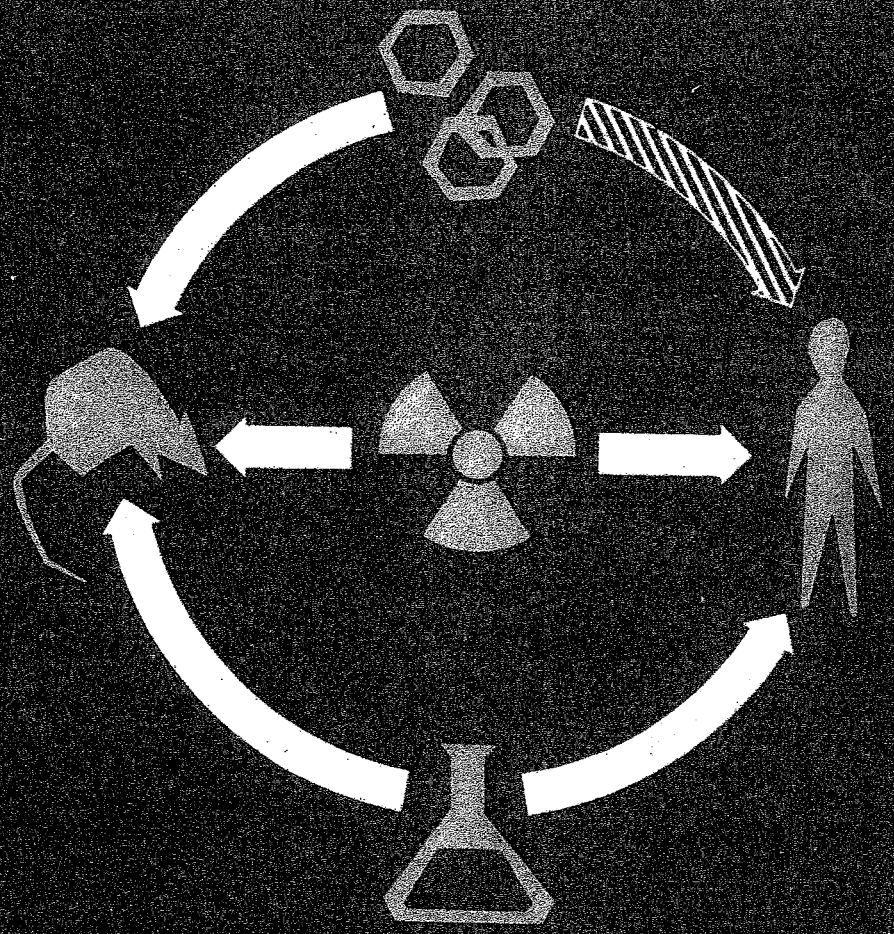
special virus - cancer program

AUGUST 1971



Etiology Area - National Cancer Institute

U. S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

Public Health Service

National Institutes of Health

SPECIAL VIRUS CANCER PROGRAM

PROGRESS REPORT #8

Program Staff
Viral Oncology, Etiology Area
National Cancer Institute*
July, 1971**

* National Institutes of Health, Public Health Service. U.S. Department of Health, Education, and Welfare, Bethesda, Maryland

** This report was prepared by senior staff of the Viral Oncology Area, Etiology, NCI and submitted to NIH as the area's Annual Report in May, 1971. It was updated in July, 1971 for the Annual Joint Working Conference, SVCP at the Hershey Medical Center, Hershey, Pa., October 24-27, 1971.

PROGRESS REPORT

OFFICE OF THE ASSOCIATE SCIENTIFIC DIRECTOR FOR VIRAL ONCOLOGY (OASDVO)

July 1, 1970 - June 30, 1971

J. B. Moloney, Ph.D.

I. VIRAL ONCOLOGY AREA - SPECIAL VIRUS CANCER PROGRAM (SVCP)

A. Introduction:

The Viral Oncology Area is responsible for planning and conducting the Institute's program of coordinated research on viruses as etiological agents of cancer. Scientists within this Area not only provide the broad operational management for intramural and collaborative research but also conduct comprehensive investigations on specific animal oncogenic viruses and their interaction with host cells and apply this information to search for viruses which may be etiologically related to the initiation and continuation of human cancer.

