hydroxylase activity (57).

In another set of studies, the glutathione S-transferases, which play a major role in the detoxification and excretion of xenobiotics, were isolated and characterized. Rat liver microsomal glutathione S-transferase was isolated and purified

to near homogeneity. It was shown to be a protein of apparent molecular weight 14,000. There are at least seven forms of cytoplasmic glutathione S-transferase. Three of the major forms (A, B, and C) have been shown to be basic proteins with a molecular weight of approximately 48,000. Immunochemical studies have shown that the microsomal glutathione S-transferase is different from the three cytoplasmic forms of the enzyme. The effect of adding glutathione and/or purified glutathione S-transferases on benzo(a)pyrene metabolism by rat liver nuclei or microsomes has been examined. The level of conjugate formation was found to increase. Analysis of metabolites by high pressure liquid chromatography showed that benzo(a)pyrene 4,5-diol was decreased by at least 80% and the 7,8-diol by 40 to 60% by conjugation. Also, the binding to DNA of the reactive metabolites of trans-7,8-dihydroxy-7,8-dihydrobenzo(a)pyrene was found to be inhibited by the addition of glutathione or glutathione S-transferases to either rat liver nuclei or microsomes. Thus, glutathione and glutathione S-transferase levels may play an important role in preventing the formation of reactive metabolites and in protecting DNA from attack by reactive metabolites of polycyclic aromatic hydrocarbons. (33).

Aromatic amines have been shown to be metabolically activated by at least two different pathways. Cytosolic enzymes can activate these compounds by conjugation with sulfate to form sulfate conjugates of hydroxamic acid derivatives. This pathway appears to be limited to rat liver and is not responsible for the activation of these compounds in extrahepatic tissues. Alternatively, the metabolic activation of arylhydroxamic acid was shown to involve the formation of reactive N-acyloxy-arylamines by N,O-acyltransfer. For aromatic amides the primary activation step is believed to involve N-hydroxylation to form arylhydroxamic acids. In rat mammary gland it has been demonstrated that the microsomes do not N-hydroxylate aromatic amides and the tissue does not form sulfate esters of arylhydroxamic acids. The soluble activating enzyme arylhydroxamic acid N,O-acyltransferase has been shown to be present in mammary tissue. The activaton of N-acyl derivatives of N-hydroxy-2-aminofluorene was previously shown to be relatively specific for the N-acyl moiety. In order to better define the relationship between metabolic activation by mammary gland N,O-acyltransferase and mammary gland tumor formation, the N-formyl, N-acetyl, and N-propionyl derivatives of N-hydroxy-2-aminofluorene were examined for both mammary gland tumorigenicity and for metabolic activation using mammary gland preparations. Twelve months after being applied locally to the mammary glands, the mammary tumor incidence observed was 53% for the N-acetyl, 41% for the N-formyl, and 33% for the N-propionyl derivatives of N-hydroxy-2-aminofluorene. N-acetyl-2-aminofluorene gave only an 8% mammary tumor incidence. A nucleic acid binding assay was used to demonstrate N,O-acyltransferase activity. Mammary gland cytosol catalyzed tRNA adduct formation to a greater extent with the N-formyl derivative. Fractionation with ammonium sulfate or separation by gel filtration chromatography demonstrated the presence of two enzymes, one specific for the N-formyl derivative and the other specific for the N-acetyl and N-propionyl derivatives. Mammary gland microsomes also catalyzed the formation of tRNA adducts, but only with the N-formyl derivative. The mutagenic activity of the N-hydroxy-2-aminofluorene derivatives in Salmonella typhimurium TA-1538 with either mammary gland cytosol or microsomes was demonstrated to be in the order N-formyl >> N-acetyl > N-propionyl. The data obtained demonstrate the presence of two distinct enzymes in rat mammary gland that activate arylhydroxamic acids. It also demonstrates that the ability of rat mammary gland to catalyze the formation of nucleic acid adducts from arylhydroxamic acids in vitro does not correlate with the tumorigenic response. Future studies are in progress to determine the role of these metabolic pathways in mammary gland tumorigenesis (89).

Development of Analytical Methodology for Detecting Chemical Carcinogens in Body Fluids and Environmental Samples: Technological or methodological breakthroughs in a field frequently herald a new advent in our understanding at the molecular or mechanistic level. A small number of grants are supported in this Branch which have taken an approach based on improvement of our analytical technology. In an effort to develop rapid, dependable, economical detection of N-nitroso compounds (60) the chromatographic and electrochemical conditions were established for the determination of all major classes of N-nitrosamines using HPLC with electrochemical detection (HPLC-EC). The mobile phases for each class were found and the appropriate electroanalytical parameters were established. It was found that using the differential pulse mode with the polarographic detector (EG & G Parc model 310) a detection limit of less than 1.0 ng was possible. Ten different nitrosamines representing four classes were studied. A linear response covering several orders of magnitude was found.

A procedure using HPLC-EC for the determination of N-nitrosodiethanolamine (NDELA) in cosmetic products was developed. An extraction procedure based upon the insolubility of NDELA in chloroform coupled with the use of Sep-Pak cartridges (Waters Assoc.) was developed for the isolation of NDELA from various hand creams, lotions and shampoos. Using the experimental conditions established above NDELA was determined in the range of 0.5 ppm to 2.0 ppm with recoveries of approximately 95-98%. Lower recoveries were found for the shampoo.

The chloroform extraction procedure was used, followed by photolysis of the aqueous extract with UV light to develop a rapid screening system for NDELA. Photolysis produces nitrite and nitrate from the NDELA. To this solution was added N,N-dimethylaniline and the pH adjusted to 1.0. This solution was heated in a water bath. If NDELA was present in the original product, a bright yellow color developed from the formation of p-nitroso-N,N-dimethylaniline. For low levels, the color was intensified by adding a few drops of ether and thus concentrating the yellow compound in the ether layer. Creams, lotions and shampoos were run. Some were obtained locally and spiked with NDELA and five were obtained from FDA and known to contain NDELA. The procedure screens samples down to 500 ppb, is rapid and quite simple. Large numbers of samples can be run in a short time. The very nature of the products themselves precludes many false positives.

Work previously reported for the determination of nitrite by differential pulse polarography was successfully extended to include nitrate in model solutions, human saliva and processed meats. The detection limit reported for nitrite was 0.3 ppb. The limit found for nitrate was slightly higher, about 20 ppb as nitrate (or 5 ppb as nitrate-nitrogen).

A comparative study using gas chromatography-thermal energy analysis (GC-TEA) and HPLC-EC for the determination of nitrosopyrrolidine in fried bacon was initiated and completed. Sample preparation used the method developed by Fazio, et al. Samples of nitrite-free bacon spiked to desired levels and nitrite-processed bacon were run. Samples were obtained from the U.S. Department of Agriculture. This work was done via a collaborative arrangement with Dr. Walter Fiddler of the U.S. Department of Agriculture, Eastern Regional Laboratory. The comparison between the GC-TEA results and those for HPLC-EC is quite good and demonstrates that HPLC-EC is a viable alternative for bacon analysis. This work was presented at the Banbury Conference, April 1982, and will be published in the proceedings of that conference.

On another front, investigators have been developing instrumentation based on physical principles that were discovered over 100 years ago by Alexander Graham Bell in order to detect very small quantities of potent carcinogens in environmental mixtures (30). Through the use of lasers and what is termed an optoacoustic effect produced when a gas is excited by modulated radiation, the investigators hope to construct instrumentation that is capable of detecting picogram quantities of dioxins and polychlorinated biphenyls.

During the last year, research has focused on three areas. First, a very high sensitivity laser schlieren microphone was built and tested for suitability as a detector for trace quantities of gases. The device is built as a double Helmholtz resonator. The infrared laser is directed into one chamber of the resonator where the eluant from a gas chromatograph is sent. The optoacoustic effect taking place in this chamber gives a pressure rise in the second chamber of the resonator. On this chamber is mounted a flexible diaphragm that responds to a periodic pressure rise by deforming to an approximately parabolic shape. The diaphragm acts as a lens whose focal length changes in time as $(A(\omega/c)^2\cos\omega t)^{-1}$ where A is an amplitude factor dependent on magnitude of the pressure signal, ω is the modulation frequency and c is the sound velocity in the diaphragm. A low power He-Ne laser reflected off the diaphragm undergoes a periodic focusing and enlargement that can be converted into an intensity modulation of the He-Ne beam with a knife edge. This modulation is detected after being frequency up-converted by chopping the He-Ne beam at a high (50 kHz) frequency. Two lock-in amplifiers are used to detect the modulation.

Initial experiments have shown that this device is useful for optoacoustic detection of trace quantities of gases. Experiments using the non-toxic gas SF_6, which should have characteristics similar to PCB's or dioxin, have shown a detection limit of 10^{-6} mole fraction of SF_6 in N_2. For practical reasons this approach is not being pursued further at present. That is, the construction of such a detector requires ultra stable mounting of all components; the apparatus for proper construction of such detector is not available in the grantee laboratory.

The second area of research has been chemical amplification of the optoacoustic signal. It is well known that chain reactions are capable of liberating great amounts of heat per radical generated since chain reactions propagate many steps before termination takes place. This principle has been found to give a chemical amlification of optoacoustic signals in H_2-$C\ell_2$ mixtures where $C\ell$ is generated by dissociation of $C\ell_2$. An optoacoustic signal gain of 3600 was found in a 20% H_2 - 70% $C\ell_2$ - 10% N_2 mixture compared with a 85% $C\ell_2$ - 15% N_2 mixture. This rather spectacular gain can be expected to be even greater depending upon the temperature of the sample and the nature of the reactants. The phenomenon is general in that it can be generated in a large number of chemical systems. It remains to explore this possibility for trace detection.

The most promising lead for high sensitivity detection of carcinogens comes from a small volume differential Helmholtz resonator. This device has a small chamber (where the high power laser irradiates the gas eluant) which is connected to a second part of the resonator that houses an electret microphone. The latter is water cooled while the part of the resonator into which the eluant is flowed can be heated. Extensive experimentation with this detection cell has shown that as little as 10 pg of SF_6 can be detected eluting from a gas chromatograph. The results from this work suggest that the most fruitful path for detection of trace quantities of TCDD or PCB's lies with this device.

Role of DNA Repair in Carcinogenesis: There are currently 34 research grants supported in this subject area with objectives focused on an understanding of the DNA repair process and the consequences of faulty repair which may lead to carcinogenesis. The research substrate material ranges from plasmids and viruses through prokaryotes and eukaryotes up to man. The agents under study include all types of chemical carcinogens and mutagens, ultraviolet light, and ionizing radiation, to the synthesis of model polynucleotides with specific lesions already in place. Highlights of the significant advances from some of the research supported under this topic follow:

The relative roles and interaction of excision and postreplication repair in predicting the sensitivity of cells to killing is being assessed by comparing reactions in cell populations whose rate and synchrony of cycling and the extent of both types of DNA repair are controlled (51). Excision repair (ER)-/postreplication repair (PRR)-proficient and ER proficient-/PRR-deficient (reversible) C3H 10T1/2 cells are utilized for studying the mechanisms of chemically induced cytotoxicity. Ample experimental data suggest that the chemical induction of mutagenesis and neoplastic transformation are correlated with the presence, and persistence, of chemical lesions in DNA. The mechanism(s) of chemically induced cytotoxicity, however, are not well understood and are not clearly correlated with damage to DNA.

From previous work on this project, it was found that the occurrence of cytotoxicity after alkylating the DNA of cells with N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) is maximal during S phase, a period when excision repair of methyl adducts fails to occur.

Cellular inactivation (toxicity) induced by MNNG increases linearly in synchronized populations of 10T1/2 cells as the time of exposure approaches the G_1/S border, suggesting that DNA excision repair reduces the cytotoxic potential of an initial level of DNA damage. The presence of MNNG-DNA adducts in the DNA template perturbs DNA replication in the ensuing S phase. The onset of DNA replication is delayed, the duration of the S phase is prolonged, and a caffeine sensitive mechanism for the completion of DNA replication is utilized to minimize cytotoxicity. The cytotoxic potential of MNNG appears to be mediated by a DNA replication-dependent mechanism. How general is this observation? The cytotoxic potential of a diverse group of chemicals has been evaluated following exposure of synchronized 10T1/2 cells in early G_1 phase. 4-Nitroquinoline-1-oxide (4-NQO), benzo(a)pyrene diol epoxide (BPDE), acetoxy-acetylaminofluorine (AcAAF), N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG), and methyl methane sulfonate (MMS) have been tested. The relative cytotoxic potential of these chemicals was found to be 4-NQO > BPDE > AcAAF > MNNG > ENNG > MMS. The range of LD_{50} exposure levels on a molar basis was 9×10^{-8}M (4-NQO) to 8×10^{-2}M (MMS).

The positive determination that a misreplicative event can result in cytotoxicity is difficult because of the uncertainty as to the nature of the DNA target(s). The chemical induction of specific locus mutagenesis is clearly associated with a DNA replication-dependent mechanism. Consequently, it was determined that equi-cytotoxic exposures of this group of chemicals were also equi-mutagenic, thereby indirectly implicating a DNA replication-dependent mechanism in the chemical induction of cytotoxicity. The induced mutation frequency at the Na/K ATPase locus in the 10T1/2 cells for MNNG, 4-NQO, BPDE, AcAAF, and ENNG was approximately 1.0×10^{-4} mutants/survivor. Only MMS diverged from the equi-cytotoxic = equi-mutagenic pattern, with an induced mutation frequency of 1.5×10^{-6} mutants/-survivor. This rate is only slightly greater than the spontaneous mutation rate of

0.8×10^{-6} mutants/survivor. The divergence of MMS from the pattern observed for the other chemicals led to studies which demonstrated that the cytotoxicity induced by MMS is the result of overt cell death due to membrane damage and is not due to a DNA replication-dependent mechanism, as is the situation for MNNG. These preliminary studies strongly suggest that cytotoxicity for the chemicals other than MMS is mediated by a DNA replication-dependent mechanism, possibly produced by the promutagenic adduct(s) and at equi-cytotoxic/equi-mutagenic exposures for this variety of chemicals, equivalent levels of some critical adduct(s) should be demonstrable in the DNA.

The methyl transferase responsible for the removal of O^6-alkylguanine in DNA continued to generate interesting research results this year particularly in the context of nitrosamine-induced carcinogenesis.

O^6-Methylguanine-DNA methyltransferase activity has been quantitatively assayed in a number of mammalian cell lines (112). These studies have shown that cell lines characterized as methylation-repair deficient (Mer^-) lack constitutive methyltransferase activity, whereas Mer^+ cell lines contain ~20,000-200,000 molecules of methyltransferase per cell. These conclusions differ from those of Waldstein et al. (Proc. Natl. Acad. Sci., 79: 5117-5121, 1982) who suggest that Mer^+ and Mer^- cells contain comparable constitutive levels of the protein but that Mer^- cells are deficient in the ability to resynthesize the methyltransferase after the constitutive activity has been exhausted.

The question of inducibility of O^6-methylguanine has been investigated by assaying extracts of mammalian cells which were treated with N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) according to protocols reported to produce increased O^6-methylguanine repair capacity or increased resistance to killing and sister chromatid exchange by the alkylating agent. Cells were treated with multiple doses of MNNG over a wide range of concentrations (0.5-50 ng/ml), and cell extracts were prepared at various times following the last dose and assayed for methyltransferase activity. No significant increase of methyltransferase activity was found in the Mer^+ human tumor cell line HeLa CCL2 or in normal rat kidney (NRK) cells by any of the protocols tested; significant decreases of activity occurred at the higher concentrations of MNNG tested for up to 24 hours following treatment. No detectable induction of methyltransferase occurred in a Mer^- HeLa S3 cell line or in Chinese hamster ovary (CHO) cells. These studies suggest that mammalian cells, unlike bacteria, cannot be induced by MNNG to respond to DNA methylation damage by increasing methyltransferase levels. However, the constitutive levels of methyltransferase (molecules/cell) present in Mer^+ mammalian cells are ~10-100 times greater than the induced levels in bacteria.

It has been known for some time that hamsters are significantly more susceptible to induction of liver tumors than rats after single dose administration of dimethylnitrosamine. It has been shown that the rate of removal of O^6-methylguanine from hamster liver DNA is much slower than that from rat liver DNA. However, the methyltransferase had not been quantitated in hamster tissues. In efforts to map the methyltransferase gene, the enzyme level in a number of hamster cell lines was surveyed and the surprising observation was made that all the permanent cell lines derived from either Chinese hamster (CHO, V79), or Syrian golden hamster (BHK 21) lack the methyltransferase. The primary culture of Syrian hamster embryos has a low level of activity (~3500 enzyme molecules/cell). The methyltransferase activity in different organs of rats and hamsters is currently being compared. Several laboratories have been pursuing the purification of O^6-methylguanine transferase.

One objective of the work under (17) has been to purify the enzyme 7,000 fold from rat liver by the use of phosphocellulose, heparin agarose, DNA cellulose, gel filtration and glycerol gradient centrifugation. This project is near conclusion. When sufficient protein is produced by purification it will be submitted to amino acid analysis. The enzyme is estimated to have a molecular weight of about 20,000.

Poly(ADP-ribose) appears to play a role in DNA repair metabolism. A study of NAD and poly(ADP-ribose) metabolism in normal human fibroblasts following UV irradiation has provided the first direct demonstration that UV treatment of cells results in a rapid increase in the intracellular content of poly(ADP-ribose) (75). Exposure of human fibroblasts to 5 J/m^2 of UV light resulted in a rapid increase of up to 1500% in the intracellular content of poly(ADP-ribose) and a rapid depletion of its metabolic precursor, NAD. When added just prior to UV treatment, the poly(ADP-ribose) polymerase inhibitor, 3-aminobenzamide totally blocked both the increase of poly-(ADP-ribose) and decrease in NAD for up to 2.5 hrs. Addition of 3-aminobenzamide at the time of maximal accumulation of poly(ADP-ribose) resulted in a decrease to basal levels with a half life of aproximately six minutes. This represents a maximum estimate of half life since it cannot be determined how rapidly 3-aminobenzamide blocks synthesis. Between 1 and 5 hrs following UV irradiation, the intracellular content of poly(ADP-ribose) remains at 150 to 200 $pmol/10^8$ cells during which time the NAD pool is decreasing at a rate of approximately 200 $pmol/min/10^8$ cells. Thus, assuming that all the NAD is being converted to poly(ADP-ribose), the half life of poly(ADP-ribose) would actually be less than one min.

By way of contrast to normal cells, fibroblasts homozygous for the xeroderma pigmentosum (XP) genotype do not deplete their NAD pool following UV irradiation. This is consistent with a mechanism in which the rate of conversion of NAD to poly(ADP-ribose) is regulated by the number of DNA strand breaks, since it is well known that XP homozygotes are very deficient in the introduction of endonucleolytic incisions into DNA following UV treatment. These investigators have observed that the rates of accumulation of poly(ADP-ribose) and depletion of NAD were increased in normal cells following UV exposure in the presence of either 1-β-arabinofuranosylcytosine or hydroxyurea. Since these agents are known to cause additional accumulation of DNA strand breaks following UV irradiation, these data provide additional evidence for a mechanism in which the rate of poly(ADP-ribose) synthesis is regulated in intact cells by the number of DNA strand breaks.

These investigators (75) have developed analytical methodology that permits the measurement of the intracellular levels of protein bound mono(ADP-ribose) derived from intact cells and tissues. This methodology involves the chemical release of ADP-ribose intact from the protein and its selective isolation from crude extracts by adsorption to a boronate affinity resin. The ADP-ribose is then quantitatively converted by a fluorescent, $1,N^6$-etheno derivative, separated from interfering substances by high pressure liquid chromatography on an anion exchange column, and quantified by fluorescence monitoring. An initial application of the method has been to measure the levels of protein-bound monomers of ADP-ribose in C3H 10T1/2 cells following treatment with MNNG. It was observed that such treatment results in an increase of up to 5 fold in the levels of protein-bound mono(ADP-ribose).