Contract supported research is conducted within the Viral Oncology Program under the Special Virus Cancer Program (SVCP) whose primary objectives are: (1) to determine whether viruses comparable to those known to induce cancers of laboratory and domestic animals are causative agents of human cancers, and (2) to develop therapeutic and preventive measures for control of human cancers when such causative agents are found. A detailed history of events leading to the development of the SVCP may be found in previous Annual Reports of the NCI. Briefly, in 1964, the Congress of the United States provided funds to the NCI for an intensified program in virus-leukemia research because many scientists were convinced that an effort to identify viruses or to detect virus expression in human tumors would contribute to the determination of the etiology of cancer. Using a new planning approach (Convergence Technique), an overall program aimed at controlling human leukemia and lymphoma was formulated. It is based on the premise that one virus is an indispensable element for the induction (directly or indirectly) of at least one kind of human cancer and that the virus or viral genome persists in the diseased individual. In 1968, the program was enlarged to encompass all types of cancer. The Program plan has undergone considerable revisions since its inception and has been reviewed regularly by the Director, NIH; Director, NCI; the National Cancer Advisory Council; the Scientific Directorate, NCI; and the Etiology Program Management Group, NCI.

During the past seven years, the Institute has developed an effective management program which has made rapid, substantial progress in understanding cancer induction by viruses. The funding level for this Program in fiscal year 1971 has been about $35 million.

B. Organization:

1) Viral Oncology Area. During this reporting period the area was reorganized in a structure that will permit the most effective use of scientists and facilities in the attainment of Program goals. All changes were implemented with existing personnel, space, and funds. By increasing the number of sections within the present three branches, additional positions of authority and responsibility were created.

On February 1, 1971 the Deputy Director for Science approved the NCI request to establish three offices and to redefine the functions of existing branches in the Office of the Associate Scientific Director for Viral Oncology. The Office of the Associate Scientific Director for Viral Oncology is organized as follows:

Office of the Associate Scientific Director for Viral Oncology

Plans and conducts the Institute's program of research and development dealing with viruses as etiological agents of cancer. Supports programmatic investigations aimed at the detection, propagation, characterization, prevention, and control of tumor viruses and/or their induced diseases.

Office of the Coordinator for Ultrastructural Studies
Immediate Office of the Coordinator
Virus Studies Section

Plans and conducts research on the morphology, ultrastructure, biological characterization and correlation of structure and function of viral agents associated with neoplasms of man and animals.

Office of Biohazards and Environmental Control
Immediate Office of the Chief
Biohazards Research Section
Environmental Control Section

Performs research in virology, aerobiology, mammalian physiology and biochemistry in order to evaluate the risk involved to the host under stress when exposed to infectious agents. Develops and recommends equipment and procedures for handling of potentially biohazardous materials and disseminates this information to the scientific community and to research laboratories throughout the world.

Office of Program Analysis and Communications

Manages computerized systems of program statistics, clinical and laboratory data interchange, and scientific information for collaborative studies with inhouse and contract scientists; applies computer techniques to data systems and retrieval problems of inhouse and contract laboratories; plans and conducts surveys; plans, implements, and maintains a controlled central data system and inventory for a multi-institutional human serum bank. Maintains a system of triannual progress reporting from research contracts; compiles and distributes summaries of current progress to program scientists. Maintains an automated system for publication searches and reference printouts on requested subjects in viral oncology; prepares a monthly publication featuring abstracts of current worldwide literature on viral oncology; compiles and publishes semiannually indexed summaries of

for establishing this new segment is as follows: since the initiation of the Special Virus Cancer Program, exploratory studies based on information and leads from the mouse-mammary tumor virus (MTV) system have been conducted to determine whether evidence could be found for an association of viruses with breast cancer of humans or of animals other than mice, the only species, thus far, in which breast cancer is known to be caused by a virus.

The similarity of the "clustering" of breast cancer within human family groups to the "clustering" that had been observed for breast cancers of mice at the turn of the century, before the development of inbred mouse strains, suggested that a virus comparable to the mouse MTV might be involved in human disease. Since the MTV was not discovered until high breast cancer strains of mice had been developed by selective inbreeding, human population groups which would most closely approximate the experimental mouse models were selected for study. These included: (1) women of the relatively highly inbred Parsi sect of Bombay, India; (2) women of this country having a history of "family clustering" of breast cancer, e.g. among the mothers, sisters, maternal grandmothers, and maternal aunts of probands to be studied; and (3) women who actually have had breast cancer, but who have survived after successful treatment by removal of one breast. Other leads from the mouse model involved the use of milk and of tissue culture-propagated breast cancer tissue, as the specimens most likely to contain sufficient amounts of an etiologically related virus for detection of electron microscopy (the only method available for the detection of a human agent at this time).

The results of the human studies accumulated through this year provide substantial electron microscopic evidence of virus-like particles resembling the RNA tumor viruses of animals (both B- and C-type particles have been observed) in the milk of women belonging to the above study groups, with much higher frequencies than in milk from normal women of the general population in two different geographical areas studied (Philadelphia, Pa. and Washington, D. C.). Whereas the control studies in the latter areas showed positive specimens with frequencies of 4.5% (7/156) and 2.3% (1/43), respectively, the frequencies in the test-population groups ranged from 37.5% (6/16) for women who have had breast cancer, to 60% (6/10) for normal women belonging to high-breast-cancer families. The frequency for normal women of the Parsi sect was 39.1%, (18/46), and the average for all test groups was 41.7% (30/72). In addition to these highly significant results of studies involving milk, similar virus-like particles have also been observed in 11% (5/45) of tissue culture explants of breast cancer tissue maintained for 60 days or more, and in a continuous cell line from a pleural effusion associated with metastatic breast cancer.

As previously reported, candidate C-type viral agents have been isolated from a spontaneous breast cancer of a rhesus monkey and from a transplantable breast cancer of a laboratory rat. Both viruses are being successfully propagated in the laboratory, and are now being further investigated. Both have been shown to contain 70S RNA as well as the RNA-dependent DNA polymerase, characteristics of the RNA tumor viruses.

Intramural Review Committees

Virus Cancer Program Coordinating Committee

Dr. Stuart Aaronson
Dr. James Duff
Dr. Peter Fischinger
Dr. Berge Hampar
Dr. David Howell, Executive Secretary
Dr. Robert Huebner
Dr. Robert Manaker
Dr. J.B. Moloney, Chairman
Dr. Wade Parks
Dr. Jeffrey Schlom
Dr. Edward Scolnick
Dr. Louis Sibal, Vice-Chairman
Dr. George Todaro
Dr. George Vande Woude

Viral Oncology Program Resources & Logistics Advisory Group

Dr. Jack Gruber, Chairman
Dr. Garrett Keefer, Executive Secretary
Dr. Peter Fischinger
Dr. Clarice Gaylord
Dr. Maurice Guss
Dr. Takis Papas
Dr. Ernest Plata
Dr. John Stephenson
Dr. David Troxler
Dr. George Vande Woude
Dr. David West
Dr. Wilna Woods

Clinical Advisory Group for Viral Oncology

Dr. Michael Blaese (DCBD)
Dr. Peter Fischinger (DCCP)
Dr. Robert Gallo (DCT)
Dr. Sylvan Green (DCCP)
Dr. Curtis Harris (DCCP)
Dr. Ronald Herberman (DCBD)
Dr. Paul Levine (DCCP)-Chairman
Dr. Franco Muggia (DCT)
Dr. Alan Rabson (DCBD)

Contract Specialists (Research Contracts Branch)

*Mr. William Caulfield
Mrs. Dorthy Coleman
**Mr. Charles Fafard
Mr. Sidney Jones
Mr. Jacques Labovitz
Mr. J. Thomas Lewin
Mr. Thomas Porter
Mr. Clyde Williams

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*Deputy Chief
**Chief

activity requires close and frequent communication between the project officer and the contract's principal investigator. The fine balance of coordination is maintained by frequent telephone conversations, written communications and by frequent visits between the project officer and the contractor. The functions of a good project officer require a lot of time which he might personally prefer to spend conducting his own research. The need, however, for existing and additional scientific personnel to continue and assume this managerial role cannot be overemphasized.

Executive Secretaries. The executive secretaries assist the segment chairmen and the project officers in the managerial duties that necessarily accompany the administration of a large research contract program. Each executive secretary is a senior scientist in his own field of specialty and thus consults with the segment chairmen, the project officers and the principal investigators representing the contractor in scientific aspects of the research being performed. A high level of scientific knowledge is necessarily required, since it is often impossible to separate scientific decisions from management decisions. Each executive secretary is specifically responsible for assuring that (1) each contract in his area receives optimal review; (2) working group members, as well as NCI and NIH officials are provided with required documentation; (3) appropriate recording of the proceedings of each meeting is made and that the information gleaned from the meetings is distributed to the proper scientific and management recipients; and (4) site visits are planned, implemented and documented as they are needed and required.

Contract Specialists. The contract specialists are the bridge between the scientists who administer research and the administrative mechanism whereby the research is implemented through the awarding and fiscal monitoring of a research contract. Within the Etiology Area, contract specialists are well conversant with scientific aspects of the program. They constantly provide invaluable advice in fiscal and legal matters to the project officers, the executive secretaries, the program segment chairmen and the Associate Scientific Director. It is the contract specialists, by combining their scientific comprehension with their administrative knowledge and skill, that allow the senior scientists to manage such a large and multidisciplined research program. This is an especially pertinent contribution to the total effort in the face of the present critical personnel shortages under which the program must and will continue.