Other investigators (140) have prepared and characterized an immuno-affinity column using antibody specifically directed against poly(ADP-ribose). This column has been successful in the isolation of those limited domains of chromatin (about 10% of the total genome) undergoing polyADP-ribosylation, from the bulk of unmodified nucleosomes. These ADP-ribosylated nucleosomes contain significant numbers of internal

DNA strand breaks compared to unbound chromatin. This is again consistent with a role for poly(ADP-ribose) in the repair of DNA whether due to alkylating agents or resulting from exposure to UV light.

A number of investigators have been successfully applying modern DNA technology in order to molecularly clone and characterize genes involved in DNA repair. Working with a simple eukaryote, the yeast S. cerevisiae, one group (43) has been cloning the genes required for the excision repair of DNA containing base damage. The genes of interest are RAD1, RAD2, RAD3, RAD4, and RAD10, all of which have been previously shown to be required for the specific incision of DNA during excision repair. Plasmids have been isolated that contain yeast DNA inserts that complement mutants defective at all 5 RAD genes listed above. These plasmids are designated pNF1000, pNF2000, pNF3000, pNF4000 and pNF100.

Plasmids pNF1000, pNF2000 and pNF3000 have been shown to contain the yeast chromosomal RAD1, RAD2 and RAD3 genes respectively. This conclusion comes from both physical (DNA hybridization) and genetic studies. The latter employed integration of the cloned DNA into the yeast chromosome by homologous recombination and subsequent tetrad analysis of the diploids generated by mating integrants to RAD^+ or rad^- strains as appropriate. The subcloning of these 3 genes is in progress, using a novel vector constructed in this laboratory. This vector (pNF2) facilitates cloning of Sau3A fragments into a unique BamHI site and their subsequent recovery if desired, from two flanking SalI sites engineered into the vector. This vector should be generally useful to a number of investigators working with cloned yeast genes.

Further studies on the RAD3 gene of S. cerevisiae have shown that this is an essential gene--a surprising and interesting observation, since we know of no excision repair gene in any other biological system that is essential for the viability of cells in the absence of DNA damage.

A plasmid designated as pNF4000 partially complements the UV sensitivity of a single rad4 mutant (rad4-3) but not of two other allelic mutants at the RAD4 locus (rad4-2 and rad4-4). They have shown that pNF4000 contains a yeast transposon, the restriction map which is very similar to that of the transposon Tyl. Deletion of a 6.0 kb fragment containing the Ty element results in loss of the UV resistance determinant.

During the past year they have also isolated a plasmid (pNF100) that complements rad10 mutants to UV resistance and likely contains the yeast RAD10 gene.

Once the subcloning of all of these RAD genes is completed they will begin tailoring the 5' noncoding regions of these genes in order to place them under the regulatory control of strong exogenous yeast and/or E. coli gene promoters, with a view to large-scale expression of the genes and isolation of their products.

Another group (62) is working towards the goal of isolating the gene in normal human cells that can complement the defect in the fibroblasts derived from patients who suffer from the clinical syndrome of xeroderma pigmentosum of the complementation group A (XPA). Progress over the last year in this area has been rapid. The group has successfully devised a selection system which is capable of selecting one in 10^7 UV-resistant cells from the total population of UV-sensitive cells.

UV resistance has been transferred to an XPA line of fibroblasts. In initial experiments it was noticed that the frequency of transfection using total DNA from

normal cells was too low to obtain UV-resistant colonies. Two strategies were used to overcome this barrier. The first involved use of TPA, a tumor promoter, to enhance the survival of transfected cells. Using TPA, they were able to obtain a number of clones that were UV-resistant and capable of unscheduled DNA synthesis using normal DNA as a donor DNA. The second strategy involved the use of the pSV_2 SV40 vector ligated to normal DNA. Using this vector-linked DNA as a transfecting source, they were able to obtain UV-resistant, unscheduled-synthesis positive, colonies from transfection assays.

In the above experiments, it was possible to use a restriction enzyme to cleave normal cell DNA. The normal DNA cleaved with this restriction enzyme retained its activity and was linked to the pSV_2 vector. This accomplished two goals. First, it gave a frequency of transformants that was within a workable range and will also allow the use of linked SV40 DNA to isolate flanking sequences which, it is suspected, confer the UV-resistance phenotype. Over the next year, work will continue on the isolation of this gene by obtaining secondary transformants of the DNA-SV40 linked transformant and by obtaining molecular clones of these DNA regions and their flanking sequences.

Another approach to complementation of the XPA phenotype has also been developed by this group (62). They have constructed a vector which contains the uvr a, b and c genes of E. coli capable of being expressed both in bacteria and in eukaryotic cells.

The uvr genes a, b and c were placed between the initiation and termination transcription signals of SV40. Each of the three genes was placed between such signals and the entire vector ligated into the pSV_2 gpt vector described by Mulligan and Bera. This vector is capable of complementation of uvr a, b and c loci of E. coli. In preliminary experiments some cells have been obtained which exhibit UV-resistant phenotypes after transfection with this vector.

Contracts Activity Summary

The contract mechanism for support of research in the Molecular Carcinogenesis program area has been rapidly phased out. Only one contract was active in FY83. This contract to DOE/Argonne National Laboratory (158) was active due to a four month no cost extension which was granted to allow the completion of a series of experiments to study the induction of different markers of differentiation in human malignant cells by tumor promoting agents and to study the alterations in lipid biosynthesis in differentiating human tumor cells. These experiments were delayed as a result of the transfer of this project from DOE/Oak Ridge National Laboratory. Using monoclonal antibodies specific for differentiated T lymphocytes, it was shown that the tumor promoter, TPA, caused human T lymphoid (CEM) leukemia cells to differentiate into mature suppressor and cytotoxic T lymphocytes. Functional tests showed that these TPA-treated cells were suppressor T lymphocytes. TPA treatment of human promyelocytic HL-60 leukemia cells, which results in the induction of macrophage-like cells, stimulates phospholipid methylation. Phospholipid methylation was also stimulated after treatment with phorbol-12,13-dibutyrate and teleocidin but not with phorbol-12,13-diacetate and 4-O-methyl-TPA which are inactive as inducers of cell differentiation and as tumor promoters. The TPA-induced phospholipid methylation was not observed in an HL-60 cell variant that is resistant to phorbol ester-induced differentiation.

MOLECULAR CARCINOGENESIS

GRANTS ACTIVE DURING FY83

Investigator/Institution/Grant Number	Title
1. ACS, George Mount Sinai School of Medicine 5 R01 CA 16890-08	Studies on Chemotherapeutic Deoxyribonucleosides
2. ADAIR, Gerald M Univ of Texas System Can Ctr 5 R01 CA 28711-02	Expression of Genetic Variation in Cultured Cells
3. AVADHANI, Narayan G University of Pennsylvania 5 R01 CA 22762-06	Cellular and Molecular Targets of Chemical Carcinogenesis
4. BECKER, Frederick F Univ of Texas System Can Ctr 2 R01 CA 20657-07	Phenotypic Analysis of Chemical Carcinogenesis
5. BECKER, Frederick F Univ of Texas System Can Ctr 5 R01 CA 20659-07	Analysis of Cellular Events in Chemical Carcinogenesis
6. BECKER, Frederick F Univ of Texas System Can Ctr 5 R01 CA 28263-03	Chromosomal Proteins in Chemical Carcinogenesis
7. BERNHARD, William A University of Rochester 5 R01 CA 32546-08	Effects of Ionizing Radiation on Nucleic Acids
8. BERTRAM, John S Roswell Park Memorial Institute 5 R01 CA 18197-06	Mechanisms of Carcinogenesis in Cell Culture
9. BLOOM, Stephen E Cornell University (Ithaca) 5 R01 CA 28953-03	Chick Embryos for Detecting Environmental Mutagens
10. BLUMER, Jeffrey L Case Western Reserve University 5 R23 CA 30067-03	Lymphocyte Carcinogen Metabolism in Acute Leukemia
11. BOUCK, Noel P Northwestern University 2 R01 CA 27306-04	Genetic Analysis of Malignant Transformation
12. BOWDEN, George T University of Arizona 2 R01 CA 26972-04	Postreplication Repair in Cultured Mammalian Cells

13. BOX, Harold C
Roswell Park Memorial Institute
5 R01 CA 29425-03
Molecular Studies of Carcinogenesis and Mutagenesis

14. BOYNTON, Alton L
Nat'l Research Council of Canada
5 R01 CA 28340-03
Assays for, and Actions of, Carcinogens and Promoters

15. BRANSCOMB, Elbert W
Univ of California (Berkeley)
1 R01 CA 31714-01
Monitoring In Vivo Somatic Mutations In Animals and Man

16. BRESNICK, Edward
University of Vermont and State Agriculture College
2 R01 CA 20711-07
Polycylic Hydrocarbon Metabolism and Carcinogenesis

17. BRESNICK, Edward
University of Vermont and State Agriculture College
5 R01 CA 23514-06
DNA Repair After Polycyclic Hydrocarbon Administration

18. BROOKES, Peter
University of London
5 R01 CA 25807-03
Biological Results of Carcinogen Induced Damage to DNA

19. BROYDE, Suse B
New York University
5 R01 CA 28038-03
Carcinogen-DNA Adducts: Linkage Site and Conformation

20. CALOS, Michele P
Stanford University
1 R01 CA 33056-01
Mutation in Human Cells at the DNA Sequence Level

21. CHEN, Fu-Ming
Tennesse State University
5 R01 CA 29817-02
Binding of Benzo(a)pyrene Metabolites to DNA

22. CHEN, Lan B
Dana-Farber Cancer Institute
5 R01 CA 29793-02
Chemical Carcinogenesis of Epithelial Cells

23. CHETSANGA, Christopher J
Univ of Michigan (Ann Arbor)
1 R01 CA 33025-01
Excision Repair of Alkylated DNA

24. CHRISTMAN, Judith K
Mount Sinai School of Medicine
2 R01 CA 25985-04
Response of Phagocytic Leukocytes to Tumor Promoters

25. CLARKE, Richard H
Boston University
5 R01 CA 17922-06
Carcinogen-DNA Complexes: Structure and Interactions

26. CLARKSON, Judith M
Univ of Texas System Can Ctr
2 RO1 CA 19281-07
Cell-Cycle Related DNA Repair Mechanisms

27. COX, Ray
Univ of Tennessee Center Health Sciences
5 RO1 CA 15189-09
DNA Repair and Chemical Carcinogenesis

28. DAVIDSON, Richard L
Univ of Illinois Medical Center
2 RO1 CA 31781-03
Mechanisms of Chemical Mutagenesis in Mammalian Cells

29. DI MAYORCA, Giampiero
University of Medicine and Dentistry of New Jersey
5 RO1 CA 25013-05
Molecular Mechanism of Chemical Carcinogenesis

30. DIEBOLD, Gerald J
Brown University
5 RO1 CA 29912-02
Optoacoustic Detection of Carcinogens

31. DIXON, Kathleen
Univ of California (Los Angeles)
5 RO1 CA 28449-03
Probing DNA Repair With SV40 Virus and Mutant Cells

32. DUKER, Nahum
Temple University
2 RO1 CA 24103-04A1
Molecular Pathology of Carcinogenic DNA Damage

33. ERNSTER, Lars
University of Stockholm
2 RO1 CA 26261-04
The Metabolism of Polycyclic Hydrocarbons and Cancer

34. FAHL, William E
Northwestern University
5 RO1 CA 25189-05
Hydrocarbon Carcinogenesis in Mouse and Human Cells

35. FARBER, Emmanuel
University of Toronto
2 RO1 CA 21157-07
Pathogenesis of Liver Cancer Induced by Chemicals

36. FARBER, John L
Hahnemann Medical College and Hospital of Philadelphia
5 RO1 CA 32610-02
Hepatocarcinogenesis: A Role for Liver Necrosis

37. FELDBERG, Ross S
Tufts University
2 RO1 CA 19419-07A1
The Nature and Repair of a New Form of DNA Damage

38. FINE, David
New England Institute For Life Sciences
1 RO1 CA 34837-01
Analysis and Detection of Carcinogenic N-Nitrosamines

39. FINK, Gerald R
Massachusetts Institute of Tech
2 RO1 CA 34429-02
Chemical Carcinogens and Frameshift Mutation in Yeast

40. FRAENKEL-CONRAT, Beatrice
Univ of California (Berkeley)
5 RO1 CA 12316-13
Alkylation of Polynucleotides In Vitro and In Vivo

41. FRANKLIN, Michael R
University of Utah
5 RO1 CA 15760-09
Modification of Procarcinogen Enzymatic Activation

42. FREEDMAN, Herbert A
Downstate Medical Center
5 RO1 CA 29052-03
H-2 Locus and Local Tumorigenesis By Methylcholanthrene

43. FRIEDBERG, Errol C
Stanford University
5 RO1 CA 12428-13
DNA Repair and its Relationship to Carcinogenesis

44. GEACINTOV, Nicholas E
New York University
5 RO1 CA 20851-06
Characterization of Carcinogen-Nucleic Acid Complexes

45. GESSNER, Teresa
Roswell Park Memorial Institute
5 RO1 CA 24127-04
Conjugations and Carcinogen Metabolism

46. GOLD, Barry I
Univ of Nebraska Medical Ctr
5 RO1 CA 29088-02
Activation and Transportation of Nitrosamines

47. GOLDFARB, Stanley
Univ of Wisconsin (Madison)
5 RO1 CA 15664-08
Cholesterol Metabolism of Hepatic Neoplasms

48. GOLDTHWAIT, David A
Case Western Reserve University
5 RO1 CA 27528-03
Chemical Carcinogenesis and DNA Repair

49. GOODMAN, Jay I
Michigan State University
5 RO1 CA 30635-02
Genetic Toxicology--The Role of Non-Random Gene Damage

50. GREENBERGER, Joel S
Dana-Farber Cancer Institute
5 RO1 CA 25412-05
Stem Cell Age and X-Ray/Chemo-therapy Leukemogenesis

51. GRISHAM, Joe W
Univ of North Carolina Chapel Hill
2 RO1 CA 24144-04A1
Toxicity in DNA Repair Deficient and Proficient Cells

52. GRISHAM, Joe W
Univ of North Carolina Chapel Hill
5 RO1 CA 29323-03
Analysis of Tumor Progression in Liver Cells In Vitro

53. GRISHAM, Joe W
Univ of North Carolina Chapel Hill
5 R01 CA 32036-02
DNA Methyl Adducts: Toxicity, Mutation, & Transformation

54. GUDAS, Lorraine J
Dana-Farber Cancer Institute
2 R01 CA 27953-04
Genetics/DNA Precursor Metabolism, Mutagenesis, Repair

55. GUENTHNER, Thomas M
Univ of Illinois Medical Center
1 R01 CA 34455-01
Toxicologic Implications of Multiple Epoxide Hydrolases

56. GUPTA, Ramesh C
Baylor College of Medicine
5 R01 CA 30606-02
Reaction of Carcinogenic Aromatic Amines With DNA

57. HANKINSON, Oliver
Univ of California (Los Angeles)
5 R01 CA 28868-04
Carcinogen Activation and Screening in Variant Cells

58. HANNA, Patrick E
Univ of Minnesota (Mnpls-St Paul)
5 R01 CA 21659-03
Carcinogen Activation Via Acyl Transfer

59. HARD, Gordon C
Temple University
2 R01 CA 24216-04A1
Experimental Pathology of Renal Carcinogenesis

60. HARRINGTON, George W
Temple University
5 R01 CA 18618-08
Electroanalytical Studies of N-Nitrosamines

61. HASELTINE, William A
Dana-Farber Cancer Institute
2 R01 CA 26716-04
DNA Damage and Repair by Environmental Carcinogens

62. HASELTINE, William A
Dana-Farber Cancer Institute
5 R01 CA 29240-03
Complementation Group A Locus of Xeroderma Pigmentosum

63. HECHT, Stephen S
American Health Foundation
5 R01 CA 23901-05
Environmental Nitrosamines--Metabolism and Carcinogenesis

64. HENDERSON, Earl E
Temple University
5 R01 CA 23999-03
Characterization of Unique Lymphoblastoid Cell Lines

65. HERRIOTT, Roger M
Johns Hopkins University
5 R01 CA 25167-03
Pyrolytic Products of Proteinous Foods As Mutagens

66. HITTELMAN, Walter N
Univ of Texas System Cancer Ctr
2 R01 CA 27931-04
Molecular Basis of Chromosome Aberrations

67. HNILICA, Lubomir S
Vanderbilt Univeristy
5 RO1 CA 26412-05
Experimental Hepatocarcinogenesis

68. HOLLENBERG, Paul F
Northwestern University
5 RO1 CA 16954-07
Hemoprotein-Catalyzed Oxygenations of Carcinogens

69. HOWARD-FLANDERS, Paul
Yale University
2 RO1 CA 26763-04
Enzymatic Repair of Damaged DNA

70. HUMAYUN, M Zafri
University of Medicine and Dentistry of New Jersey
2 RO1 CA 27735-04
Mutagenesis by Carcinogens: A Molecular Approach

71. HYLEMON, Phillip B
Virginia Commonwealth University
5 RO1 CA 17747-09
Bile Acids and Large Bowel Carcinogenesis

72. ISSENBERG, Phillip
Univ of Nebraska Medical Ctr
1 RO1 CA 29197-01A2
Environmental Occurrence of Some Hydroxy Nitrosamines

73. JACOBS, Lois J
Univ of Wisconsin (Madison)
5 RO1 CA 30450-02
Quantitative Mutagenesis Studies in Human Fibroblasts

74. JACOBSON, Myron K
North Texas State University
5 RO1 CA 23994-06
Alteration of NAD Metabolism by Chemical Carcinogens

75. JACOBSON, Myron K
North Texas State University
5 RO1 CA 29357-03
Poly(ADP-Ribose) Metabolism in Xeroderma Pigmentosum

76. JEFCOATE, Colin R
Univ of Wisconsin (Madison)
5 RO1 CA 16265-09
DNA Modification by Polycylic Hydrocarbons

77. JENSEN, David E
Temple University
5 RO1 CA 31503-02
Nitrosocimetidine--DNA Methylation and Cellular Response

78. JENSEN, Ronald H
Univ of California (Berkeley)
1 RO1 CA 31549-01
Detection of Somatic Cell Mutations in Humans

79. JONES, Peter A
Children's Hospital of Los Angeles
1 RO1 CA 33592-01
5 Azacytidine Induced Differentiation

80. KALLENBACH, Neville R
University of Pennsylvania
5 R01 CA 24101-05
Specificity in Frameshift Mutagenesis

81. KALLENBACH, Neville R
University of Pennsylvania
5 R01 CA 31027-02
Frameshift Mutagenesis by Covalently Reacting Mutagens

82. KAN, Lou-Sing
Johns Hopkins University
2 R01 CA 27111-04
Model Alkylated Decanucleotide DNA Helices

83. KAUFFMAN, Shirley L
Downstate Medical Center
5 R01 CA 17569-08
Lung Preneoplastic Hyperplasia and Chemical Carcinogens

84. KAUFMAN, David G
Univ of North Carolina Chapel Hill
5 R01 CA 20658-06
Chemical Carcinogenesis and Cell Proliferation

85. KAUFMAN, David G
Univ of North Carolina Chapel Hill
1 R01 CA 32238-01
Factors Influencing Initiation of Hepatocarcinogenesis

86. KENNEDY, Ann R
Harvard University
2 R01 CA 22704-05
Radiation and Chemical In Vitro Malignant Transformation

87. KERR, Sylvia J
University of Colorado Health Science Center
5 R01 CA 12742-11
Study of Methylations in Neoplasia

88. KIMBALL, Paul C
Battelle Memorial Institute
5 R01 CA 33554-02
Chemical Cocarcinogenesis in the Rat: Gene Activation

89. KING, Charles M
Michigan Cancer Foundation
5 R01 CA 23386-06
Mechanistic Approaches to Carcinogenesis

90. KOESTNER, Adalbert
Michigan State University
5 R01 CA 32594-02
Neurooncogenesis by Resorptive Carcinogens

91. KOHEN, Elli
Papanicolaou Cancer Res Inst
5 R01 CA 21153-05
Intracellular Enzyme Kinetics and Carcinogenesis

92. KULESZ-MARTIN, Molly
Roswell Park Memorial Institute
5 R01 CA 31101-02
Quantitative Carcinogenesis in Cultured Epithelial Cells