Working Groups. The program segment working groups are the basic operational units for the SVCP. They are composed of both Federal and non-Federal scientists representing expertise in the varying areas of research involved in the program. Each working group is chaired by a senior NCI scientist. Although the primary function of the working groups is to provide advice to the Institute on the research and scientific aspects of the program, the participation of the members of the working groups is more extensive

CONSULTANTS

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Dr. Patrick Lai, Yale University, New Haven, CT
Dr. Boris Lapin, Sukhumi Institute of Experimental Pathology & Therapy, Sukhumi, USSR
Dr. Edwin Lennette, Department of Public Health, Berkeley, CA
Dr. Arleen C. Levin, Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL
Dr. Arnold Levine, Princeton University, Princeton, NJ
Dr. Diana Lopez, University of Miami, School of Medicine, Miami, FL
Dr. Tom Maniatis, Cold Spring Harbor Laboratories, Cold Spring Harbor, NY
Dr. Charles McGrath, Michigan Cancer Foundation, Detroit, MI
Dr. William McGuire, University of Texas Health Science Center, San Antonio, TX
Dr. Dan Medina, Baylor University College of Medicine, Houston, TX
Dr. Robert C. Nowinski, Fred Hutchinson Cancer Research Center, Seattle, WA
Dr. Fred Rapp, Pennsylvania State University, Hershey, PA
Dr. William Riggsby, University of Tennessee, Knoxville, TN

program segment chairman to the Program Chairmen's review group where it is evaluated for relevance, priority and need to attainment of SVCP objectives and goals.

The SVCP is continuously changed, refined, reviewed, and evaluated by the Science Management Team and its key scientific advisors.

In summary, program review is effected at three levels, all of which are highly integrated and interrelated:

(1) The Contract is the basic functional unit and receives almost constant monitoring by the project officer and technical review by the working group who makes recommendations regarding goals and objectives to the Segment Chairman.

(2) Program segment working groups, as the basic operational units, review the contract performance for technical and scientific competence and adherence to contract workscope, for its contribution to segment goals and makes recommendations to the respective segment chairman concerning each of the segments activities.

(3) Program segment chairmen meeting jointly once a month, comprise the basic integrative unit and determine segment priorities within overall total program objectives and goals.

Contract Review. Contracts are reviewed according to their type.

Type I. The majority of the contracts in SVCP are of this type. These are research and development contracts which are generally limited to one or two areas of research and may include a service aspect. The dollar range is usually between $50,000 and $500,000 per year.

Type II. These are large and/or multi-faceted contracts which involve a number of research areas or have other special features. These are generally $1,000,000 and over and require special review consideration.

Type III. These are routine procurement contracts which cover the purchase of services and/or materials and contain essentially no research.

There are three major review groups: the Segment Working Group, the Program Segment Chairmen and the Etiology Program Management Group.

Progress Reports. As a valuable aid, in the review process, to all key members of the staff, the Program Analysis and Communications Section coordinates and handles progress reports on all SVCP contracts. Policy regarding progress reports was set forth in a memorandum dated November 6, 1968, from the Associate Scientific Director to the Segment Chairmen as follows:

b. The Virus Cancer Program (VCP)

Background. At the present time the Virus Cancer Program is administered by the Collaborative Research Branch with five Sections: the Breast Cancer Virus Studies Section; the Clinical Studies Section; the Co-carcinogenesis Studies Section; the DNA Virus Studies Section; and the RNA Virus Studies Section. Virus-cancer research is now managed by the heads of each Section under the general supervision of the Branch Chief, the Associate Chief, and the Office of the Associate Director; Section heads no longer conduct intramural research.

Advice on overall Program direction is now provided by the VCP Advisory Committee, consisting of seven members including the Chairman. The members are chosen entirely from the biomedical community on the basis of their qualifications and expertise in the cancer field, in particular cancer virology. The Committee has met on several occasions to advise on areas for expanded research and development, and the application of research findings to the control of cancer in man. The Committee's efforts are separate from the review and approval of individual contracts.