93. LAISHES, Brian A
Univ of Wisconsin (Madison)
5 RO1 CA 24818-05
Proliferation Control During Hepatocarcinogenesis

94. LAPEYRE, Jean-Numa
Univ of Texas System Cancer Ctr
5 RO1 CA 31487-02
Regulation and Enzymology of DNA Methylase in Cancer

95. LARCOM, Lyndon L
Clemson University
5 RO1 CA 21479-06
Biological Effects of DNA-Protein Crosslinks

96. LIEBERMAN, Michael W
Washington University
5 RO1 CA 20513-07
Chemical Carcinogen-Induced DNA Repair in Human Cells

97. LIEBERMAN, Michael W
Washington University
5 RO1 CA 31734-02
Methylation of DNA During Repair of Carcinogen Damage

98. LIEHR, Joachim G
University of Texas Health Science Center (Houston)
5 RO1 CA 27539-03
Mechanism of Estrogen-Induced Renal Carcinogenesis

99. LINDAHL, Ronald G
Univ of Alabama (University)
2 RO1 CA 21103-04
Gene-Enzyme Relationship of Liver Aldehyde Dehydrogenase

100. LIPSKY, Michael M
Univ of Maryland (Baltimore)
5 RO1 CA 28951-03
Multi-Stage Renal Carcinogenesis in Rats

101. LOEB, Lawrence A
University of Washington
5 RO1 CA 24998-05
Genetic Miscoding by Metals

102. LOMBARDI, Benito
University of Pittsburgh
5 RO1 CA 23449-06
Choline Deficiency, Oval Cells, and Hepatocarcinogenesis

103. LOTLIKAR, Prabhakar D
Temple University
5 RO1 CA 31641-02
Modulation of Mycotoxin Carcinogenesis by Glutathione

104. LOWE, Nicholas J
Univ of California (Los Angeles)
5 RO1 CA 25970-03
UV Light, Epidermal Polyamine, and DNA Synthesis

105. MACMANUS, John P
Nat'l Research Council of Canada
1 RO1 CA 31898-01
Incidence and Quantitation of a Tumor Protein

106. MAHER, Veronica M
Michigan State University
5 R01 CA 21253-06
Interaction of Carcinogens with DNA--Repair of Lesions

107. MARCHOK, Ann C
Oak Ridge National Laboratory
5 R01 CA 30529-02
Prenoplastic Markers In Specific Lesion Cells

108. MCCORMICK, J Justin
Michigan State University
5 R01 CA 21289-05
In Vitro Transformation of Human Cells by Carcinogens

109. MEEHAN, Thomas D
Michigan Molecular Institute
5 R01 CA 25106-03
Specificity in BAP Diol Epoxide Covalent Binding to DNA

110. MEEHAN, Thomas D
Michigan Molecular Institute
1 R01 CA 31705-01
Physical Interactions of BAP Diol Epoxides with DNA

111. MILO, George E
Ohio State University
5 R01 CA 25907-03
Chemical Carcinogen Induced Neoplastic Transformation

112. MITRA, Sankar
Oak Ridge National Laboratory
1 R01 CA 31721-01
DNA Repair and Nitrosamine-Induced Carcinogenesis

113. MULLINS, Dail W Jr
Univ of Alabama (Birmingham)
5 R01 CA 30547-02
The Role of Poly (ADP-Ribose) Polymerase in DNA Repair

114. NAKANISHI, Koji
Columbia University
5 R01 CA 11572-14
Structural and Bioorganic Studies of Bioactive Compounds

115. OLIVE, Peggy L
Johns Hopkins University
5 R01 CA 28793-03
Mutagenicity and DNA Damage Using Spheroids

116. OLSON, Jack W
University of Kentucky
5 R01 CA 31099-02
Hepatocarcinogenesis and Ornithine Decarboxylase

117. OLSON, Wilma K
Rutgers The State University of New Brunswick
5 R01 CA 25981-03
Carcinogenesis by Hydrocarbons: A Molecular Approach

118. PALL, Martin L
Washington State University
1 R01 CA 33503-01
Tandem Gene Duplication and Carcinogen Screening

119. PEGG, Anthony E
Pennsylvania State University
Hershey Medical Center
5 R01 CA 18137-08
Persistence of Alkylated DNA in Carcinogenesis

120. PIETTE, Lawrence H
University of Hawaii (Manoa)
5 R01 CA 10977-18
ESR Studies of Biological Free Radical Mechanisms

121. PLANCK, Stephen R
University of Arizona
5 R23 CA 30466-02
Enzymology of Mammalian DNA Replication and Repair

122. PRAKASH, Satya
University of Rochester
5 R01 CA 32514-02
Repair of DNA Damaged by Psoralen + 360 nm Irradiation

123. RANDERATH, Kurt
Baylor College of Medicine
5 R01 CA 32157-02
32P-Labeling Test for Nucleic Acid Damage by Carcinogen

124. RICH, Alexander
Massachusetts Institute of Tech
5 R01 CA 29753-03
Chemical Carcinogenesis and DNA Stucture

125. ROGAN, Eleanor, G
Univ of Nebraska Medical Ctr
5 R01 CA 25176-03
Binding of Aromatic Hydrocarbons to Nucleic Acids

126. ROSENSTEIN, Barry S
University of Texas Health
Science Center (Dallas)
1 R23 CA 33920-01
Repair of 290-320 nm Induced Non-Dimer DNA Damage

127. ROSSMAN, Toby G
New York University
5 R01 CA 29258-03
Mutagenesis by Metals of Environmental Significance

128. SARMA, D S
University of Toronto
5 R01 CA 23958-05
DNA Repair/Replication in Chemical Carcinogenesis

129. SCHENDEL, Paul F
Univ of Connecticut (Storrs)
5 R01 CA 32182-02
Mismatch Repair in Mutagenesis by Alkylating Carcinogens

130. SEDWICK, W David
Duke University
5 R01 CA 31110-02
Antifolate-Induced Misincorporation of UDR in Human Cells

131. SELL, Stewart
University of Texas Health
Science Center (Houston)
5 R01 CA 34139-02
Radioimmunoassay of Alphafetoprotein

132.	SHIM, Sang C Korea Advanced Institute of Science and Technology 5 R01 CA 21729-05	Photochemistry of 5,7-Dimethoxycoumarin
133.	SICILIANO, Michael J Univ of Texas System Cancer Ctr 5 R01 CA 28909-03	Genetics of Chemical Carcinogenesis in Fish
134.	SINCLAIR, Peter R Dartmouth College 5 R01 CA 25012-05	Liver Cell Culture for Study of Carcinogen Activation
135.	SIRICA, Alphonse E Univ of Wisconsin (Madison) 5 R23 CA 29401-03	Isolation of "Prenoplastic" Cell Populations
136.	SIRICA, Alphonse E Univ of Wisconsin (Madison) 5 R01 CA 30102-02	Hepatic Oval Cells in Culture and In Vivo
137.	SIROVER, Michael A Temple University 5 R01 CA 29414-03	Regulation of DNA Repair in Chemical Carcinogenesis
138.	SLAGA, Thomas J Univ of Texas System Cancer Ctr 1 R01 CA 34890-01	In Vitro Transformation of Epidermal Cells
139.	SMUCKLER, Edward A Univ of California (San Francisco) 5 R01 CA 21141-07	Pathology of Chemical Carcinogenesis
140.	SMULSON, Mark E Georgetown University 2 R01 CA 25344-04	Carcinogens and Chromatin Structure and Function
141.	SOLT, Dennis B Northwestern University 7 R01 CA 34160-01	Sequential Analysis of Oral Carcinogenesis
142.	SOROF, Sam Institute for Cancer Research 5 R01 CA 05945-20	Macromolecules in Chemical Carcinogenesis
143.	TEEBOR, George W New York University 5 R01 CA 16669-08	Repair of Radiation-Induced Carcinogenic Damage to DNA
144.	TESSMAN, Irwin Purdue University West Lafayette 5 R01 CA 22239-05	Effect of Ultraviolet Light on Cellular Processes

145. TOPAL, Michael D
Univ of North Carolina Chapel Hill
5 R01 CA 28632-03
Effects of Carcinogen Modification of DNA Precursors

146. WALBORG, Earl F, Jr.
Univ of Texas System Cancer Ctr
2 R01 CA 27377-04
Membrane Glycoproteins During Hepatocarcinogenesis

147. WALKER, Graham C
Massachusetts Institute of Tech
5 R01 CA 21615-07
Mutagenesis and Repair of DNA

148. WEBBER, Mukta M
University of Colorado Health Sciences Center
5 R01 CA 28279-03
Human Prostatic Growth Regulation and Carcinogenesis

149. WHALEN, Dale L
Univ of Maryland (Baltimore)
5 R01 CA 26086-03
Kinetics of Alkylation of RNA Components by Epoxides

150. WHITLOCK, James P, Jr
Stanford University
5 R01 CA 24580-03
Chemical Carcinogens and their Cellular Receptors

151. WHITLOCK, James P, Jr
Stanford University
1 R01 CA 32786-01
Carcinogen-Metabolizing Enzymes: Action in Variant Cells

152. WINKLE, Stephen A
Rutgers State University
1 R01 CA 34762-01
Cooperative, Selective Carcinogen, Drug Binding to DNA

153. YAGER, James D, Jr.
New York University
7 R01 CA 32175-01
Error-Prone DNA Repair in Hepatocarcinogenesis

154. YAGER, James D, Jr.
New York University
1 R01 CA 33750-01
DNA Sequence Changes During Hepatocarcinogenesis

155. YANG, Chung S
University of Medicine and Dentistry of New Jersey
5 R01 CA 16788-09
Monooxygenase: Properties and Carcinogen Activation

156. YANG, Nien-Chu C
University of Chicago
2 R01 CA 10220-13A2
Chemistry of Biologically Active Oxiranes

157. YU, Fu-Li
Rockford School of Medicine
5 R01 CA 30093-03
Aflatoxin B1 and Nucleolar RNA Synthesis

158. HUBERMAN, Elizer
Department of Energy/Argonne
National Laboratory
Y01-CP-70222

Malignant Cell Transformation
and Mutagenesis Induced by
Carcinogenic Chemicals

SUMMARY REPORT

CHEMICAL RESEARCH RESOURCES

The Chemical Research Resources program of the Branch endeavors to make available to the cancer research community those critical resources which are difficult or impossible for most investigators to obtain on their own, but which are necessary for the pursuit of studies on the chemical and physical aspects of carcinogenesis. Eight resource contracts totalling $2.26 million in FY 83 dollars presently comprise this program. There are no grants included. A major effort of this program has involved the synthesis and distribution of chemical carcinogens, derivatives, and metabolites for use as authentic research standards. Labeled analogs of vitamin A, known as retinoids, which have shown promise in studies conducted for the Biological and Chemical Prevention program are also synthesized under contract and made available for pharmacologic and metabolic investigations. Also included under the resource category are two initiatives which support the Biological and Chemical Prevention program. These include a contract for the synthesis of kilogram quantities of retinoids for subsequent testing in chemoprevention and toxicity assays and a contract for the tracheal organ culture bioassay of new retinoids developed by the chemoprevention program. The Research Resources program also monitors an instrument loan program, involving NCI-owned thermal energy analyzers, which are placed in laboratories literally around the world for studies on the environmental occurrence and relevance of N-nitroso compounds such as nitrosamines and nitrosamides.

The Research Resources program currently has six contractors who are involved in the synthesis of compounds, either carcinogen standards or chemopreventive agents. These contractors develop suitable routes for the unequivocal organic synthesis of compounds designated by the NCI project officer. Methods are developed to produce adequate quantities of well-characterized compounds of high purity (generally greater than 98%). Compounds are analyzed by a meaningful combination of techniques to assess purity and confirm structure. These may include UV, IR, NMR, mass spectrometry, HPLC, TLC, fluorescence and elemental analysis.

At the American Health Foundation (2) the objective is to synthesize one gram quantities of key metabolites of benzo(b)fluoranthene (B(b)F), benzo(j)fluoranthene (B(j)F), and benzo(k)fluoranthene (B(k)F) for distribution to the research community through the NCI Chemical Carcinogen Reference Standard Repository. The benzofluoranthenes are among the most prevalent of the carcinogenic environmental polynuclear aromatic hydrocarbons, but in contrast to other hydrocarbons such as benzo(a)pyrene, relatively little is known about the mechanism by which they cause cancer. It is hoped that, as these standards are made available, research will be stimulated in this area. The trans-8,9-dihydroxy-8,9-dihydrobenzo(k)fluoranthene (B(k)F-8,9-diol) has been prepared. This is a proximate mutagen, but not a proximate carcinogen of B(k)F. Also available now is the trans-9,10-dihydro-9,10-dihydroxybenzo(b)fluoranthene (B(b)F-9,10-diol) which is a stronger mutagen and tumor initiator than B(b)F but appears to be only a minor metabolite of B(b)F. The major dihydrodiol metabolite of B(b)F, trans-1,2-dihydro-1,2-dihydroxybenzo-(b)fluoranthene (B(b)F-1,2-diol) has also been synthesized. Syntheses of other diols are also in progress.

At the Midwest Research Institute (MRI) (8), in Kansas City, Missouri, there is a large effort invested in the synthesis of NCI-selected nonlabeled and labeled

(^{3}H, ^{14}C) polynuclear aromatic hydrocarbon (PAH) derivatives other than the benzofluoranthenes. The parent compounds of interest for new synthesis work include benz(a)anthracene, benzo(a)pyrene, benzo(e)pyrene, cyclopenta(c,d)pyrene, chrysene, dibenzanthracene and 3-methylcholanthrene. Derivatives of the following types are prepared as research trends dictate: phenols; quinones; epoxides; dihydrodiols; diolepoxides; alkyl and hydroxyalkyl substituted parent hydrocarbons; nitro-PAH derivatives; PAH-DNA adducts; and sulfate, glucuronide, and glutathione conjugates. The Midwest Research Institute (MRI) maintains a Radiochemical Repository for the NCI under this contract. Shipments of isotopically labeled PAH metabolites are prepared and monitored for authorized recipients as directed by the NCI project officer.

During the last year, 32 polynuclear aromatic hydrocarbon derivatives were synthesized, characterized, and shipped to the NCI Chemical Carcinogen Reference Standard 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); K-region phenols, dihydrodiols, and ^{3}H-labeled derivatives of indeno(1,2,3-c,d)pyrene; non-K-region A-ring phenols, dihydrodiols, and the epoxide of 7,12-dimethylbenz(a)anthracene; methylpyrene derivatives; multifunctional derivatives of BP (e.g., 9-phenol-4,5-dihydrodiol); and re-synthesis of labeled and unlabeled BP phenols, dihydrodiols and epoxides.

The Radiochemical Repository has, from its inventory of 41 compounds, shipped 256 ^{3}H- and ^{14}C-labeled PAH metabolite samples to 128 authorized investigators in the United States, Japan, England, France, Australia, Chile, Canada, Sweden, Finland, Switzerland, India, and Germany during the last year.

The derivative classes of PAH that will be prepared in the future are listed below (samples of many of these classes are now available in the Repository; however, not for all of the PAH that are of active research interest):

- Metabolites of parent PAH currently in the Repository (e.g., phenols, epoxides, quinones, dihydrodiols, triols, tetrols, dihydrodiolepoxides, derivatized methyl and nitro analogs and conjugates);
- 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);
- Sulfate, glucuronide, and glutathione conjugates of selected PAH phenols, alcohols, dihydrodiols, quinones, and epoxides;
- Re-synthesis 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;
- 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;
- 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;
- Mono-, di-, and trimethyl derivatives and metabolites of a selected group of parent PAH (e.g., chrysene, phenanthrene, fluoranthene, pyrene, dibenz(a,h)anthracene, benzo(a)pyrene, benzo(e)pyrene, and benz(a)anthracene);
- 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;

- Nitro PAH derivatives of selected PAH of wide environmental distribution with special emphasis on PAH that appear on the EPA priority pollutant list.

Companion contract efforts at MRI (1) and at SRI International (5) provide for the re-synthesis 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 the future re-syntheses in order to maintain a continuing supply. Each contractor has specific parent PAH compounds for which responsibility is assigned for the preparation of derivatives. A second objective for these contractors is the syntheses of compounds from other chemical classes that are needed in the Repository: nitrosamines, aromatic amines, additional parent polynuclear aromatic hydrocarbons, aflatoxins, steroid derivatives, and physiologically active natural products to name a few. These two contracts will be recompeted during the coming fiscal year.

All nonlabeled compounds prepared by the four previously mentioned contractors are forwarded to the Chemical Carcinogen Reference Standard Repository operated for the NCI by IIT Research Institute (4). Other items of inventory are derived from surplus, re-analyzed chemicals that are tested by the National Toxicology Program and other chemicals which are purchased commercially and re-analyzed. Most commercial purchases are made as a result of a need to obtain a given chemical for in vitro testing. The Repository participates in a program for the Coordinator for Environmental Carcinogenesis, Office of the Director, Division of Cancer Cause and Prevention, in which selected chemicals are submitted as blind-coded samples for in vitro testing and subsequent evaluation as candidates for in vivo testing.

In addition, analytical standards are supplied, as part of an interagency agreement with the USDA, to 13 laboratories participating in the USDA Food Safety and Quality Assurance Recognized Laboratory Program for Nitrosamine Analysis. These laboratories test the products of commercial meat packers for contamination by nitrosamines. The Repository has, from time to time, provided assistance to other Federal government agencies as well as state and local agencies by providing environmental standards needed in research or monitoring.

During the past year, 300 shipments were made to the research community at large. These shipments contained a total of 1760 custom packaged samples usually with 5 to 100 milligrams of material. Samples were furnished with analytical documentation and safety data sheets. General information on the handling and disposal of carcinogens has been provided in response to inquiries. 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 materials.

On April 1, 1983 the Chemical Research Resources program introduced a user's fee, or payback system, for samples distributed under the program. A price structure was developed which includes cost centers for the chemical cost, the handling/packaging cost and the shipping cost. Because of the great expense involved in developing a synthesis route for a new chemical, the NCI will still be significantly involved in the support of these contract efforts. The repository contractors (IITRI and MRI) will bill the requestors for shipment and deduct the net

income from their operating costs. The NCI will then cover the balance of each months operating cost to the contractors.

Retinoid candidates which are to be tested by contractors in the Biological and Chemical Prevention program (this Branch) are synthesized in kilogram amounts by the Southern Research Institute (7). The retinoids are synthesized in large amounts for long-term evaluation of the chemoprevention of cancer in animals, as well as for teratological and toxicological end points. Large amounts of all-trans-N-(4-hydroxyphenyl)retinamide (>2.5 kg.), all-trans-N-butylretinamide (>0.6 kg.), and all-trans-N-propylretinamide (560 g.) and smaller amounts of all-trans-N-((4-pivaloyloxy)phenyl)retinamide and of N-(2,3-dihydroxypropyl)retinamide were synthesized for these biological evaluations. Small amounts of several all-trans- and 13-cis-retinamides were provided for teratological studies. A specimen of all-trans-4-oxoretinoic acid was synthesized for a biological study, and additional 13-cis-retinoic acid was prepared. Multistep syntheses of three congeners of the aryltriene analog (1E,3E)-1-(4-carboxyphenyl)-2-methyl-4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-1,3-butadiene of all-trans-retinoic acid were completed and supplied for initial toxicological studies.

At SRI International (3) radiolabeled retinoids are prepared for evaluation of metabolic and pharmacologic action in biological systems by the research community. These labeled compounds are now also provided to requestors on a pay-back basis. The contractor currently offers an inventory of approximately 15 compounds. Considerable time was devoted this year to the preparation of unlabeled starting materials needed for the synthesis of tritium labeled, high specific activity, all trans-retinoic acid, tritiated beta-carotene and two different stilbenoids. Approximately 50 requests for labeled materials were filled during the last year.