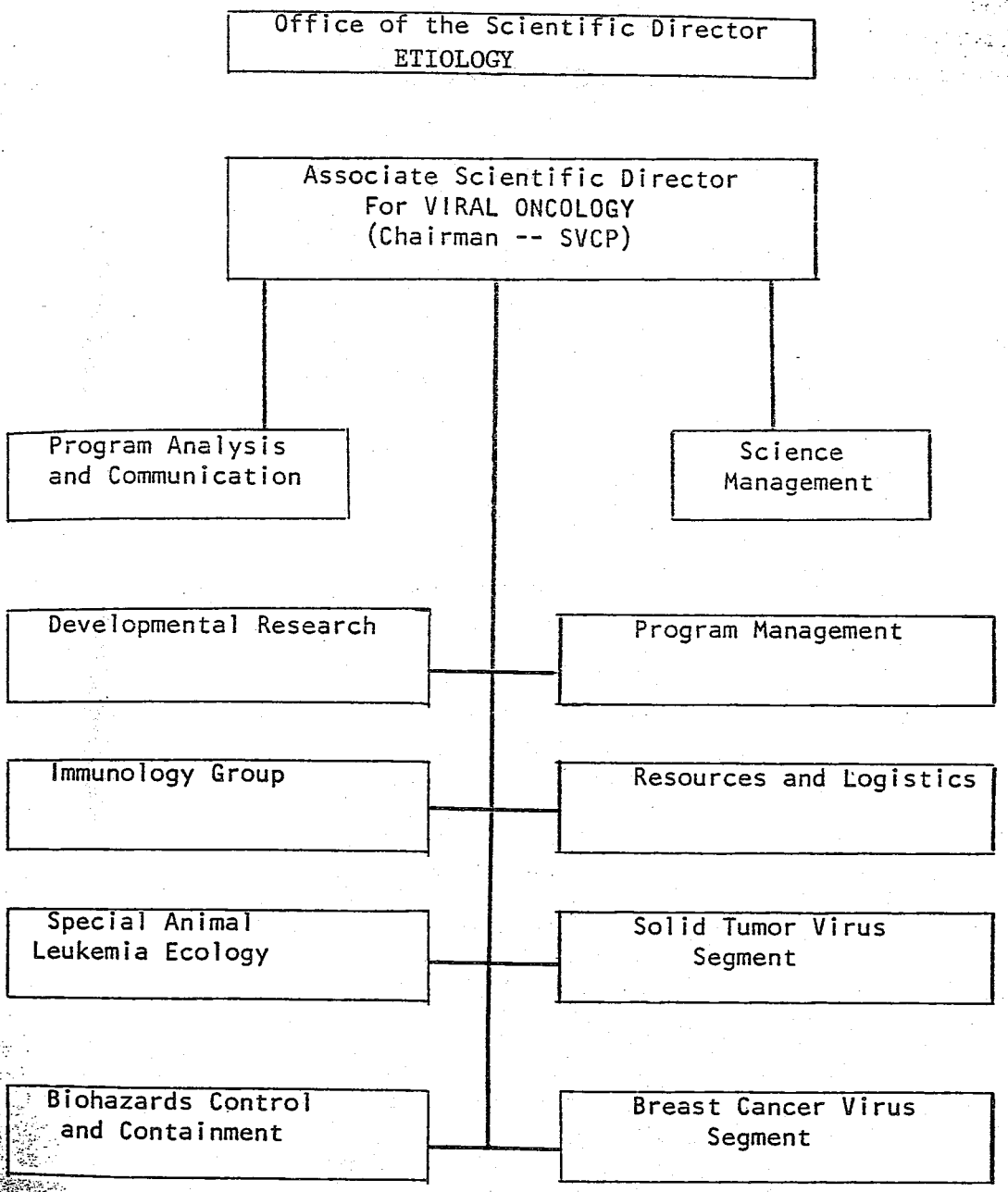
Review of Contracts. Projects within the total Program are reviewed at many levels:

(1) Each contract is reviewed for relevance, priority and need to the total Program by the Virus Cancer Program Coordinating Committee (VCPCC). This Committee, composed of members of the Office of the Associate Director, the Collaborative Research Branch, and senior intramural investigators, reviews solicited and unsolicited proposals developed within the Program. Consensus is obtained by open vote and priority is established by scoring on secret ballot.

(2) Each contract is reviewed for scientific excellence and technical competence by Scientific Review Committee A or B. These committees, first chartered in FY 1975, consist of up to 25 members each, including the Acting Chairman. The members were appointed by the Director, NCI, to serve overlapping terms of four years on the basis of their outstanding qualifications in the fields of microbiology, molecular biology, immunology, biochemistry, virology, and clinical medicine, as related to cancer. The committees are composed entirely of non-NCI scientists. Although intramural scientists participate as needed in the review process, they are not voting members. The committees operate entirely under the regulations set forth by the OMB, DHEW, NIH, and NCI, with an Executive Secretary who is responsbile for the review and complete documentation of each project.

(3) Each contract is continually monitored for performance by (a) the Associate Director's Science Management Team; (b) the Collaborative Research Branch; (c) CRB Section Heads; (d) Project Officer(s). Project Officers are intramural senior laboratory investigators who serve as advisers to principal investigators on scientific matters.

ORGANIZATIONAL CHART -- SPECIAL VIRUS CANCER PROGRAM



(2) Coordinating Committee review. (Coordinating Committee determines relevance, priority, need and uniqueness.)

(3) Project Plan drafted. (Project Plan document, Justification for Non-Competitive Procurement, prepared by Program staff member and Contract Specialist.)

(4) Technical review. (Technical review of proposal performed by Scientific Review Committee.)