One other resource contract which supports studies of the Biological and Chemical Prevention program is an effort at IIT Research Institute (6) which provides for the bioassay of retinoid activity by the tracheal organ culture system. New retinoid compounds prepared by research syntheses by contractors or submitted (gratis) by BASF, Germany or Hoffmann-LaRoche, New Jersey are evaluated using the hamster tracheal organ culture assay which determines 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 mucous secreting cells similar to those observed in vitamin A normal animals. Eighty-two retinoids in all were received from Dr. Marcia I. Dawson, SRI International (NO1-CP-05600) (thirty); Dr. William H. Okamura, University of California, Riverside (NO1-CP-05715) (fifteen); Dr. John McMurry, Cornell University, (NO1-CP-05716) (twenty four); BASF Aktiengesellschaft, Germany (twelve); and Dr. K. Darrell Berlin, Oklahoma State University, Stillwater, OK (one). A total of 158 assays (one retinoid dose response per assay) were performed using approximately 5200 hamster tracheas, and their ability to reverse keratinization was compared to all-trans-retinoic acid, the reference substance. Dose-response curves were made for these newly synthesized retinoids and all-trans-retinoic acid and the 50% effective dose (dose effective in suppressing keratinization in one-half of the cultures) determined.

Eight retinoids from SRI International, four from Cornell University, three from the University of California-Riverside and four from BASF had appreciable activity in the 10^{-10} M to 10^{-11} M range.

RESEARCH RESOURCES

CONTRACTS ACTIVE DURING FY83

Investigator/Institution/Contract No	Title
1. BODINE, Richard S Midwest Research Institute NO1-CP-05719	Synthesis of Selected Chemical Carcinogen Standards
2. HECHT, Stephen S American Health Foundation NO1-CP-15747	Synthesis of Derivatives of Polynuclear Aromatic Hydrocarbons
3. KAEGI, Hans H SRI International NO1-CP-05601	Synthesis of Radiolabeled Retinoids for Metabolic and Pharmacologic Studies
4. KEITH, James N IIT Research Institute NO1-CP-05612	Chemical Carcinogen Standard Reference Repository
5. REIST, Elmer J SRI International NO1-CP-05614	Synthesis of Selected Chemical Carcinogens
6. SCHIFF, Leonard J IIT Research Institute NO1-CP-05610	Bioassay of Retinoid Activity by Trachael Organ Culture System
7. SHEALY, Y Fulmer Southern Research Institute NO1-CP-26009	Synthesis of Kilogram Amounts of Retinoids for Chemoprevention and Toxicity Studies
8. WILEY, James C Midwest Research Institute NO1-CP-05613	Synthesis of Derivatives of Polynuclear Aromatic Hydrocarbons

SUMMARY REPORT

SPECIAL PROJECTS

This category consists of 104 grants with FY 83 funding of approximately $15.10 million. The grants include 87 R01 (Research Project) grants, 12 P01 (Research Program Projects) grants, 4 R13 (Conference) grants, and 1 R23 (New Investigator Research Award) grant. The R01 grants are principally concerned with the role of tumor promoters in carcinogenesis, endocrine-related biochemistry of cancer, interspecies comparisons in carcinogenesis, and the development of cell culture systems and of biological models for use in carcinogenesis research. All of the P01 grants assigned to this Branch are included in this category; principally these include a spectrum of studies on molecular events in chemical carcinogenesis, endocrine-related biochemistry of cancer, endogenous nitrosation, and inhalation carcinogenesis.

Grants Activity Summary

Until recently, the phorbol esters and related plant diterpenes appeared to be unique compounds since they were the only agents capable of acting as tumor promoters on mouse skin when applied at concentrations as low as 10^{-6}M; these compounds also exert their effects in cell culture at very low concentrations (about 10^{-9}M). Sugimura, Fujiki, and their colleagues have recently found, however, that the compound teleocidin and related indole alkaloids are as potent as 12-O-tetradecanoylphorbol-13-acetate (TPA) as tumor promoters on mouse skin. In studies done in collaboration with these investigators, Weinstein and coworkers have found that despite marked differences in chemical structure these compounds appear to act through the same receptor system as the phorbol ester tumor promoters. It was found that teleocidin induces several biological effects similar to those of TPA in cell culture. Both TPA and teleocidin enhanced transformation of a clone of Fischer rat embryo cells (CREF) by a temperature-sensitive mutant of adenovirus type 5 (H5ts125); enhanced the cloning efficiency in agar of E11 cells, a clone of H5ts125-transformed Sprague-Dawley rat embryo cells; inhibited melanogenesis in murine B-16 melanoma cells; inhibited myogenesis in myoblast cultures established from normal human skeletal muscle; and stimulated choline release from prelabeled phospholipids of C3H10T1/2 mouse cells. In general, TPA and teleocidin were equipotent in inducing these biological effects and were most active in the 3 to 10 ng/ml range, i.e., approximately 10^{-8} to 10^{-9}M. These studies provide further evidence that teleocidin represents a new class of tumor-promoting agents with properties similar to, if not identical with, those of the phorbol ester tumor promoters. These findings also suggest that cell culture systems can be used to identify new types of tumor-promoting agents in addition to the diterpene phorbol esters. It was also found that both lyngbyatoxin A and dihydroteleocidin B induce increased prostaglandin release and choline turnover in HeLa cells at concentrations (6-20 ng/ml) and with a time course similar to that of TPA. These findings have led these investigators to postulate that the three dimensional conformations of the phorbol ester and indole alkaloid tumor promoters share somewhat similar hydrophilic and hydrophobic domains, allowing them to interact with the same set of membrane-associated receptors and thus exert similar biologic effects (96).

Previously a factor in normal human serum that inhibits the receptor binding of (^{3}H)-phorbol-12,13-dibutyrate (PDBu) was identified. During the past year, this inhibitor has been studied in detail. It was found that the serum factor inhibited (^{3}H)-PDBu binding in both intact monolayer cultures of the rat embryo cell

line CREF N and in a subcellular system containing membranes from these cells. Inhibition occurred at both 37° and 4°C and was rapid and reversible. An analysis of (^{3}H)-PDBu binding in the presence of the serum factor indicated that inhibition of (^{3}H)-PDBu binding by the serum factor was noncompetitive. Using gel filtration to separate the serum factor from free (^{3}H)-PDBu, evidence was obtained that the serum factor does not act by binding or trapping the (^{3}H)-PDBu. Unlike the phorbol ester tumor promoters, the serum factor alone did not stimulate the release of choline or arachidonic acid from cellular phospholipids, nor did it inhibit the binding of (^{125}I)-labeled epidermal growth factor to cellular receptors. The factor did, however, antagonize the inhibition of epidermal growth factor binding induced by PDBu. Sera from pregnant women were, in general, more inhibitory of (^{3}H)-PDBu binding than were those from nonpregnant women, which were more inhibitory than those from men. During these studies, it was found that CREF N cells responded to being grown in the presence of PDBu by partial down regulation of the phorboid receptor. The 50% effective dose for down regulation was 8 nM PDBu, and the maximum effect occurred after 6 hours. Taken together, these results indicate that the serum factor inhibits (^{3}H)-PDBu binding by a direct physical effect at the level of the phorboid receptors or their associated membranes. It would appear that if this factor acts in vivo, then it might antagonize certain effects of this class of tumor promoters. The normal physiologic role of this factor and its possible effects on carcinogenesis remain to be determined (96).

Because of the high membrane content of cellular mitochondria, and because of the possible role of mitochondrial changes in tumor formation, the effects of tumor promoters on mitochondrial function were studied. It was found that TPA is a potent inhibitor of mitochondrial respiration in both normal and methycholanthrene-transformed C3H10T1/2 mouse fibroblasts. This inhibition is seen at concentrations of tumor promoter in the range of 10^{-8}M, occurs within a few minutes after exposure of the intact cells, and is not seen with a biologically inactive analog. The effect appears to be exerted through inhibition of the function of an oligomycin-sensitive ATPase. It is possible, therefore, that alterations in mitochondrial function are associated with the process of tumor promotion (96).

Since TPA can modulate the differentiation of a wide variety of types of cells in tissue culture, the effects of this compound on the profile of newly synthesized proteins related to keratinocyte differentiation in the intact mouse epidermis were determined. TPA was applied to the skin of the intact mouse, and either 3 or 24 hours later, skin fragments were pulse-labeled in vitro with ^{35}S-methionine for 4 hours. The epidermal proteins were extracted and separated by two-dimensional gel electrophoresis. Over 200 individual proteins were resolved in acidic gels. At least 10 of these showed major (by a factor of 5 or more) increases or decreases in response to TPA; 8 of these appear to be keratin proteins. Two-dimensional gel profiles of basic proteins synthesized by mouse epidermis resolved over 100 individual proteins. Only one of these showed a significant change in response to TPA. This 41 kd protein increased more than 100-fold within 24 hours after the application of TPA. Treatment of mouse skin with mezerein, a plant diterpene structurally related to TPA, produces an almost identical change in the pattern of proteins produced. Four agents that induce hyperplasia but are not potent tumor promoters, ethylphenylpropiolate, acetic acid, turpentine oil and the Ca^{++} ionophore A23187, modulate the synthesis of only three of the keratin proteins. Thus the changes in protein profiles induced by TPA and mezerein are not simply the consequence of hyperplasia. In addition, application to mouse skin of a gluco-corticoid that is a potent inhibitor of tumor promotion inhibits most of the changes in protein profiles induced by TPA. Taken together, these results indicate that TPA

and mezerein induce early and marked changes in the profile of specific epidermal proteins. It seems likely that some of these changes are directly related to the process of tumor promotion (96).

In previous studies from Weinstein's laboratory, variants of Friend erythroleukemia (FEL) cells that are resistant to TPA inhibition of erythroid differentiation were isolated. In recent studies, fluorescence polarization of the probe 1,6-diphenyl-1,2,5-hexatriene was used to compare membrane lipid dynamics in clones of FEL cells displaying sensitivity or resistance to TPA. Two resistant clones yielded higher fluorescence anisotropy values, indicative of decreased lipid fluidity, as compared to two sensitive clones. This difference in fluorescence anisotropy was abolished by treatment of the sensitive clones with cholesteryl hemisuccinate. The treatment also removed another phenotypic difference between the sensitive and resistant clones. Cell adherence to plastic culture dishes in the presence of TPA, a property of sensitive but not of resistant clones, was markedly diminished when the former were enriched with the cholesteryl ester. No significant differences were observed in the binding of (^{3}H)-PDBu to high affinity saturable receptors between sensitive versus resistant cells. These results implicate membrane lipids as determinants of the actions of phorbol ester tumor promoters. It is presumed that the resistant clones have acquired changes that are distal to the interaction between phorbol esters and their receptors. In more general terms, these results suggest that specific alterations in the lipid composition of cell membranes, as a function of nutritional factors, might alter the sensitivity of tissues to tumor promoters in the intact animal (96).

In the area of estrogen metabolism, Fishman and coworkers have extended their findings of a clear increase in 16-hydroxylation of estradiol in women with breast cancer to the mouse model. Using special modifications and minaturization of the radiometric techniques used in their human studies, these investigators have measured tritium release from (16α)estradiol in 10 strains of laboratory mice. The strains had a spectrum of spontaneous tumor rates ranging from nearly 100% in the C$_3$H/OUJ to near 0% in the C57Br/CdJ. There was an invariant correlation between in vivo 16-hydroxylase activity, and the predicted tumor appearance rates. Studies are now underway to determine whether this enzyme activity profile is genetically predetermined or is influenced by the expression of the mouse mammary tumor viruses (25).

A product of the 16α-hydroxylase pathway which is elevated in breast cancer is 16α-hydroxyestrone. The biological activity of this metabolite has already been shown to be disproportionate to its very modest receptor affinity. It has now been observed that 16α-hydroxyestrone, unique among all natural estrogens, can bind covalently to primary amino groups via a nonenzymatic Heyn's rearrangement in vitro. If this reaction is shown to occur in vivo, a mechanism becomes available whereby a covalent adduct of this estrogen can influence cellular biology to provide for its unusual uterotropic activity and possible oncogenic properties (25).

During the past year, considerable progress has been made in the immunochemical detection, measurement, and localization of estrogen receptor in human breast cancers and in normal reproductive tissues from several mammalian sources. An immunoradiometric (IRMA) sandwich assay for estrophilin was developed with two monoclonal antibodies (D547, D75) prepared against estrogen receptor from MCF-7 human breast cancer cells. This assay and an immunocolorimetric (ICMA) modification of the same assay have now been tested on more than 82 breast cancer cytosols. The correlation between receptor levels determined by these methods and by routine

steroid-binding techniques, such as sucrose density gradient centrifugation and treatment with Dextran-coated charcoal, was found to be excellent. The ICMA assay is currently being tested in several laboratories and should be available in the near future (33).

Selkirk and coworkers have studied the metabolic and macromolecular binding and mammalian cell mutagenesis of benzo(a)pyrene (B(a)P) in non-transformed and chemically transformed rat liver epithelial cells for the purpose of observing changes in the activation and detoxification pathways for polycyclic hydrocarbons as a result of malignant transformation. Metabolic assays showed that non-transformed parent cells (IARC-20) were able to turn over more B(a)P during the five-day experiments than any of the malignant clones (IAR-6-2; IARC-2-19, dimethyl-nitrosamine transformed; IAR-2-28 N-methyl-N-nitro-N-nitrosoguanidine transformed; IARC-27; spontaneous transformant). Formation and disappearance of each oxygenated metabolite over a five-day period was determined to monitor variations and portions of the metabolic scheme and especially to observe conjugation products to water-soluble derivatives as final detoxification products. In all cases, 9,10-dihydroxy-dihydrodiol was the major component with 7,8-dihydro-dihydroxydiol (the "normal" diol-epoxide precursor), the second most abundant product. As expected, phenolic and quinone products were less than 10% of the total metabolites, and these derivatives were shown to be major substrates for glucuronide conjugation. Conjugation of diols was not observed in tumor cells, and toxicity studies were generally in direct proportion to degree of metabolism indicating malignantly transformed cells possess no additional enzymatic steps to protect against alkylation. However, macromolecular binding did not directly parallel metabolism as one would have predicted from the metabolism results. For example, the non-malignant line (IARC-20) yielded an equivalent DNA/nuclear protein-binding ratio to IARC-6-1 but metabolized twice as much B(a)P. In contrast, primary hamster fibroblasts produced twice the DNA binding as IARC-20 with approximately equivalent metabolism. The DNA/nuclear protein-binding ratios of the other chemically transformed liver lines were varied and although the data suggested an overall correlation between metabolism and binding, there appear to be too many variable steps in the metabolic scheme for a linear relationship to be derived. This knowledge is especially critical for research utilizing short-term assays for mutagenesis and transformation, since there is a tendency in the literature to extrapolate results between species and directly up to human risk assessment. These results showed that large differences in metabolic competence are possible in diverse cell types (80).

Studies have been completed on human metabolism and macromolecular binding of B(a)P to bladder, skin, bronchus, and esophagus grown in explant culture. Samples were taken at autopsy within four hours after death. Once established, the explants were incubated with tritiated B(a)P for 24 hours and the metabolites extracted and analyzed by high pressure liquid chromatography. Fibroblasts were also grown from two patients and incubated with B(a)P in the same fashion. Metabolite profiles were qualitatively the same for explants and fibroblasts with similar product ratios, although fibroblasts were much less active in overall B(a)P turnover. DNA binding showed a broad variance between patients and tissues with the relative distribution being widest in bladder, followed by skin, bronchus, and esophagus, respectively (80).

Formaldehyde is a known nasal carcinogen in rats. Studies investigating the genotoxic effects of in vivo formaldehyde exposure are underway. A procedure for obtaining rat nasal mucosa cells has been developed. In early studies, rats were

exposed to 15 ppm formaldehyde or air and DNA isolated from the nasal mucosa cells. DNA from formaldehyde exposed rats exhibited slower alkaline elution rates than DNA from control rats, suggesting crosslinking of the DNA by formaldehyde. If rats are given a 15 ppm formaldehyde exposure and then allowed to recover for seven days, the elution rate of the DNA is not different from control DNA rates. A recent experiment suggests a six-hour recovery may be sufficient to return DNA elution rates to control values. Other studies have used the S-1 nuclease assay of DNA damage and repair in transformed mouse epidermal cells. Formaldehyde did not appear to produce single strand breaks at concentrations up to 2560 μM. In fact, the experiments suggested that exposure to formaldehyde prevented the unwinding of DNA. This may be attributed to crosslinking of DNA with DNA or with protein as others have observed. In further experiments, it was shown that formaldehyde prevented alkaline unwinding of DNA damaged by 4-nitroquinoline-N-oxide (1).

Studies have been conducted on the nature of the mutagenic material produced when fava beans (*Vicia faba*) are treated with nitrite under simulated gastric conditions. The present interest in fava beans derives from the possible relevance to cancer of the association of dietary fava beans, plus nitrate exposure, in a Columbian population which is at high risk for gastric cancer. The mutagenicity of extracts of nitrite-treated fava beans was established by testing in S. typhimurium TM677. Microsomal activation was not required. These and related studies would appear to suggest that the mutagen produced upon treatment of fava beans with nitrite under simulated gastric conditions is an activated N-nitroso compound, possibly an N-nitrosourea. Studies on the isolation and characterization of the fava bean promutagen continue (86).

Researchers at Columbia University have confirmed and have extended their earlier findings concerning the involvement of mitochondrial DNA when mammalian cell cultures are exposed to B(a)P or to its ultimate carcinogenic metabolite, 7β, 8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene (BPDE). The present comments relate only to those portions of the Columbia University report concerned with the separation of nuclear DNA (nDNA) and mitochondrial DNA (mtDNA) and to the distribution of carcinogen adducts in nDNA and mtDNA in studies involving the use of C3H10T1/2 mouse embryo cells. Upon gel electrophoretic separation of nDNA and mtDNA from C3H10T1/2 cells, it was found that mtDNA was about 5% of nDNA in rapidly growing C3H10T1/2 cells (1% in the case of confluent cells). Mitochondrial DNA is reported as constituting 1 to 5% of total cellular DNA. Studies on the distribution of carcinogen adducts in nDNA and in mtDNA were based on the exposure of C3H10T1/2 cells to transforming doses of either (^{3}H)BP or (^{3}H)BPDE, whereupon the radioactivity in the mtDNA fraction was found to be several times that in the nDNA. The calculated specific activity of the mtDNA was 50 to 100 times greater than the specific activity of the nDNA. The latter findings confirm these investigators' earlier findings to the effect that the extent of carcinogen modification of mtDNA exceeds that of nDNA (95).