(5) Project Plan and Review Summary Sheet approved. (Contract Specialist routes Project Plan document and Review Summary Sheet for signatures.)

(6) Negotiation and award. (Contract Specialist negotiates contract with help of Project Officer as required.)

Scientific Staff, Office of the Associate Director, Viral Oncology Program

Dr. J.B. Moloney, Associate Director
Dr. L.R. Sibal, Deputy Associate Director
Dr. D.M. Howell, Assistant to the Deputy Associate Director
Dr. H.J. Hearn, Scientific Coordinator for Viral Oncology, Frederick Cancer Research Center (FCRC)

Science Management Team, Office of the Associate Director, VOP

Dr. J.T. Duff
Mr. Charles Fafard
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Administrative Staff, Office of the Associate Director, VOP

Mr. John Miller, Administrative Officer, DCCP
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Solid Tumor Virus Segment

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Dr. James Duff, Vice Chairman
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Dr. Charles Boone, NCI
Dr. Maurice Green, St. Louis University
Dr. Leonard Hayflick, Stanford University
Dr. Karl Hellstrom, University of Washington
Dr. Edwin Lennette, Calif. Dept. of Public Health
Dr. Joseph Melnick, Baylor University
Dr. Herbert Rapp, NCI
Dr. Wallace Rowe, NIAID, NIH
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Immunology Group

Dr. Paul Levine
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Dr. Ernest Plata, Executive Secretary
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Dr. Tibor Borsos, NCI
Dr. Ronald Herberman, NCI
Dr. Maurice Hilleman, Merck Institute
Dr. Barry Bloom, Einstein College of Medicine
Dr. Arthur Brown, University of Tennessee
Dr. George Santos, Johns Hopkins University

Resources and Logistics Segment

Dr. Robert Holdenried, Chairman
Dr. George Todaro, Vice Chairman
Dr. Roy Kinard, Executive Secretary
Dr. James Duff, NCI
Dr. K. Arnold Fowler, NCI
Dr. Adi Gazdar, NCI
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Miss Marie Purdy, NCI

Type C Viruses. While some RNA tumor viruses exist primarily as infectious particles, most are in a repressed form integrated into the cellular chromosomes. These endogenous viruses are derepressed by x-irradiation, chemicals, and infection by other viruses. However, some endogenous viruses replicate very poorly in the cells that harbor them (xenotropic) and require special techniques of cell fusion of growth in a mixed culture. Often they can productively infect only cells from animals foreign to the host species. Another class of endogenous viruses (ecotropic) preferentially infect and grow in cells from their own host species. There are also endogenous viruses which are defective for replication and can only be recognized by the presence of their proteins or nucleic acids.

Related RNA viruses readily undergo genetic recombination in cells. Such exchanges occur between endogenous and infectious viruses of the same species and between viruses of different species. Interference between xenotropic and ecotropic viruses does not occur; interference does exist among members of the same class of viruses. Thus, replication of xeno- and ecotropic viruses in the same cell can result in phenotypic alterations involving the antigenic coat and host range of the progeny viruses. Recent studies show that genetically stable recombinant viruses also occur. Such recombinants may be important as the real oncogenic variants, since highly oncogenic strains of sarcoma and leukemic viruses have been observed as a result of genetic recombination. The rapidly transforming virus, the Friend spleen focus-forming virus (a defective virus), is an example of a recombinant between an eco- and xenotropic mouse type C virus. Some of these type C viruses readily transform cells in culture and most produce cancer in animals in days rather than months after infection. These highly oncogenic viruses contain two types of genes: those involved with viral replication and virion structural proteins, and those specifically responsible for cell transformation.

Recently, it has become possible to separate the transformation gene sequences from the replication gene sequences. Essentially this is done by breaking DNA transcripts of viral RNA into pieces and absorbing out those pieces that have replication sequences by a series of nucleic acid hybridizations, leaving only the transformation sequences. Thus, preparing specific cDNA probes from mixtures of sarcoma and helper viruses permitted selecting of virus mixtures highly enriched for sarcoma virus-specific RNA. K-MSV and H-MSV strains have been found to contain a unique set of rat sequences which have become associated in covalent linkage to MuLV information. These rat sequences, called 'sarc' genes, apparently code for the transforming functions of sarcoma viruses.

The transforming sequences from different highly oncogenic viruses have been purified and it has been reported that these transforming sequences are widespread in the normal cells of the animals from which they are derived. However, these sequences represent only a small part of the complete viral RNA and apparently form no protein product that is contained in the viral particle. Consequently, until the transforming sequences were purified, the probes that were used to detect viral activity could not detect either transforming sequences or their products. With the new transformation-specific probes it is now possible to detect viral activity where none could

3) LIST OF CONSULTANTS TO THE SPECIAL VIRUS CANCER PROGRAM

FISCAL YEAR 1971

Dr. Norman Anderson, Atomic Energy Commission

Dr. Arthur Axelrod, University of Toronto

Dr. David Baltimore, Massachusetts Institute of Technology

Dr. Joseph Beard, Duke University

Dr. Trygve Berge, American Type Culture Collection

Dr. Maurice Black, Flower and Fifth Avenue Hospital, N.Y.C.

Dr. Paul Black, Harvard Medical School

Dr. Barry Bloom, Albert Einstein College of Medicine

Dr. R. G. Bond, University of Minnesota

Dr. Michael Brennan, Michigan Cancer Foundation

Dr. Arthur Brown, University of Tennessee

Dr. Joseph Burchenal, Sloan-Kettering Institute

Dr. Ben Burmester, U.S.D.A., East Lansing, Michigan

Dr. Bruce Calnek, Cornell University

Mr. Mark Chatigny, Naval Biological Laboratories

Dr. Thomas Chin, Communicable Disease Center, Kansas City

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Dr. Sam Dales, Public Health Research Institute, N.Y.C.

Dr. Friedrich Deinhardt, Presbyterian-St. Luke's Hospital

Dr. Kenneth DeOme, University of California, Berkeley

Dr. Giampero di Mayorca, Eppley Institute, University of Nebraska

type C virus isolates of murine, feline, and primate origin, were also demonstrated. The interspecies antigenic determinants of p10 were shown to be as broadly cross-reactive as those exhibited by the major type C virus structural polypeptide, p30.

Several low molecular weight proteins of endogenous viruses of the RD114/baboon group were compared with the gag gene-coded translational products of endogenous type C viruses of murine origin. The p10 proteins of each virus group are immunologically and biochemically related, while the p12 proteins of RD114/baboon viruses were demonstrated to share antigenic determinants with murine viral p15. Moreover, highly type-specific phosphoproteins, p15 of RD114/baboon viruses and p12 of murine viruses, possess analogous biochemical properties. These findings, along with previous studies indicating immunologic cross-reactivity between their major internal antigen, p30, demonstrate that each of the gag gene-coded proteins of murine type C viruses has an analogue in viruses of the RD114/baboon group. The immunologic and biochemical relatedness of their gag gene translational products supports the concept of a common progenitor in the evolution of these endogenous viruses.

Type C RNA viruses have been shown to exist as endogenous viruses within the cellular genomes of many mammalian species. A major research effort is aimed at devising immunological approaches for detection of endogenous virus expression in human tissues. Techniques have been successfully developed for the detection and partial purification of several type C virus-coded proteins from virus-negative cells of a variety of species. Efforts have focused on the search for viral antigens in primate tissues. It has been possible to show that species representing each of the major genera of Old World primates express antigens related to the major structural proteins of the baboon-RD114 virus group. These antigens were shown to have diverged sufficiently so as to be immunologically distinguishable. Differences in the levels of expression of endogenous virus-coded proteins in tissues of a variety of primate species suggest differences in cellular regulation of virus expression by these species. With highly sensitive immunological techniques now available, analogous approaches are being utilized in the search for evidence of endogenous virus expression in higher apes and man.

The biological functions of gp70 (env gene) were studied: gp70 itself was found to interfere, hemagglutinate, and induce neutralizing antibody. Cell attachment, interference, and the neutralizing antigen site were all on the protein portion of the molecule; carbohydrate was necessary only for hemagglutination. The analysis of surface glycoprotein molecules of RNA tumor viruses with regard to specific antigenic and biological functions stimulated the question of whether protection from leukemia could be achieved by active immunization. The finding of interspecies reactive determinants on gp70 molecules allows the possibility that other species may be protected even if the causative virus was not isolated. Recently, the presence of RNA tumor virus envelope glycoproteins on the surface of normal cells has received considerable attention. This interest relates to the larger question of whether the expression of endogenous viral antigens have a functional role in cellular differentiation. The most detailed studies of tumor virus glycoproteins (gp70) have been done in inbred strains of mice. Virion-

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Dr. John Higginson, International Agency for Research on Cancer

Dr. Maurice Hilleman, Merck Institute for Therapeutic Research

Dr. Yashar Hirshaut, Sloan-Kettering Institute

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Dr. Roy Luginbuhl, University of Connecticut

Dr. Edwin Lennette, California State Department of Public Health

Dr. Robert McAllister, Children's Hospital of Los Angeles

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reflect a significant relationship between cell activity and viral growth. Thus, the factors governing mammary tumorigenesis are highly complex but are closely correlated with factors which regulate the expression of mammary tumor virus expression.