SPECIAL PROJECTS

GRANTS ACTIVE DURING FY83

Investigator/Institution/Grant Number	Title
1. ALBERT Roy E New York University 5 P01 CA 26724-04	Inhalation Carcinogenesis of Environmental Agents
2. ALBERTINI, Richard J University of Vermont and State Agriculture College 5 R01 CA 30688-02	Direct Mutagenicity Testing in Man
3. BAIRD, William M Purdue University West Lafayette 5 P01 CA 30234-02	Molecular Mechanisms of Carcinogen-DNA Interactions
4. BARKA, Tibor D Mount Sinai School of Medicine 5 R01 CA 24023-03	Tumor Promoters, Growth and Differentiation
5. BAXTER, C Stuart University of Cincinnati 5 R01 CA 24022-03	Cell Culture Studies of Environmental Promoters
6. BELMAN, Sidney New York University 5 R01 CA 18536-06	Role of Cyclic Nucleotides in Tumor Promotion
7. BENFIELD, John R City of Hope National Med Ctr 5 R01 CA 28045-03	Esophageal and Pancreatic Carcinogenesis
8. BENFIELD, John R City of Hope National Med Ctr 5 R01 CA 29373-02	Model of Bronchogenic Lung Cancer
9. BERRY, David L US Agricultural Res Service 1 R01 CA 28968-01A1	Mode of Action of Phorbol Esters in Epidermal Cells
10. BIRT, Diane F University of Nebraska Med Ctr 1 R01 CA 33368-01	Urinary Bladder Cancer Promotion by Dietary L-tryptophan
11. BOKKENHEUSER, Victor D St Luke's-Roosevelt Institute for Health Sciences 5 R01 CA 25763-08	Bacteria and Steroid Metabolism

12. BRANSCOMB, Elbert W
Univ of California (Berkeley)
5 R01 CA 30613-02
Somatic Point Mutation Monitoring

13. BRASITUS, Thomas A
Columbia University
5 R01 CA 28040-03
Colonic Epithelial Cell Plasma Membranes

14. BRESNICK, Edward
University of Vermont and State Agriculture College
7 R01 CA 36105-01
Comparison of Hamster Trachea and Human Bronchus

15. BRUEGGEMEIER, Robert W
Ohio State University
5 R01 CA 28578-03
Biotransformations of Estrogens and Cancer

16. BUTEL, Janet S
Baylor College of Medicine
1 R01 CA 33369-01
Tumor Promotion and Murine Mammary Cancer

17. CHU, Ernest H
Univ of Michigan (Ann Arbor)
1 R13 CA 34165-01
Workshop in China on Mutation, Cancer and Malformation

18. COSTLOW, Mark E
St Jude Children's Res Hospital
5 R01 CA 25170-03
Prolactin Receptor Regulation in Cultured Mammary Cells

19. CURPHEY, Thomas J
Dartmouth College
5 R01 CA 30650-02
Pancreas and Liver Carcinogen Metabolism in Three Species

20. DIAMOND, Leila
Wistar Inst of Anatomy & Biology
5 R01 CA 23413-05
Tumor Promoter and Cell Differentiation

21. DIAMOND, Leila
Wistar Inst of Anatomy & Biology
5 R01 CA 30446-02
Hydrocarbon Activation by Cells

22. DIGIOVANNI, John
Wistar Inst of Anatomy & Biology
1 R01 CA 33403-01
Mechanism of Skin Tumor Promotion by Chrysarobin

23. ESTENSEN, Richard D
Univ of Minnesota (Mnpls-St Paul)
5 R01 CA 22195-06
PMA--A Cocarcinogen as a Lymphocyte Mitogen

24. FIALA, Emerich S
American Health Foundation
5 R01 CA 31012-02
Disposition of Hydrazines: Species and Strain Effects

25.	FISHMAN, Jack Rockefeller University 5 P01 CA 22795-06	Specialized Center for Cancer Endocrinology
26.	FOX, C Fred University of California 1 R13 CA 36092-01	DNA Damage and Repair
27.	FRANKEL, Fred R University of Pennsylvania 5 R01 CA 17301-08	Mammary Cancer and the Nuclear Estradiol Receptor
28.	FRANTZ, Andrew G Columbia University 2 R01 CA 11704-12A1	Prolactin and Other Peptides
29.	FREEMAN, Aaron E Center for Neurologic Study 5 R01 CA 30220-02	Organoid In Vitro Model of Liver Carcinogenesis
30.	GORSKI, Jack Univ of Wisconsin (Madison) 2 R01 CA 18110-08	Prolactin Synthesis in Normal and Neoplastic Tissue
31.	GOULD, Michael N Univ of Wisconsin (Madison) 5 R01 CA 30295-02	Human vs Rodent Mammary Mediated Mutagenesis Assay
32.	GRANDJEAN, Carter J Midwest Research Institute 5 R01 CA 27934-03	Diallylnitrosamine Carcinogenesis: Species Differences
33.	GREENE, Geoffrey L University of Chicago 5 R01 CA 02897-27	Steroids and Growth
34.	GRIFFITH, O Hayes University of Oregon 5 R01 CA 11695-14	Photoelectron Microscopy of Cell Membranes
35.	GUENGERICH, F Peter Vanderbilt University 5 R01 CA 30907-03	Purified Human Enzymes and Carcinogen Metabolism
36.	GURPIDE, Erlio Mount Sinai School of Medicine 5 R01 CA 15648-10	Steroid Dynamics in Human Endometrial Cancer
37.	HAM, Richard G Univ of Colorado (Boulder) 5 R01 CA 30028-02	Defined Medium for Human Mammary Epithelial Cells
38.	HECHT, Stephen S American Health Foundation 1 R01 CA 33285-01	Cocarcinogenicity of Ethanol with Nitrosamines

39.	HICKS, Ruth M University of London 5 RO1 CA 31082-02	Carcinogenesis in Human Bladder Tissues
40.	HILL, Donald L Southern Research Institute 5 RO1 CA 30296-02	Carcinogen Metabolism in Sensitive and Resistant Species
41.	HOLLANDER, Vincent P Mount Sinai School of Medicine 7 RO1 CA 32631-01	Endocrine Factors in the Development of Plasmacytoma
42.	HOLLANDER, Vincent P Mount Siani School of Medicine 7 RO1 CA 32581-01	Studies on Hormonally Sensitive Tumors
43.	HOMBURGER, Freddy Bio-Research Institute 5 RO1 CA 24696-03	Syrian Hamster Model of Pancreatic Carcinogenesis
44.	HOSEIN, Barbara H New York Blood Center 1 R23 CA 34621-01	Human Epidermal Differentiation Reversibly Blocked by PM
45.	IP, Margot M Roswell Park Memorial Institute 1 RO1 CA 33240-01	Dietary Fat and Promotion of Mammary Carcinogenesis
46.	JACOBSEN, Linda B Purdue University West Lafayette 1 RO1 CA 33441-01	Promotion and Progression of Liver Cells In Vitro
47.	KAUFMAN, David G Univ of North Carolina Chapel Hill 5 RO1 CA 31733-02	Promotion of Chemical Carcinogenesis in Uterine Tissue
48.	KAUFMAN, David G Univ of North Carolina Chapel Hill 1 RO1 CA 32239-01	Species Comparison of Uterine Carcinogenesis
49.	KLEIN-SZANTO, Andres J University of Texas System Cancer Center 7 RO1 CA 34690-01	Importance of Dark Cells in Skin Carcinogenesis
50.	LEHRER, Robert I University of California (LA) 5 RO1 CA 30526-02	Blood Cell Receptors for Tumor-Promoting Phorbol Esters
51.	LI, Jonathan J Univ of Minnesota (Mnpls-St Paul) 5 RO1 CA 22008-06	Estrogen Carcinogenicity and Hormone Dependent Tumors

52.	LILLY, Frank Yeshiva University 1 P01 CA 31855-01	Mechanisms of Chemical Lymphomagenesis
53.	LING, Gilbert N Pennsylvania Hospital 5 R01 CA 16301-08	Water in Cancer and in Normal Tissues
54.	MAGUN, Bruce E University of Arizona 5 R01 CA 29290-03	Mechanisms of Tumor Promotion In Vivo and In Vitro
55.	MALKINSON, Alvin M Univ of Colorado (Boulder) 1 R01 CA 33497-01	Promotion of Lung Tumors by BHT and Glucocorticoids
56.	MANDEL, H George George Washington University 1 R01 CA 32306-01	Effects of Tumor Promoters on Mammalian Embryogenesis
57.	MC GRATH, Charles M Michigan Cancer Foundation 5 R01 CA 25482-03	Hormonal Control of Metastasis
58.	MC GUIRE, William L University of Texas Health Science Center (San Antonio) 5 R01 CA 11378-13	Mechanism of Hormonal Control of Mammary Carcinoma
59.	MEITES, Joseph Michigan State University 5 R01 CA 10771-15	Neuroendocrine Control of Mammary and Pituitary Tumors
60.	MICHALOPOULOS, George K Duke University 5 R01 CA 30241-02	Cell Culture and Transplantation of Human Hepatocytes
61.	MILLER, Elizabeth C Univ of Wisconsin (Madison) 2 P01 CA 22484-06	Biochemical Studies in Chemical Carcinogenesis
62.	MILLER, Jon P SRI International 5 R01 CA 24588-03	Effects of Tumor Promoters on Protein Kinases
63.	MIRVISH, Sidney S University of Nebraska Med Ctr 5 P01 CA 25100-03	N-Nitroso Compounds
64.	MOOLTEN, Frederick L Boston University 5 R01 CA 23534-05	Protective Immunity to Chemical Carcinogens

65. MOSSMAN, Brooke T
University of Vermont and
State Agriculture College
1 RO1 CA 33501-01
Role of Minerals as Cofactors in Bronchogenic Carcinoma

66. MUFSON, Robert A
New York University
1 RO1 CA 33874-01
Phorbol Esters a Phospholipid-CA^{++} Dependent Kinase

67. OFNER, Peter
Tufts University
5 RO1 CA 29513-03
Androgens in Prostatic and Epididymal Culture

68. OYASU, Ryoichi
Northwestern University
1 RO1 CA 33511-01
Experimental Urinary Bladder Carcinogenesis

69. PARDEE, Arthur B
Dana-Farber Cancer Institute
5 PO1 CA 22427-05
Molecular Analysis of Malignant Transformation

70. PARSA, Ismail
Downstate Medical Center
5 RO1 CA 30354-02
Interspecies Comparisons of Pancreas Carcinogenesis

71. PARSONS, Donald F
New York State Dept of Health
5 RO1 CA 29255-03
Squamous Cell Carcinoma--Invasion Mechanisms

72. PIKE, Malcolm C
Univ of Southern California
1 RO1 CA 33512-01
Hormones in the Etiology of Breast and Prostate Cancer

73. POUR, Parviz M
University of Nebraska Med Ctr
1 RO1 CA 34473-01
Improvement of a Prostatic Cancer Model

74. PURDY, Robert H
Southwest Foundation for
Research and Education
5 RO1 CA 24629-05
Mutagenic and Carcinogenic Potential of Estrogens

75. ROSEN, Jeffrey M
Baylor College of Medicine
5 RO1 CA 16303-08
Hormonal Regulation of Breast Cancer

76. SCARPELLI, Dante G
Northwestern University
1 RO1 CA 34051-01
Metabolism of Pancreatic Carcinogens: Species Differences

77. SCHECHTER, Joel E
Univ of Southern California
5 RO1 CA 21426-06
Rathke's Pouch-Derived Tumors: Effects of Hormones

78. SCHUT, Herman A
Medical College of Ohio (Toledo)
5 R01 CA 30514-02
In Vitro Carcinogenesis Studies in Colon and Esophagus

79. SCOTT, Robert E
Mayo Foundation
5 R01 CA 21722-06
Membrane Pathology in Carcinogenesis

80. SELKIRK, James K
Oak Ridge National Laboratory
5 R01 CA 30355-02
Comparative Dynamics of Benzo(a)pyrene Metabolism

81. SINGER, Bea A
Gordon Research Conferences
1 R13 CA 32017-01
Gordon Research Conference on Mutagenesis, 1982

82 STONER, Gary D
Medical College of Ohio (Toledo)
5 R01 CA 28950-03
Carcinogenesis Studies in Cultured Rat Esophagus

83. STONER, Gary D
Medical College of Ohio (Toledo)
5 R01 CA 30133-02
Carcinogenesis Studies in the Human Bronchus

84. STUART, Robert K
Johns Hopkins University
5 R01 CA 30491-02
Tumor Promoters and Regulation of Hematopoiesis

85. TANG, Frank Y
University of Rochester
5 R01 CA 25455-03
Regulation of Mammary Gland Growth and Regression

86. TANNENBAUM, Steven R
Massachusetts Institute of Tech
2 P01 CA 26731-04
Endogenous Nitrite Carcinogenesis in Man

87. TROSKO, James E
Michigan State University
5 R01 CA 21104-05
Mutation and Derepression of Genes in Carcinogenesis

88. TS'O, Paul O
Johns Hopkins University
5 P01 CA 16043-06
Biomedical Risks Caused by Nucleic Acid Perturbation

89. VARSHAVSKY, Alexander J
Massachusetts Institute of Tech
1 R01 CA 33297-01
Gene Amplification and Tumor Promotion

90. VESSELINOVITCH, Stan D
University of Chicago
5 R01 CA 25522-05
Role of Sex Hormones in Hepatocarcinogenesis

91. VESSELINOVITCH, Stan D
University of Chicago
2 R01 CA 25549-04
Synthetic Steroids and Hepatocarcinogenesis

92. VOLSKY, David J
University of Nebraska Med Ctr
1 R01 CA 33386-01
Epstein-Barr Virus and Tumor Promotion

93. WALKER, Bruce E
Michigan State University
5 R01 CA 27535-03
Tertogenicity of Transplacental DES in Mice

94. WEBER, George
Indiana University-Purdue University (Indianapolis)
5 P01 CA 13526-11
Correlated Study of Metabolic Regulation in Neoplasia

95. WEINSTEIN, I Bernard
Columbia University
5 P01 CA 21111-06
Molecular Events in Chemical Carcinogenesis

96. WEINSTEIN, I Bernard
Columbia University
5 R01 CA 26056-04
Cellular and Biochemical Effects of Tumor Promoters

97. WEISBURGER, John H
American Health Foundation
5 R01 CA 30658-02
Strain Differences in Carcinogenesis

98. WENDER, Paul A
Stanford University
5 R01 CA 31841-02
Synthetic Studies on Tumor Promoters and Inhibitors

99. WENNER, Charles E
Roswell Park Memorial Institute
2 R01 CA 13784-10A1
The Effect of Cocarcinogens on Cellular Membranes

100. WILLIAMS, Jerry R
George Washington University
5 R01 CA 31015-02
Mechanisms of Procarcinogenic Metabolism

101. WILLIAMS, Jerry R
George Washington University
1 R01 CA 33482-01
Cellular Hypermutability in Cancer Promotion

102. WITSCHI, Hanspeter R
Oak Ridge National Laboratory
1 R01 CA 33795-01
Enhancement of Lung Tumor Formation: Cell Kinetics

103. WOLF, George D
Federation of American Societies for Experimental Biology
1 R13 CA 31915-01
Conference on Micronutrients: Vitamin A and Retinoids

104. WOTIZ, Herbert H
Boston University
5 P01 CA 28856-03
The Role of Hormones and Binding Proteins in Cancer

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 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) serves as a focus for extramural efforts in biometry, epidemiology, diet/nutrition and smoking and health within the Division of Cancer Cause and Prevention (DCCP). The programs were brought together in one branch to facilitate multidisciplinary approaches to research in these special areas of carcinogenesis research. While Biometry has relevance to research in all of biology, it also contributes specifically to each of the other SPB programs. Both the Diet/Nutrition and the Smoking and Health programs benefit directly from an interface with the Epidemiology and Biometry Programs which are, 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. 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.

Biometry: Although primarily comprised of research grant activities, contracts and/or interagency agreements are being utilized to determine the feasibility of linking existing data sources to provide resources to the extramural 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 and 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 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: The extramural Epidemiology Program is currently supported entirely by the research grants mechanism. Research areas of interest 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.

Biochemical Epidemiology: Specific program emphasis in the area of Biochemical Epidemiology was initiated during this fiscal year. Our efforts to stimulate additional research in this area were marked by the issuance of a request for applications and a $1.5 million budget reserve from Division funds for the period 1983-1986. The response was enthusiastic. At lease 11 applications will be funded concerned with a wide variety of cancers and even more varied laboratory procedures. Few responses were concerned specifically with the development or validation of new laboratory procedures which show promise of epidemiologic usefulness, a problem which will need to be addressed in future years.

Smoking and Health: This program was begun in 1968 solely as a contract effort. Since its transfer to SPB in 1979, the program has largely focused on attempts to identify groups of individuals at high risk to tobacco-related diseases, and to better understand the toxicology and pharmacology of smoking related exposures. The program currently utilizes both the contract and grant mechanisms for support of its activities. Several meritorious research grant proposals, including an epidemiologic study of smoking in relation to hepatocellular carcinoma and a multidisciplinary program project on tobacco-specific nitrosamines, have been funded. In addition, an RFP to investigate smoker compensation when changing smoking materials (cigarettes having various tar and nicotine delivery levels) has been issued and is expected to be funded during this fiscal year.

Branch Notes: Several important events have occurred during the past year. In August 1982, two requests for applications (RFAs) were published to stimulate epidemiologic research. One of these was directed toward Rare Cancers and the other toward Biochemical Epidemiology. The response to both announcements was excellent, with 22 responses citing the rare tumors announcement and 41 responses citing biochemical epidemiology. For review of applications responding to these announcements, Special Study Sections were assembled by Dr. Michael Alavanja and Dr. Ann Schluederberg, Executive Secretaries of the Epidemiology and Disease Control Study Section, which reviews most of the research applications assigned

to the Epidemiology Program. Eight applications responding to each announcement were disapproved. At least eleven applications from the Biochemical Epidemiology announcement were judged to be more meritorious and will be funded. At least two applications from the rare cancers group, both relating to male breast cancer, will be funded. The infusion of new ideas resulting from the announcements is important to the Epidemiology Program.

Two additional RFAs were published in the areas of Questionnaire Derived Historic Dietary Information and the Pharmacological Role of Nicotine in Disease. Again, response was adequate with 29 responses citing the first and 6 citing the latter of these RFAs. The responses were reviewed by special study sections and it now appears that five (of 15 approved) and two (of 4 approved), respectively, will be funded during this fiscal year.

In the period since the preparation of our last annual report three workshops have been held. The workshops were chaired by members of the DCCP Board of Scientific Counselors and provided advice to program staff on the need for, and best approach to, new initiatives of interest to the Branch. The first, in July 1982, (chaired by Dr. Allan Conney) was designed to provide background information which would be useful in developing a new initiative in the area of Biochemical Epidemiology research. From the discussions, it became apparent that additional research was felt to be needed in this area. The group requested that staff direct specific attention to developing and validating additional laboratory procedures suitable for epidemiologic use, providing adequate characterization of existing tests prior to their use in large scale studies, and applying laboratory measures of epidemiologic parameters in full scale field studies. When the RFA was written, a clause permitting pilot and feasibility studies was included to indicate interest in test development. Although the response to the Biochemical Epidemiology RFA was excellent, only two or three applicants proposed studies in the area of test development. A weakness cited for several applications was the attempt to use a procedure in a large scale study before there was evidence of its suitability; thus, it appears that further steps will be needed to encourage test development and the collaboration of epidemiologists and laboratory scientists in validation and pilot testing phases.

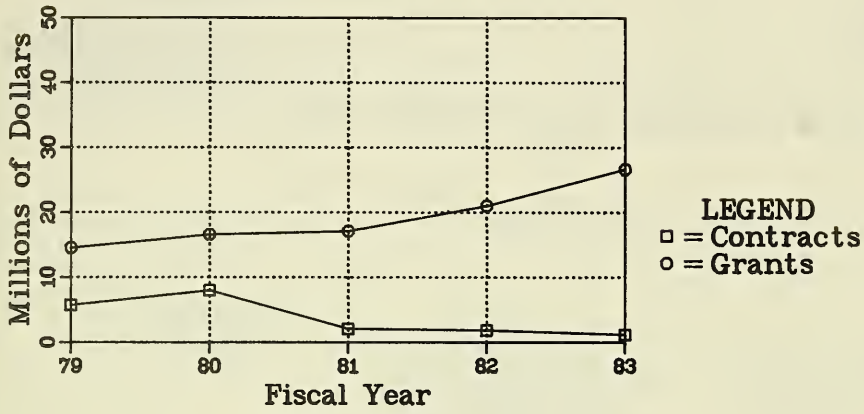
During the same July workshop, discussion also focused on the inadequacies of existing methods of dietary assessment to yield quantifiable epidemiologic parameters. No in-depth discussion was possible because of time constraints and the perceived need for additional expertise. Suggestions were made that more objective measures based on laboratory procedures be developed. Research relating to dietary parameters should be concerned not only with measurement of exposure to substances of interest, but also with individual variation in handling the exposure. It was decided that further follow-up of these suggestions should depend upon responses to the Biochemical Epidemiology announcement. While several responses demonstrated interest in diet and in serum/diet correlations, no research proposals with the primary objective of developing new laboratory techniques relating to dietary parameters were received. Further efforts in this area will be necessary in the future.

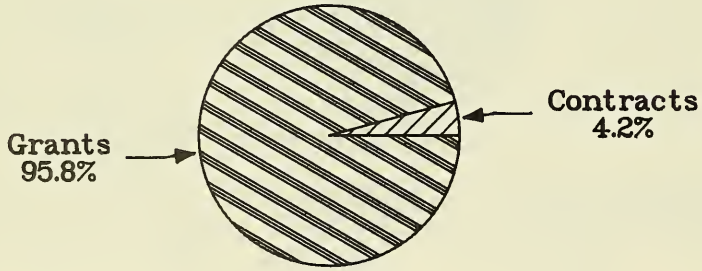
In January 1983, a multidisciplinary group interested in Childhood Tumors was convened (chaired by Dr. Louise Strong) to consider the need for additional research stimulation in this area. The consensus was that no need for an RFA currently exists. However, problems relating to the relative rarity of childhood neoplasia, and the availability of materials for study, were recognized.

A series of workshops was suggested to resolve problems concerning 1) the identification of cases, 2) the identification of high risk families and 3) the availability of specimens for laboratory study.

A problem which has required much attention this year has been the burgeoning epidemic of Acquired Immunodeficiency Syndrome (AIDS), with its associated opportunistic infections and malignancies (Kaposi's sarcoma). It is disappointing that relatively little funding for epidemiologic research will result from the Divison of Cancer Cause and Prevention's research investment of $1.46 million. Methodologic problems at the interface between chronic and infectious disease requirements underlie the disapproval of many epidemiologic responses. Further efforts will be made in the immediate future to address this situation. The Branch will embark on a series of working group meetings to help define new approaches and will attempt to allocate additional staff resources to this area.

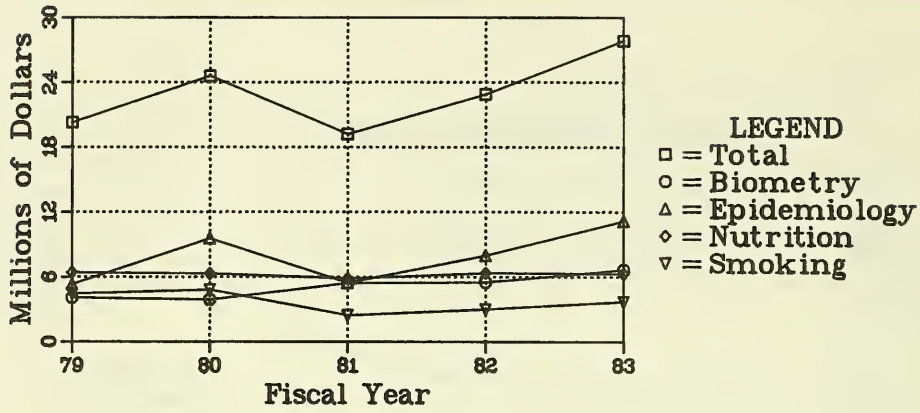
SPECIAL PROGRAMS BRANCH

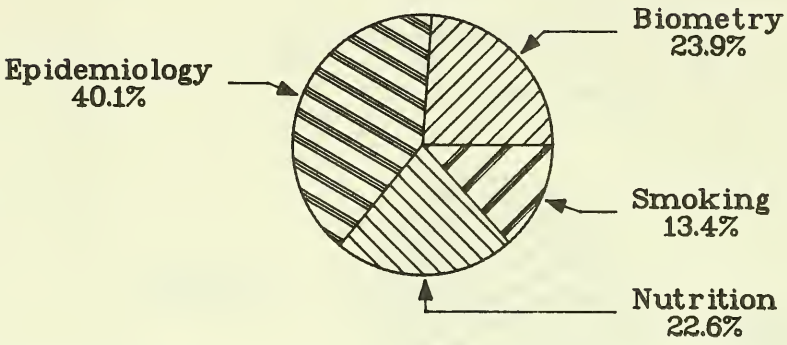




FISCAL YEAR 1983 ESTIMATES	$ (Millions)	% of Program
Contracts	1.18	4.2
Grants	26.66	95.8
Total	$ 27.84	100.0

SPECIAL PROGRAMS BRANCH





FISCAL YEAR 1983 ESTIMATES		$ (Millions)	% of Program	% of Branch
Biometry:	Grants	6.31	94.7	---
	Contracts	0.35	5.3	---
	Subtotal	6.66	---	23.9
Epidemiology:	Grants	11.17	100.0	---
	Contracts	0.00	0.0	---
	Subtotal	11.17	---	40.1
Diet & Nutrition:	Grants	6.29	100.0	---
	Contracts	0.00	0.0	---
	Subtotal	6.29	---	22.6
Smoking & Health:	Grants	2.89	77.7	---
	Contracts	0.83	22.3	---
	Subtotal	3.72	---	13.4
Total, Special Programs Branch		$ 27.84		100.0%

SUMMARY REPORT

BIOMETRY PROGRAM

Description: The extramural Biometry Program currently supports 44 grants funded at $6.31 million, and two Interagency Agreements at a funding level of $0.35 million in the areas of biostatistics and environmental and genetically oriented carcinogenesis. Four research grants resulted from an FY 83 RFA issued by this program area. Activities in the Biometry Program are developmental (innovative) in nature and share a common denominator -- their reliance on recent advances in computer technology (both hardware and software). The Program is successfully encouraging collaboration among biostatisticians, oncologists, geneticists, epidemiologists and members of related disciplines in their quest for insight into the unique problems of analysis of carcinogenesis data and the carcinogenic process itself.

Research Accomplishments: Fifty-five percent (24) of the activities in this program stress the development of biostatistical methodology related to topics such as survival analysis, clinical trials, competing risks, and dose-response phenomena, many of which involve mathematical modeling. These basic topics are being studied in detail and with great sophistication. Survival analysis, which in the past was considered primarily as determining the probability of death given disease/event, now includes 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 nonparametric 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 (7, 35, 37). 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 coalescence of factors in given situations and begin to see avenues for future exploration.

Clinical trials are of great interest to the statistician who views them as decision-making problems (4, 8, 25). The aim, of course, is to test one or more treatments against a standard treatment. Much work in this area involves determination of stopping procedures; to stop when an explicit trade off can be made between the additional information which could be obtained if a trial is allowed to continue and the cost of keeping patients on an inferior treatment. Specific ongoing work involves the design of new methods of randomization to avoid bias caused by heterogeneity of patient populations (43); nomograms for calculating the number of patients needed when survival is an end point (30), and sequential stopping procedures using censored survival data (15). In essence, the statistician is working with the oncologist to devise methods for determining which of several treatments will yield longer patient survival coupled with improved quality of life. The problems being addressed are those which have been detected in previous trials and need to be resolved before new trials are initiated.

The areas of competing risk and dose response are being investigated in much the same manner. Problems are identified, conflicting or confounding factors determined, theoretical work initiated, tested and finally made available to applied biostatisticians for use in handling analysis of laboratory/epidemiologic data (8, 27).

As the search for competing risks and confounding factors in the natural history of cancer intensifies, there is growing recognition of the need for familial (pedigree) information to augment 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 25 percent (12) of the grants, including two large Program Project Grants (P01) (22, 23). Meanwhile, some of the theoretical statistical projects noting the mathematics involved in cell marker analysis have become active in the area. Strict demarcations between biostatistical/computing and genetics within the program no longer exist. For example, the recent developments in multiparameter flow cytometry technology (computer techniques for recognition and classification of human chromosomes) has made biomathematical modeling extremely important in helping the scientist understand the steady state behavior of the multiparameter problem and estimate changes in the various parameters, e.g., cell volume, nuclear size, dry mass and RNA content (44). Likewise, statisticians have been developing mathematical models to show that familial concentration of cancer seen by oncologists is not due to chance alone (5). These models allow for a common environment in nuclear families; variable age at onset of disease; marital, parental, offspring environmental correlations; and mortality risk factors. Even though it is assumed that most cancer is attributable to environmental agents (either present in the atmosphere or ingested in the diet), since all exposed persons do not develop cancer, it may also be assumed that genetic factors must be considered. Some of these factors may be the ability of an individual to metabolize mutagenic agents, as well as promoters, carcinogens, etc.; the ability of the individual to repair induced damage; and the immunocompetence of the individual to recognize, and react to, potential cancer cells. In this case, multifactorial modeling seeks to explain the causation of chromosomal aberrations detected in the laboratory. That is, are the abberations due to genetic defects alone or to interaction with specific external agents.

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 (31) and a Mexican-American population in Laredo, Texas (38), 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 (12, 32, 33, 34). Cases are identified and, subsequently, genealogies constructed. One newly funded international study is ascertaining breast cancer cases among twins to make use of the twin method of data analysis (13). In these studies 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 beginning to develop risk factor profiles and/or biological markers for disease detection. Although the genetic grants within the Biometry Program are considered primarily pedigree studies, more are becoming involved at the cellular level. Findings from these studies will complement studies on the natural history of cancer as cell biologists and biostatisticians continue to formulate mathematical models of tumor development.

The remaining 20 percent (8) of the grants include two large multidisciplinary projects (19, 44), one large data base acquisition project (26) and a Program Project Grant (P01) (3). 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.

Projections: Growth of the Biometry Program will continue at a rate acutely dependent upon the quality and the appropriate peer review of the applications received in their respective specialized areas. Theoretical statistics will need continued support to enable persons with biomathematical expertise to recognize and/or explore new and ongoing topics. Emphasis will be placed on the application of new statistical methodologies to cancer data. There will be increased emphasis on use of new computer hardware/software in graphics and image processing to advance the fields of pattern recognition, statistical theory and modeling. Genetic studies will continue to receive strong support, the Program Project now funded in FY 83 relates to two of the genetic grants already in existence. Combined, these activities should prove to be extremely productive in the coming year. Studies funded as a result of the Special Program Branch's RFA on "Accuracy" of Questionnaire Derived Historic Dietary Information will be a new element in the next fiscal year's program.

SPECIAL PROGRAMS BRANCH

GRANTS ACTIVE DURING FY 1983

BIOMETRY PROGRAM

Investigator/Institution/Grant Number	Title
1. ACTON, Ronald T.* University of Alabama 5 R01 CA 30467-02	Genetic Analysis of Melanoma
2. BLOIS, Marsden S.* University of California 5 R01 CA 26655-03	Natural Language Access to Clinical Data Bases
3. CORREA, Pelayo Louisiana State University 5 P01 CA 28842-02	Etiologic Studies of Gastric Carcinoma
4. DUDEWICZ, Edward J.* Syracuse University 7 R01 CA 35186-01	A Model for Medical Treatments Evaluation
5. ELSTON, Robert C. Louisiana State University 5 R01 CA 28198-04	Statistical Genetic Analysis for Cancer Families
6. FAREWELL, Vernon T. Fred Hutchinson Cancer Research Ctr. 1 R01 CA 32913-01	Statistical Methods for Medical Data
7. HARRINGTON, David P. University of Virginia 1 R01 CA 32693-01	Nonparametric Statistical Tests for Censored Cancer Data
8. KLOTZ, Jerome H. University of Wisconsin 5 R01 CA 18332-09	Statistical Problems in Clinical Cancer Research
9. KOZIOL, James A.* University of California, San Diego 2 R01 CA 26666-04	Topics in Biostatistics
10. LACHENBRUCH, Peter A. University of Iowa 5 R01 CA 24089-06	Estimation of Prognosis Using SEER Data
11. LAGAKOS, Stephen W. Harvard University 5 R01 CA 33041-02	Biostatistical Methods for Carcinogenicity Experiments

12.	LYNCH, Henry T.* Creighton University 5 R01 CA 27831-03	Epidemiologic-Biologic Study of Colon Cancer Families
13.	MACK, Thomas M. University of Southern California 1 R01 CA 32262-02	Determinants of Cancer Within Disease-Discordant Twins
14.	MANTEL, Nathan American University 7 R01 CA 34096-01	Cancer Research: Statistical Methods
15.	MEHTA, Cyrus R. Dana-Farber Cancer Institute 5 R01 CA 33019-02	New Statistical Methods for Cancer Data
16.	MIKE, Valerie* Memorial Sloan-Kettering Cancer Ctr. 1 R13 CA 29801-01	Conference on Biostatistics in Clinical Oncology (Meeting)
17.	MILLER, Kenneth J. Rensselaer Polytechnic Institute 5 R01 CA 28924-02	Computer Assisted Analyses of Carcinogenicity
18.	MOOLGAVKAR, Suresh H. Institute for Cancer Research 5 R01 CA 22780-05	Biomathematical Approaches to Cancer
19.	MOOLGAVKAR, Suresh H.* Institute for Cancer Research 5 RO 1 CA 25588-02	Temporal Evolution of Cancer
20.	MOOLGAVKAR, Suresh H. Institute for Cancer Research 5 R01 CA 30671-03	Malignant Melanoma Multifactorial and Stochastic Models
21.	MYERS, George C. Duke University 5 R01 CA 23399-05	Certification of Cancer Related Deaths
22.	NEEL, James V. University of Michigan 5 P01 CA 26803-04	Program Project: The Study of Human Mutation Rates
23.	NICHOLS, Warren W. Institute for Medical Research 1 P01 CA 33624-01	Epidemiologic Lab Investigation of Cancer-Prone Children
24.	PAFFENBARGER, Ralph S. Stanford University 2 R01 CA 25264-05	Early Predictors of Site-Specific Cancers

25.	PAGANO, Marcello Dana Farber Cancer Center 5 ROl CA 28066-03	Statistical Computing and Clinical Trials of Cancer
26.	PHILLIPS, Roland L. Loma Linda University 5 ROl CA 14703-09	Epidemiology of Cancer in Adventists -- A Low Risk Group
27.	PIERCE, Donald A.* Oregon State University 5 ROl CA 27532-03	Statistical Methodology for Response-Time Data
28.	PUNNETT, Hope H.* St. Christopher's Hospital for Children 5 ROl CA 19834-04	Genetic Constitution and Cancer Predisposition
29.	SCHNEIDER, Robert* University of California, Davis 5 ROl CA 14916-09	Animal Neoplasm Registry
30.	SCHOENFELD, David A.* Dana Farber Cancer Center 5 R23 CA 25162-03	Regression Analyses Techniques for Cancer Research
31.	SKOLNICK, Mark H. University of Utah 5 ROl CA 28854-03	Genetic Epidemiology of Cancer in Utah Genealogies
32.	STRONG, Louise C. University of Texas 5 ROl CA 27925-03	Genetic Etiology and Consequences of Childhood Cancer
33.	STRONG, Louise C. University of Texas 1 ROl CA 32064-02	Identification of Genes Predisposing to Childhood Cancer
34.	SWIFT, Michael R. University of North Carolina 5 ROl CA 14235-11	Neoplasia-Predisposing Genes of Man
35.	TARTER, Michael E. West Coast Cancer Foundation 5 ROl CA 28142-03	Modern Functional Representation in Cancer Research
36.	TSUTAKAWA, Robert K. University of Missouri 5 ROl CA 29765-03	Statistical Analysis of Cancer Mortality Rates
37.	WEI, Lee-Jen George Washington University 5 ROl CA 34428-02	Some Nonparametric Statistical Inference Problems

38. WEISS, Kenneth M.
University of Texas
Health Science Center
5 ROl CA 19311-07
Genetic Epidemiology of Cancer

39. WHITE, Colin
Yale University
5 ROl CA 30931-03
Systematic Analysis -- Connecticut Cancer Incidence Trends

40. WHITTEMORE, Alice S.
Stanford University
5 ROl CA 23214-05
Effects of Multiple Exposures: Quantitative Aspects

41. WILLIAMS, Barbara J.
Gonzaga University
5 ROl CA 33110-02
Statistical Methods for Multiple Event Time Data

42. WOODS, James S.
Battelle Memorial Institute
1 ROl CA 29900-01A2
Cancer Incidence and Phenoxy Herbicide Exposure

43. ZELEN, Marvin
Dana Farber Cancer Center
5 ROl CA 23415-06
Statistical Models of Biomedical Phenomena

44. ZIMMERMAN, Stuart O.
M.D. Anderson Hospital
and Tumor Institute
2 ROl CA 11430-17
Biomathematics and Computing in a Cancer Institute

*Grants Active During FY 83 but Funded with Previous Year's Funds

CONTRACTS ACTIVE DURING FY 1983

45. AZIZ, Faye
Social Security Administration
YOl CP3 0501
Test of the Continuous Work History Sample of SSA as a Probe for Cancer in the Workplace

46. SCHEUREN, Frederick
Internal Revenue Service
YOl CP2 0510
Feasibility of Coding Occupation from the IRS Form 1040

SUMMARY REPORT

DIET AND NUTRITION PROGRAM

Description: The extramural Diet and Nutrition Program is responsible for those aspects of nutrition research related to cause and prevention of cancer in humans. It does not include those parts of the NCI-wide Diet/Nutrition and Cancer Program (DNCP) dealing with special nutritional needs of patients or tumor-bearing animals. Currently, there are 58 grants in the Special Program Branch's Diet and Nutrition Program, supported at the level of $6.29 million. The program supports epidemiologic as well as laboratory investigations searching for etiologic factors related to diet and nutrition. These include mechanistic 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, the program supports studies which focus on specific dietary factors (i.e., nutrients or micronutrients), aspects of 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 factors. Diet could influence cancer in several ways: through undernutrition, by affecting the immune system; 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 carcinogen detoxification systems.

A number of published epidemiologic, as well as laboratory studies have suggested that retinoids may inhibit cancer, while fats enhance and ascorbic acid reduces cancer risk. However, in a recently completed case control study, it was observed that the risk of prostate cancer rose with increased ingestion of retinoids, animal fats, and vitamin C, while ingestion of cruciferous vegetables had no influence (13). The latter finding is at odds with that of Hirayama who observed a lower incidence among men who frequently ate green and yellow vegetables. It has been suggested that specific nutrients may diminish cancer risk at certain sites, but may enhance risk or have no effect on other sites. A given nutrient may well be site-specific in effect. Furthermore, nutrients may react differently in different environments inducing enzymes that may increase cancer risk for one system but diminish it for another.

Biennial follow-up through a mail questionnaire to a large cohort of nurses aged 30-55 in 1976 is continuing to assess the magnitude of the associations between a variety of factors (exogenous estrogens and other hormones, cigarette smoking, hair dyes, and nutrient intake) and site-specific cancer rates (49).

Reorientation of a study, implemented in 1958 to determine risk of cardiovascular disease, has resulted in an attempt to identify dietary and other risk

factors associated with cancer. This study offers an unusual opportunity for long-term follow up of a population that was well characterized initially and has been followed in the past with a high degree of completeness (43).

Two multidisciplinary studies were initiated recently. In one, advantage is being taken of the existence of the multiethnic population of Hawaii to carry out studies of dietary methodologies in epidemiologic research as well as etiologic studies of cancer of various sites in relation to dietary exposures to specific foods and their nutrient components (22). The other study will assess the relationship between selenium status and cancer mortality by means of a population-based study in the People's Republic of China (7). The intent is to compare site-specific and sex-specific (age-standardized) cancer mortality rates with selenium status. Selenium interactions with other nutrients, such as vitamin E, beta-carotene/vitamin A, vitamin C and vegetable fat will also be evaluated. The contribution of selenium nutrition towards cancer mortality rates will be compared with the presumed independent contributions of protein (total, animal), fat (total, animal), dietary fiber, zinc, riboflavin, and cholesterol. The contribution of these nutrients to cancer risk will be assessed both by nutrient intake estimation and by clinical measures.

A number of investigators are currently studying the influence of dietary fat on chemically induced tumors. In one study, the antioxidants, butylated hydroxytoluene (BHT) and propyl gallate, inhibited dimethylbenzanthracene (DMBA) induced mammary carcinogenesis. The two antioxidants were added to a high polyunsaturated fat diet and fed to rats. Neither substance, when added in the diet at 0.3%, showed any protection against a direct-acting carcinogen (not requiring metabolic activation to be carcinogenic), nitrosomethylurea (NMU). This finding suggests that the two antioxidants alter the metabolism of precarcinogens such as DMBA.

In experiments designed to evaluate the influence of dietary fat on NMU-induced mammary carcinogenesis in Fischer rats, it was found that an increase in fat intake enhanced mammary carcinogensis (10). The magnitude of the increase depended on the type of fat. Further analysis revealed that the total oleic and linoleic acid intake correlated positively with mammary tumor incidence.

It has been recently demonstrated that feeding of a choline devoid (CD) diet to rats promotes chemically induced liver carcinogenesis (45). Inclusion of phenobarbital in a CD diet resulted in a synergistic effect. In rats not exposed to a carcinogen, feeding of the CD diet alone stimulated liver cell proliferation. A combination of the CD diet and phenobarbital had an inhibitory effect. From these observations, it was hypothesized that barbiturates selectively inhibit the proliferation of non-initiated cells shifting the proliferative stimulus of the CD diet toward amplification of the initiated cells. Further studies involving other modifiers of the CD diet promotion, such as altering the quality of dietary fat, BHT, and a hypolipedemic drug, would be useful in characterizing factors responsible for modifications.

When iodine labeled Bowman-Birk inhibitor (a protease inhibitor present in soybeans) was fed to mice and rats, over 80% of the inhibitor was excreted in the feces. The material in the feces was capable of combining with proteases, trypsin or chymotrypsin. From these data, it was concluded that one of the effects of the ingestion of protease inhibitors was the inactivation of the major protein digestive enzymes, trypsin and chymotrypsin. This would lead to a lower level of

processing of ingested proteins which instead would be excreted undigested in the feces. This partial removal of proteins as a nutrient may be responsible for the anticarcinogenic effect of protease inhibitors (51).

It is known that, although vitamin E supplementation alone has no prophylactic effect against tumorigenesis, it potentiates the ability of selenium to inhibit the chemically induced mammary neoplasia. Recent studies indicate that vitamin E potentiated this inhibitory effect only when it was present during the promotion or proliferative phase. High levels of vitamin E significantly depressed lipid peroxidation, whereas selenium had a negligible effect. A combination of vitamin E and selenium did not result in further inhibition compared to vitamin E alone. These findings suggest that the suppression of lipid peroxidation by excess vitamin E alone is not sufficient to prevent tumor formation. However, vitamin E may provide a more favorable climate against oxidant stress, thereby potentiating the anticarcinogenic action of selenium via some other mechanism (18).

Selenium fed as sodium selenite inhibited both NMU and DMBA induced mammary carcinogenesis in the rat. Five ppm selenite was found to provide maximal protection against tumor occurrence. Organic selenium as selenomethionine was not as effective as selenite; it was toxic at 6 ppm in the diet, whereas selenite was not. This finding suggests that reductive metabolism of pharmacologic amounts of an inorganic form (Se+4) of selenium is a necessary aspect of its anticarcinogenic activity (50).

The postulate that amines in germinated barley act as precursors for N-nitrosodimethylamine (NDMA) in dried barley malt was confirmed by first germinating raw barley and exposing the resulting malt roots to diluted nitrous acid and quantitating the NDMA formed. The characterization of N-nitrosocompounds formed from Amadori compounds and the reaction mechanism leading to the formation of N-nitrosamines from the tertiary amine alkaloids, hordenine and gramine are also currently under investigation (41).

An ongoing study which focuses on the role of creatinine, present in high concentrations in salted and dried fish and cooked meats such as bacon, as a potential precursor to NMU formation in vivo upon interaction with nitrite will, it is hoped, provide a better understanding of the etiology of stomach cancer (26).

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. Epidemiologic studies aimed at assessing the "accuracy" and reproducibility of historical dietary information by comparing current information obtained by questioning individuals or their surrogates with actual records (data reflecting past dietary intake) of the same individuals recorded at some earlier point in time will be encouraged.

A 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.

Additional research areas on which emphasis needs to be placed include the development of short-term tests for identifying inhibitors of carcinogenesis in food and natural products and further exploration of the effects of nutrient interactions on carcinogenesis.

SPECIAL PROGRAMS BRANCH

GRANTS ACTIVE DURING FY 1983

DIET/NUTRITION PROGRAM

Investigator/Institution/Grant Number	Title
1. ABRAHAM, Samuel Children's Hospital Medical Center 5 RO1 CA 29767-03	Effect of Dietary Fat on Mammary Neoplasia
2. ASANO, Tomoaki University of Notre Dame 5 RO1 CA 28276-03	Experimental Carcinogenesis by Dietary Nitrite
3. BIRT, Diane F.* University of Nebraska 5 RO1 CA 24549-03	Influence of Dietary Selenium on Pancreatic Cancer
4. BLACK, Homer S. Baylor College of Medicine 5 RO1 CA 20907-04	Effects of Dietary Factors on UVL-Carcinogenesis
5. BURKE, James P. Pennsylvania College of Podiatric Medicine 1 RO1 CA 32256-01A1	Relationship of Zinc to Cellular Membrane Composition
6. CAMPBELL, T. Colin* Cornell University 5 PO1 CA 26755-03	Nutrition and Cancer
7. CAMPBELL, T. Colin Cornell University 1 PO1 CA 33638-01	Dietary Selenium and Cancer
8. CHA, Young-Nam* Johns Hopkins University 5 RO1 CA 27594-03	Mechanism of Antimutagenesis by Anticarcinogens
9. CORWIN, Laurence M.* Boston University 5 RO1 CA 26604-03	Effect of Vitamin E and Lipids on Tumorigenicity
10. DAO, Thomas L.* Roswell Park Memorial Institute 5 RO1 CA 26597-03	Dietary Fat and Mammary Carcinogenesis
11. DRAPER, Harold H.* University of Guelph 5 RO1 CA 28242-03	Toxicity and Metabolism of Malondialdehyde

12.	GARRETT, Carlton T. George Washington University 1 ROI CA 31324-01A1	Gene Expression in Nutritionally Promoted Cancer
13.	GRAHAM, Saxon State University of New York 5 POI CA 11535-13	Social Epidemiology and Control of Cancer
14.	GRAY, James I.* Michigan State University 5 ROI CA 26576-03	Formation of N-Nitroso Compounds in Processed Food
15.	HAMILTON, Stanley R.* Johns Hopkins University 5 ROI CA 29714-02	Role of Beer and Ethanol in Experimental Colon Cancer
16.	HEINIGER, Hans-Jorg* Jackson Laboratory 5 ROI CA 19305-06	Cholesterol in Normal and Malignant Lymphocytes
17.	HSIEH, Dennis P.* University of California, Davis 5 ROI CA 27426-03	Comparative Toxicology of Carcinogenic Mycotoxins
18.	IP, Clement C. Roswell Park Memorial Institute 2 ROI CA 27706-04	Selenium Supplement and Dietary Fat in Breast Cancer
19.	JANGHORBANI, Morteza* Massachusetts Institute of Technology 5 ROI CA 27917-03	Dietary Bioavailability of Selenium in Man
20.	KING, M. Margaret Oklahoma Medical Research Foundation 5 ROI CA 34143-06	Dietary Fat and Mammary Carcinogenesis
21.	KOLONEL, Laurence N. University of Hawaii 5 ROI CA 26515-04	Case-Control Study of Lung Cancer and Dietary Vitamin A
22.	KOLONEL, Laurence N. University of Hawaii 1 POI CA 33619-01	Epidemiologic Studies of Diet and Cancer in Hawaii
23.	MACKENZIE, Cosmo G.* University of Colorado 5 ROI CA 27861-03	A Nutritional Control of Cancer
24.	MEYSKENS, Frank L.* University of Arizona 5 POI CA 27502-03	Vitamin A Program Project
25.	MILNER, John A. University of Illinois 5 ROI CA 29462-03	Dietary Arginine and Tumor Growth and Development

26.	MIRVISH, Sidney S. University of Nebraska 1 R01 CA 30593-01A1	Significance of Nitrosourea Formation from Creatinine
27.	MUSEY, Paul I.* Emory University 5 R01 CA 24616-03	The Effect of Diet on Estrogen Biosynthesis and Metabolism
28.	NEWBERNE, Paul M.* Massachusetts Institute of Technology 5 R01 CA 25382-03	Zinc, Nitrosamine, and Esophageal Cancer
29.	NEWBERNE, Paul M. Massachusetts Institute of Technology 5 R01 CA 26917-03	Dietary Fat in Colon Carcinogenesis
30.	NEWELL, Guy R. University of Texas 1 R01 CA 34048-01	Nutrition Methodology for Cancer Studies
31.	PARIZA, Michael W. University of Wisconsin 5 R01 CA 29618-03	Structure and Origin of Mutagens in Fried Beef
32.	PAULING, Linus C. Linus Pauling Institute of Science 5 R01 CA 26541-02	Diet and Breast Cancer in Mice
33.	PAWLOWSKI, Norman E. Oregon State University 5 R01 CA 25766-05	Mechanisms for Biological Activity of Cyclopropenes
34.	PETHICA, Brian A.* Clarkson College of Technology 5 R01 CA 26379-03	Dietary Fiber--the Physical Chemistry of Lignins
35.	ROEBUCK, Bill D.* Dartmouth College 5 R01 CA 26594-03	Modulation of Pancreatic Carcinogenesis by Diet
36.	ROGERS, Adrianne E. Massachusetts Institute of Technology 2 R01 CA 25538-04	Dietary Fat, Prolactin and Mammary Cancer
37.	ROSS, Morris H. Institute for Cancer Research 5 R01 CA 16442-09	Regulation of Tumor Susceptibility
38.	ROTHMAN, Kenneth J. Harvard University 5 R01 CA 29666-03	Case-Control Study of Laryngeal-Hypopharyngeal Cancer
39.	RUDOLPH, Frederick B. Rice University 5 R01 CA 14030-11	Regulation of Metabolism by Purine Interconversions

40.	SARKAR, Nurul H. Sloan-Kettering Institute for Cancer Research 5 ROl CA 25679-03	Effect of Diet on Murine Mammary Tumorigenesis
41.	SCANLAN, Richard A. Oregon State University 5 ROl CA 25002-13	Nitrosamines in Foods
42.	SELIVONCHICK, Daniel P. Oregon State University 5 ROl CA 30087-03	Membrane Protein Composition: Cyclopropanoid Fatty Acid
43.	SHEKELLE, Richard B. Rush-Presbyterian-St. Luke's Medical Center 1 ROl CA30983-01A2	Diet, Alcohol, Tobacco and Risk of Cancer in Men
44.	SHILS, Maurice E. New York Academy of Medicine 5 ROl CA 32241-02	New York/New Jersey Regional Center for Clinical Nutrition Education
45.	SHINOZUKA, Hisashi University of Pittsburgh 2 ROl CA 26556-04	Diet Modification and Promotion of Liver Carcinogenesis
46.	SIDRANSKY, Herschel* George Washington University 5 ROl CA 26557-03	Nutritional Influence on Chemical Carcinogenesis
47.	SINNHUBER, Russell O.* Oregon State University 5 ROl CA 20990-05	Protein Effects of Aflatoxin Carcinogenesis in Trout
48.	SMITH, George S. University of California (LA) 5 ROl CA 26164-03	Dietary Restriction, Cancer and Immune Functions
49.	SPEIZER, Frank E. Channing Laboratory 5 ROl CA 26560-04	Prospective Study of Diet and Cancer in Women
50.	THOMPSON, Henry J. University of New Hampshire 5 ROl CA 28109-03	Nutrition and Mammary Carcinogenesis
51.	TROLL, Walter New York University Medical Center 5 ROl CA 16060-12	Inhibition of Tumor Promotion by Protease Inhibitors
52.	VISEK, Willard J.* University of Illinois 5 ROl CA 28629-03	Hormones, Dietary Fat and Mammary Carcinogenesis

53. WADE, Adelbert E.*
University of Georgia
5 ROl CA 29583-02

Effect of Dietary Fat Type on Chemical Carcinogenesis

54. WARREN, Guylyn R.*
Montana State University
5 ROl CA 26647-03

Mutagenic/Carcinogenic Agents in Body Fluids of Children

55. WEISBURGER, John H.
American Health Foundation
5 POl CA 29602-02

Nutritional Carcinogenesis

56. WEST, Dee W.*
University of Utah
5 ROl CA 25580-03

Diet and Colon Cancer in Man: The Effects of Fiber

57. WHITTEMORE, Alice S.
Stanford University
1 ROl CA 33940-01

Reliability of Long-term Dietary Recall

58. ZAMIR, Lolita O.*
State University of New York
5 ROl CA 32131-02

Biochemical Intermediates of Aflatoxin Biosynthesis

*Grants Active During FY 83 but Funded with Previous Year's Funds.

SUMMARY REPORT

EPIDEMIOLOGY PROGRAM

Description: During this fiscal year there were 56 research grants in the extramural Epidemiology Program with an approximate funding of $11.17 million. Most of these grants were regular research projects; only four were program project grants. One of the program projects has metabolic epidemiology as its focus. The others are concerned with the relationship of hormonal factors to cancer etiology or the role of hepatitis B virus in primary liver cancer. Over the past several years, research emphasis has shifted from deep involvement with common neoplasias, such as cervical cancer, toward a search for risk factors relating to malignancies occurring with intermediate rates such as ovarian cancer, multiple myeloma, brain tumors, malignant melanoma, leukemia and lymphoma. There is increased interest in the role of environmental exposures including pesticides, animal viruses and prior medical therapy. Some of the changing emphasis has resulted from the evolution of programs such as Diet and Nutrition and Biometry. Some change is due to reorganization of the NCI with resulting modification of grant referral guidelines. For example, much breast cancer epidemiology is now supported by regular research project grants administered by the Breast Cancer Program; but some of the shift reflects the increasing availability of large, well established cancer registries, which make it feasible to investigate less common malignancies, and to the general interest in environmental safety which emerged in the late 1970s. To some extent, the pattern of research reflects the state of technologic development. To wit, investigations into the role of genital type herpes simplex virus await the development of laboratory procedures which are specific to the virus and practical for large scale use. Endocrinologic epidemiology has made significant advances, thereby leading research into more promising avenues. Indeed, advances at the interface between epidemiology and laboratory science have led to the formation of a new program area, Biochemical Epidemiology, in this fiscal year.

Research Advances: There is now epidemiologic evidence to support a strong association between several different viral agents and human cancer. These include the Epstein-Barr virus association with nasopharyngeal cancer in China and with Burkitt's lymphoma in Africa, the human T cell leukemia virus with some T cell leukemia in the Caribbean and in Japan, the genital type herpes simplex with cancer of the uterine cervix in the U.S., and hepatitis B virus with primary liver cancer in Africa and Asia.

Worldwide correlations between the prevalence of hepatitis B carriers (individuals whose blood tests for hepatitis B surface antigen are positive) and the risk of primary liver cancer are now documented. A newly reported study of Taiwanese civil servants carries these correlations another step -- to show that young men who migrated from various regions of China to Taiwan continue 20-30 years later to carry antigen titers which correlate well with current hepatoma mortality rates in their native provinces (3). In general, the hepatitis carrier rate projected for China on the basis of Taiwan immigrees suggests a gradient from lower rates in the northwest to higher rates in the southeast below the natural barrier imposed by the Yangtze River. This, taken together with studies of hepatitis virus subtypes and evidence of high levels of maternal-child transmission during infancy, suggests strong genetic and cultural influences on hepatitis B-hepatoma risk. The strength of the maternal-child link is evidenced by the first case report of death

from primary liver cancer of a seven year old child followed prospectively for hepatitis B infection status. The child was negative for hepatitis B at one month of age but by his seventh month had contracted chronic hepatitis B infection from his carrier mother. This child and his parents are members of a large cohort (10,670 women) who underwent screening for hepatitis B status between 1973 and 1978. Sixteen and a half percent of the women were carriers; these carriers and their children remain under surveillance to determine the extent of cancer risk for individuals with chronic hepatitis (3).

A cellular model has been postulated for the pathogenesis of primary hepatic carcinoma. This model is based on data collected in several different geographic areas from people varying in age from infants free of hepatitis infection to individuals with chronic hepatitis B infection, chronic liver disease and/or cancer. The model hypothesizes two types of liver cells, a somatic cell which is well differentiated, capable of only limited additional cell divisions to produce well differentiated cells in which the hepatitis virus can replicate. This viral replication within a somatic cell leads to its death. Substances released by the dying cell stimulate a second, less differentiated type of liver cell, to divide. This less differentiated cell has the capacity to reproduce itself, producing additional undifferentiated or differentiated cells. While the virus cannot replicate itself within the undifferentiated cells, part of the viral DNA can become integrated into the liver cell DNA causing the undifferentiated cell to be susceptible to transformation. However, for persistence of the transformed cells and the development of a malignant clone of cells, continued death of differentiated cells is required. This model has held up through early analyses against experimental data and is undergoing further testing (4).

The bimodal age distribution for Hodgkin's disease has suggested the possibility of more than one etiology, with Hodgkin's disease among children and young adults arising as a rare consequence of infection with an agent which is widely distributed, as occurred with poliomyelitis before immunization was available. Studies of Hodgkin's disease in the Boston area reveal that the disease in children is associated with lower socioeconomic status for the parents. Earlier studies of Hodgkin's disease in young adults show that higher socioeconomic status during childhood confers risk of Hodgkin's disease. This reversed age/socioeconomic risk pattern is characteristic of a disease arising as a rare complication of infection with an agent which is widely disseminated and is similar to the polio pattern (34).

Reporting on a series of 33 cases of rhabdomyosarcoma contrasted with 99 controls matched from North Carolina birth certificates for age, sex and race, Grufferman and colleagues find that lower socioeconomic status is associated with increased risk of rhabdomyosarcoma during childhood. They point out similarities between their findings and several reports of epidemiologic studies of childhood leukemia. Points of similarity, other than socioeconomic status of parents, include increased exposure to preventable infections and more asthma and allergy among family members. Increased risk of rhabdomyosarcoma was also associated with chemical exposures, including a smoking father, and with adverse gestational experiences; i.e., (a) maternal infections during pregnancy, b) prolonged gestation, and c) difficulty during delivery. The small numbers observed and the multiple comparisons made in analysis may have led to spurious associations and for these reasons, a follow-up study based upon larger numbers of children is underway (17).

Numerous epidemiologic studies have documented risk factors for breast and genital cancers in U.S. women consistent with the probability that higher than usual exposure to estrogen leads to elevated cancer risk. This suspicion is reinforced by animal studies in which estrogens serve either as complete carcinogens or promoters for breast cancer. On the other hand, estrogens have been credited with important functions in maintaining tissue integrity and lowering the risk of coronary heart disease and osteoporosis, quite aside from their important role in maintaining sexual identity and reproductive function. The enigma of estrogen's role as a cancer risk factor is increased by the fact that events early in life, such as age at menarche and age at first childbirth, appear to influence risk of cancer decades later.

The complexity of the relationship between cancer risk and estrogens and the overwhelming importance of cancers of the breast and genital system for women has stimulated research over a very broad range which serves not only to improve knowledge of cancer risk, but also to provide a more basic understanding of the relationship between hormone levels and common events important for the health of of women in general. Because international comparisons show that breast cancer mortality rates are correlated with meat consumption patterns, it has been hypothesized that high levels of dietary meat lead to early development of critical levels of adipose tissue and early menarche for girls in western countries. Seventh Day Adventists (SDAs), as well as residents of less developed countries, are at lower risk of breast cancer than U.S. women in general. It was thought the vegetarian diet might delay menarche and explain the lower breast cancer risk among vegetarian Seventh Day Adventists. A study of 23 vegetarian and 26 non-vegetarian girls, age 14-17 years, has failed to support that idea. The vegetarians had never eaten meat and the nonvegetarians had eaten meat, fish, or poultry weekly. The age at menarche of the two groups was not significantly different. Furthermore, no important differences were found in height, weight, proportion of total calories consumed as protein, fat or carbohydrate; or in mean plasma or urine levels of prolactin, progesterone or estrogen measured during follicular or luteal phases of the menstrual cycle. Thus, meat in the diet of children does not appear to be a determinant of adolescent hormonal status (20).

Another study of 511 nulliparous women (15-19 years) and 347 parous women (aged 30-39) compared hormone levels of women by age at menarche and also investigated the relationship between anovulatory cycles, body weight and hormone status. Body weight expressed as Quetlet's index had very little influence on hormone levels, leading the investigators to conclude that among young women, body weight is not a reliable index of hormonal status. However, age and age at menarche both were correlated with estrogen level. Estrogen levels for follicular and luteal phases of the menstrual cycle increased to age 35; thereafter the follicular levels continued to increase while the luteal levels did not. Women who began menstruating early displayed estrogen levels which were higher by about 5% per year of extra menstrual experience; the higher estrogen levels for women with early menarche were observed in women of all ages enrolled in the study. Thus, women experiencing menarche at early age have not only longer exposure to estrogens, but apparently, a lifelong exposure to higher levels (34).

Although dietary meat and body fat in young adulthood do not appear to influence endogenous estrogen levels, other lifestyle factors may. Several studies have observed a borderline protective effect for breast cancer associated with cigarette smoking. Analysis of hormone levels among 106 women aged 25-49 years,

whose smoking status was ascertained as of the time that blood and urine specimens for hormone determinations had been collected, revealed lower estrogen levels during the luteal phase for smokers. Smokers ovulated more frequently than non-smokers as determined by pregnanediol levels in luteal phase specimens. The data suggest that reduction of luteal phase estrogens occurs during ovulatory cycles. On the basis of these findings, the authors postulate that if a protective effect for breast cancer is associated with cigarette smoking, it may be mediated through the lifetime deficit in estrogen exposure leading to early menopause. They note reports of increased risk of osteoporosis/osteoporotic fractures in smoking women as further suggestion that smoking may influence estrogen levels in women (34).

For several years, epidemiologic studies of oral contraceptive use have reported deleterious effects such as increased morbidity and mortality from blood clots, coronary occlusion and stroke. Beneficial effects such as diminished instances of benign breast disease and scattered suggestions that use of oral contraceptives may decrease the risk of ovarian cancer are now emerging which will assist in weighing benefits vs. risks associated with oral contraceptive use. Dr. Daniel Cramer and associates in Boston provide a more definitive report of ovarian epithelial cancer risk based on the study of 144 white women under 60 years of age, compared with 139 similar women selected from the general population of the greater Boston area. Significantly decreased risk of ovarian cancer was associated with the use of oral contraceptives among women who were 40 through 59 years of age at the time of the study. The greatest protective effect was observed in older parous women and in women who had discontinued the use of oral contraceptives more than 10 years previously. No protective effect was observed for women less than 40 years of age when diagnosed as having cancer of the ovary (34).

Projections: An exciting facet of hepatitis-hepatoma research is the potential for primary prevention. Control of insect vectors which may transmit the virus together with administration of immune globulin to infants in hepatitis B carrier households coupled with administration of hepatitis B vaccine should offer long lasting protection against both chronic hepatitis and primary hepatic cancer attributable to hepatitis B infection (4).

While research continues to define the nature of the association between the viruses cited above and their candidate malignancies, the possibility of viral association with still other neoplasias is being studied; for example, Epstein-Barr virus and Hodgkin's disease in young people, herpes and papilloma viruses and cancers of the anogenital area; the human T cell leukemia virus (and other viruses) and the Acquired Immunodeficiency Syndrome/Kaposi's sarcoma.

Several studies supported by the program are nearing completion and should be reporting results within the next year or two. These include three studies of individuals engaged in occupations exposed to animal viruses and in some cases, to pesticides, as well as several studies of malignant melanoma, histiocytic lymphoma, renal cancer and aplastic anemia.

The single most dramatic event reported by the Epidemiology Program in 1982 was the death of a 7 year old Taiwanese child from primary hepatic cancer. The child was born of a carrier mother, was free of disease in early infancy, contracted hepatitis by his seventh month, became chronically infected and died of hepatoma. This documents that childhood as well as adult liver cancer deaths, can be attributed, at least in part, to hepatitis B infection, a preventable situation.

BIOCHEMICAL EPIDEMIOLOGY

The increasing dependence of epidemiologic studies on laboratory procedures to improve the specificity of risk parameters is a trend expected to continue. An exciting development for the program has been its increasing involvement with biochemical epidemiology and the emerging emphasis on the development of new biochemical markers.

Program emphasis in biochemical epidemiology was initiated in 1982, marked by the issuance of a request for applications and a $1.5 million budget reserve. The response was enthusiastic and excellent. At least 11 applications will be funded concerned with a wide variety of cancers and even more varied laboratory procedures. Few responses were concerned specifically with the development or pilot testing of laboratory procedures which show promise of epidemiologic usefulness, a problem which will need to be addressed. The content and scope of funded applications will be considered in determining the need for additional announcements. As a group, the applications reflected strong interest in dietary factors relative to cancer risk. There were no proposals which would improve the laboratory procedures used for assessing diet in cancer epidemiologic studies, another problem area.

SPECIAL PROGRAMS BRANCH

GRANTS ACTIVE DURING FY 1983

EPIDEMIOLOGY PROGRAM

Investigator/Institution/Grant Number	Title
1. AMSEL, Jonathan* University of Pennsylvania 2 ROl CA 23544-03	Case-Control Study of Malignant Melanoma
2. ASAL, Nabih R. University of Oklahoma 5 ROl CA 31059-02	Risk Factors in Kidney Cancer
3. BEASLEY, R. Palmer University of Washington 3 ROl CA 25327-03S2	Hepatocellular Carcinoma Risk in Hepatitis B Carriers
4. BLUMBERG, Baruch Institute for Cancer Research 5 POl CA 06551-19	Cancer Clinical Research at the Fox Chase Center
5. BUFFLER, Patricia A. University of Texas Hlth. Sci. Ctr 1 ROl CA 32584-01	CNS Tumors and Occupational Exposures
6. COMSTOCK, George W. Johns Hopkins University 5 ROl CA 24758-04	Cancer Studies in Washington County, Maryland
7. DALING, Janet R. University of Washington 5 ROl CA 32010-02	Epidemiology of Anal Cancer
8. DAVIS, Scott Fred Hutchinson Cancer Center 5 R23 CA 29395-02	A Case-Control Study of Hodgkin's Disease
9. DODD, Roger American Red Cross 5 ROl CA 31002-03	Hepatitis B Surface Antigen as a Risk Factor for Hepato-cellular Carcinoma
10. DONHAM, Kelley J. University of Iowa 5 ROl CA 28626-03	The Epidemiology of Leukemia in Rural Iowa
11. ENGLUND, Anders International Union Against Cancer 5 R13 CA 05096-21	Program of the International Union Against Cancer

12.	EVANS, Alfred S. Yale University 5 R01 CA 30433-02	Epidemiological Studies of EBV in Hodgkin's Disease
13.	FIALKOW, Philip J. University of Washington 5 R01 CA 16448-09	Human Cancer--Origin and Genetic Markers
14.	FISCHMAN, Harvey R. Johns Hopkins University 5 R01 CA 30410-02	Cancer Mortality Among Workers in the Meat Industry
15.	FRANKEL, Jack W. Life Sciences Biomedical Research Institute, Inc. 7 R01 CA 32953-01	Neurofibromatosis: Study of Prenatal Diagnostic Test
16.	FRIEDMAN, Gary D. Kaiser Foundation Research Institute 5 R01 CA 19939-07	Surveillance for Drugs That May Be Carcinogenic
17.	GRUFFERMAN, Seymour Duke University 2 R01 CA 21244-03A1	The Epidemiology of Childhood Rhabdomyosarcoma
18.	GRUFFERMAN, Seymour* Duke University 2 R01 CA 22104-04	The Epidemiology of Multiple Myeloma
19.	GUTENSOHN, Nancy M. Harvard School of Public Health 5 R01 CA 31747-02	Hodgkin's Disease and Pre-Diagnostic EBV-Antibody Status
20.	HENDERSON, Brian E. University of Southern California 2 P01 CA 17054-07	Cancer Center Epidemiology and Biostatistics Support
21.	HENDERSON, Brian E. University of Southern California 5 R01 CA 32197-02	The Role of Estrogens and Vitamin A in Disease Prevention
22.	HERBST, Arthur L. University of Chicago 3 R01 CA 20084-06S1	Exogenous Maternal Hormones and Cancer in Daughters
23.	HERBST, Arthur L. University of Chicago 1 R01 CA 32012-01	Cancer and Health Risks in DES Exposed Daughters
24.	HOCHBERG, Fred H. Massachusetts General Hospital 5 R01 CA 22533-05	Epidemiology of Brain Tumors

25. HUTCHISON, George B.
Harvard University
5 ROl CA 22849-06

Second Cancers in Patients with Hodgkin's Disease

26. JOHNSON, Carl J.
Medical Care & Research Foundation
3 ROl CA 32565-01S1

Evaluation of Low-Level Plutonium and Cancer

27. KOLONEL, Laurence N.
University of Hawaii
5 ROl CA 30119-02

A 50,000 Member Cohort Study of Alcohol and Cancer

28. KURLAND, Leonard T.*
Mayo Foundation
5 ROl CA 25441-03

Study of Males Exposed in Utero to Diethylstilbestrol

29. LAWRENCE, Charles E.*
New York State Dept. of Health
5 ROl CA 24367-03

Endometrial Cancer Epidemiology and Control

30. LEVITON, Alan
Childrens' Hospital & Medical Ctr.
2 ROl CA 21819-06

Classification and Etiology of Childhood Brain Tumors

31. LITVAK, Jorge*
Pan American Health Organization
1 R13 CA 30307-01

Cancer Epidemiology in Latin America (Meeting)

32. LOPEZ-S, Arthur*
Louisiana State University
3 ROl CA 23205-03S1

Lung Cancer and Vitamin A

33. MACK, Thomas M.
University of Southern California
5 ROl CA 23927-04

Case-Control Study of Malignant Melanoma

34. MACMAHON, Brian
Harvard University
5 POl CA 06373-22

Cancer Epidemiology and Prevention Research Center

35. MACMAHON, Brian
Harvard University
5 ROl CA 29723-02

An Epidemiological Study of Renal Adenocarcinoma

36. MARMOR, Michael
New York University
1 ROl CA 33205-01

Risk Factors for Kaposi's Sarcoma in Homosexual Men

37. 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

38. MEADOWS, Anna
Children's Hospital of Philadelphia
5 ROl CA 29275-03
Heredity and Environment in Childhood Cancer

39. MOSS, Andrew R.
University of California (SF)
5 ROl CA 34188-02
Testicular Cancer and Prenatal DES Exposure

40. NASCA, Philip C.
New York State Dept. of Health
5 ROl CA 26194-03
Epidemiologic Study of Childhood Gliomas

41. PRESTON-MARTIN, Susan
University of Southern California
5 ROl CA 28215-04
Epidemiology of Tumors of the Parotid Gland

42. REEVES, William C.*
Gorgas Memorial Institute of Tropical and Preventive Medicine
3 ROl CA 25419-03S1
Cervical Cancer Epidemiology in Panama

43. ROSS, Ronald K.
University of Southern California
5 ROl CA 24082-04
Immunoblastic Lymphadenopathy and Histiocytic Lymphoma

44. ROSS, Ronald K.
University of Southern California
5 ROl CA 25669-05
Epidemiology of Cancer of the Renal Pelvis and Ureters

45. SAMET, Jonathan M.
University of New Mexico
5 ROl CA 27187-03
Lung Cancer Etiology in New Mexico's Hispanics and Anglos

46. SPEIZER, Frank E.*
Peter Bent Brigham Hospital
3 ROl CA 23645-05S1
A Prospective Cohort for Risks in Breast Cancer

47. STARK, Alice
New York State Dept. of Health
5 R23 CA 29713-02
Cancer Incidence and Death from All Causes in Farmers

48. SZKLO, Moyses
Johns Hopkins University
5 ROl CA 24757-04
Epidemiology of Aplastic Anemia in Baltimore

49. SZKLO, Moyses
Johns Hopkins University
5 ROl CA 26500-04
Epidemiological and HLA Study of Leukemia

50. SZKLO, Moyses
Johns Hopkins University
1 ROl CA 33822-01
Epidemiologic and Immunogenetic Study of Macroglobulinem

51.	VERNICK, Leonard J. Illinois Cancer Council 1 R01 CA 34044-01	Cholecystectomy and Subsite-Specific Large Bowel Cancer
52.	WALLACE, Robert B. University of Iowa 5 R01 CA 15104-09	Anovulation and Epidemiology of Hormone-Responsive Tumors
53.	WEISS, Noel S. Fred Hutchinson Cancer Research Ctr. 5 R01 CA 23350-04	Epidemiology of Myeloma and Lymphocytic Leukemia
54.	WEISS, Noel S. University of Washington 5 R01 CA 30279-02	Vasectomy as a Risk Factor for Testicular Cancer
55.	WILLETT, Walter L. Peter Bent Brigham Hospital 1 R01 CA 33008-01	Prospective Study of Selenium and Cancer in Women
56.	WYNDER, Ernst L. American Health Foundation 1 P01 CA 32617-01	Interdisciplinary Studies in Cancer Epidemiology

*Grants Active During FY 83 but Funded with Previous Year's Funds.

SUMMARY REPORT

SMOKING AND HEALTH PROGRAM

Description: The extramural Smoking and Health Program (SHP) in the Division of Cancer Cause and Prevention is involved in efforts to understand and mitigate the deleterious effects of smoking on health. The program is presently supporting seven grants, funded at $2.89 million, three Interagency Agreements and one contract at a funding level of $0.83 million. Of the research grants active in the program, two resulted from specific RFAs issued by this program area.

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 histological 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 commercial cigarettes by the consumer. Current program emphasis is focused on epidemiological and toxicological aspects of the problem.

Research Accomplishments: The Smoking and Health Program continues to support research on identification of carcinogens in tobacco products.

The relationship between tobacco nitrate and tobacco-specific nitrosamines in mainstream smoke has been examined. Potassium nitrate was added to standard reference (1R1) cigarettes by the syringe injection technique and the mainstream smoke was analyzed for tobacco-specific nitrosamines. The results clearly indicate that tobacco nitrate is a contributing factor to the levels of N'-nitrosonornicotine (NNN), N'-nitrosoanabasine (NAB), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and N'-nitrosoanatabine (NAT) observed in mainstream smoke (5).

Preliminary experiments have been conducted on the detection of tobacco-specific nitrosamines in smoker's blood. Blood (150 ml) from a heavy smoker was analyzed after extraction by GC-TEA. Levels of tobacco-specific nitrosamines were below the detection limit of this method (approximately 7.5 ng/150 ml) (5).

A bioassay of NNK in Syrian golden hamsters, with promotion by inhalation of cigarette smoke, was recently completed. Four groups of 20 hamsters each were given single subcutaneous injections of either 10.0, 3.3, 1.0, or 0 mg of NNK in trioctanoin. One week later, smoking was commenced and continued for 62 weeks. Control groups were treated with solvent or NNK followed by sham smoking. Tumors of the lung, nasal cavity, and trachea were observed in all NNK treated groups. Smoking did not cause a significant increase in tumor incidence. It was noted, however, that a significant incidence of tumors was observed in the hamsters treated with only a single dose of 1.0 mg of NNK (5).

A dose-response study in F-334 rats of NNN, NNK, and NAT has been completed. Animals were given subcutaneous injections three times weekly of the nitrosamines

for 20 weeks and then observed until moribund. Total doses were approximately 9, 3, and 1 mmole/kg body weight. Histopathological evaluation is currently in progress. Preliminary results indicate that the lowest dose of NNK caused as high an incidence of tumors of the nasal cavity, lung, and liver as previously observed with much higher doses (4).

The Environmental Protection Agency recommended banning the use of maleic hydrazide-diethanolamine formulations (MH-30) for treatment of tobacco as of October 1981 (Federal Register, Sept. 16, 1981, 46, No. 17945999). In order to monitor the expected decrease of N-nitroso-diethanolamine (NDELA) in commercial tobacco products, representative amounts of products (three types of cigarettes, chewing tobacco and fine cut snuff) were analyzed for levels of NDELA. The decrease between the years 1981 and 1983 ranged from 14% (cigarette) to as high as 56% (fine cut snuff) (5).

Epidemiology studies utilizing histologically confirmed lung cancer cases in nonsmokers are progressing on schedule. Based on cases and controls ascertained to date, the study should obtain a total of 225 screened and confirmed lung cancer cases in nonsmoking females and the same number for males as projected in the protocol for the current study period (6).

A prospective study, utilizing a self-administered questionnaire is also proceeding on schedule. At the current rate of enrollment in the study, it is anticipated that approximately 100,000 participants will be in the study by the end of calendar year 1983. Analysis of the data for possible health effects of the newer lower tar and nicotine cigarettes is underway (9).

The manufacture of a low-yield reference cigarette (LYRC) has been completed and cigarettes are now in cold storage. Chemical and biological testing for reference purposes is now underway. The target date for initiating distribution of the reference cigarette is February 1984 (10).

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 additional work needs to be performed.

Review of responses to the Request for Applications (RFA), entitled "The Pharmacological Role of Nicotine in Diseases Related to Tobacco Products," is nearing completion. Work on studies funded under this initiative is expected to be underway before the end of the current fiscal year. Responses from a Request for Proposals (RFP), entitled "Cigarette Smoke Yield and Smoker Compensation," are in the review process, and a contract to study this problem is also expected to be awarded during this fiscal year.

Epidemiology studies utilizing histologically confirmed lung cancer cases in nonsmokers, with properly matched controls, will continue to be supported during the coming year (6). Additional information in this area should be very valuable in determining the extent of health effects in humans exposed to conditions of involuntary smoking. Identification of individuals at elevated risk of developing tobacco-related disease continues to be of high priority. Continuation of the existing prospective epidemiologic study provides the potential for profiling characteristics which may contribute to susceptibility (or resistance) to smoking-related illness.

Activities carried out under the Interagency Agreement with Oak Ridge National Laboratories (9) will continue as a resource to the NCI Smoking and Health Program. Analyses of major whole smoke components in domestic cigarettes will be made available for reference to other Divisions of the NCI and, upon request, to the scientific community at large.

The histopathologic evaluation of tissues from animal inhalation experiments conducted in this program will be completed (8). These results should yield valuable information on the relationship of whole cigarette smoke dose and nicotine concentrations to smoking-related diseases.

SPECIAL PROGRAMS BRANCH

GRANTS ACTIVE DURING FY 1983

SMOKING AND HEALTH PROGRAM

Investigator/Institution/Grant Number	Title
1. BENOWITZ, Neal L. University of California (SF) 1 R01 CA 32389-01	Nicotine and Tar Intake During Cigarette Smoking
2. CASTONGUAY, Andre American Health Foundation 1 R01 CA 32391-01	Tobacco-Specific Nitrosamine: RIA for DANA-Adducts
3. COLE, Philip University of Alabama 5 R01 CA 29968-02	Hepatocellular Carcinoma and Cigarette Smoking
4. HECHT, Stephen S. American Health Foundation 2 R01 CA 21393-07	Metabolism of the Carcinogen Nitrosonornicotine
5. HOFFMANN, Dietrich American Health Foundation 5 P01 CA 29580-02	Experimental Tobacco Carcinogenesis
6. JANERICH, Dwight T. New York State Dept. of Health 5 R01 CA 32088-02	Epidemiology of Lung Cancer in Nonsmokers
7. MCCOY, George D. Case Western Reserve University 5 R01 CA 32126-02	Role of Ethanol in the Etiology of Head and Neck Cancer

CONTRACTS ACTIVE DURING FY 1983

Investigator/Institution/Contract Number	Title
8. AUERBACH, Oscar Veterans Administration Medical Center Y01 CP8 0201	Inhalation Bioassay of Cigarette Smoke in Male Beagle Dogs
9. FRIEDMAN, Gary Kaiser Foundation Research Inst. N01 CP0 5681	Surveillance of the Health Effects of Tobacco Products

Investigator/Institution/Contract Number	Title
9. GUERIN, Michael Energy, Department of Y01 CP6 0206	Collection, Separation, and Elucidation of the Components of Cigarette Smoke and Smoke Condensates
10. TSO, T.C. Agriculture, Department of Y01 CP2 0201	Development, Production and Evaluation of Low-Yield Reference Cigarettes
11. WYNDER, Ernst American Health Foundation N01 CP0 5684	Epidemiology of Smoking-Related Diseases

☆ U.S. GOVERNMENT PRINTING OFFICE: 1983-381-132:3189