The free glycoprotein of MMTV was detected in the circulating blood of tumor-bearing mice in concentrations that correlate with tumor load. In the strains used for this study, each gram of tumor led to the production of 500 ng of glycoprotein; blood levels dropped dramatically within 8 days after the tumor was surgically removed. Subsequent assays for the antigen predicted relapse in the animals with complete accuracy. This model for cancer diagnosis and prognosis was recently applied to studies on breast cancer patients. Plasma levels of the reverse transcriptase from MPMV were found to correlate with the state of this disease.

The Mason-Pfizer monkey virus (MPMV) was initially isolated from a breast tumor of a rhesus monkey. Subsequent isolates have also been obtained from normal rhesus monkeys. MPMV differs in both its structure and the antigenic determinants of its major structural protein from those of any known RNA tumor virus. However, MPMV, like mammalian type C viruses, contains a glycoprotein whose molecular weight is approximately 70,000. An interspecies immunoassay utilizing antiserum to MPMV to precipitate baboon viral gp70 has been shown to detect antigenic determinants shared by glycoproteins of MPMV, RD114, and baboon type C viruses. These findings indicate that MPMV may have originated by recombination with endogenous primate type C viral genetic sequences.

New Viruses. Two isolates of type C viruses were obtained from Mus cervicolor. One of these is immunologically related to the woolly/gibbon group of infectious type C viruses. The other isolate shares antigenic determinants with type C virus of M. musculus. In addition, a new class of endogenous virus was isolated from Mus cervicolor and Mus caroli. These viruses are morphologically distinct from all other RNA tumor virus classes and are unrelated by immunologic and nucleic acid homology to all murine type B and C viruses.

Different viruses have been isolated from various primate species by co-cultivation of tissues with heterologous cells. These isolates include type C viruses from several species of baboons (Papio) and from the closely related genus, Theropithecus. In addition, a virus has been isolated from a langur monkey cell line. This isolate is related to MPMV, but can be readily distinguished from MPMV by its host range, antigenic properties, and by nucleic acid hybridization. Various isolates from squirrel monkey cell lines have also been obtained which are endogenous to these monkeys, and are present in multiple copies in the cellular DNA of all squirrel monkey cells and tissues.

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C. Scientific Activities - Progress Highlights:

There are now over 100 viruses which are known to cause virtually all kinds of cancer in every major group of animals including non-human primates. The probability is very high that viruses responsible for at least some human cancers will be found. When such agents are identified and successfully grown in the laboratory, it should be possible to develop therapeutic and preventive measures for the control of these diseases.

Viruses of one type (Type C) are known to cause leukemias, lymphomas, and sarcomas in chickens, mice, and cats. Particles, which closely resemble Type C viruses, can be found in human patients affected with these same kinds of cancers.

A monolayer culture releasing C-type particles has been established from cells of a pleural effusion of a child with Burkitt's lymphoma of the American type. The line is in its 28th subculture and continues to release virus. Studies have failed to identify this agent with known animal tumor viruses. This is the first human cell culture producing C-type virus in relative quantity. This makes it possible to produce antisera specific to a human C-type virus to be used for determination of the distribution of human C-type virus antigens in tumors.

Viruses of a second type (Type B) produce cancers of the breast tissue of mice. Thus far, this is the only animal species in which breast carcinoma has been proven to be caused by a virus. Recently, Type B particles resembling the mouse agent, have been found in human breast cancers and in milk concentrates from patients with breast cancer. Attempts to grow these viruses, if indeed they are definitely shown to be viruses, in the laboratory have just begun. Viruses of a third type (Herpes-type) which are structurally similar but unrelated to other known herpesviruses, have been shown to be associated with the following human malignancies: Burkitt's lymphoma, nasopharyngeal carcinoma, and uterine and penile carcinomas.

Hamster cells transformed by UV-irradiated herpesvirus type 2 grew as tumors when inoculated into newborn hamsters. This suggests that at least one virus which ordinarily is cytolytic may be oncogenic when an induced genetic defect blocking normal cycle of viral maturation with cytolysis results in the retention of the virogene in the replicating host cell.

By using techniques developed from animal tumor virus studies, every effort is being made to determine whether these viruses cause human malignancies. During the course of this work, it should be recognized that information on the biological, biophysical, and biochemical characteristics of cancer-causing viruses has provided a broad base of knowledge applicable to the isolation and identification of human agents and prevention and control of the disease in man. Some important new discoveries that have been made are: (1) Certain oncogenic viruses are unable to produce malignancies unless a "helper" virus is present, thus

suggesting that an interplay exists between two viruses. (2) Certain tumor-inducing chemicals, irradiation, (carcinogens) may act as co-factors in activating latent, oncogenic viruses within cells. (3) Certain tumor viruses contain unique enzymes which are required in the replication of viruses in cells. These and other important developments will be discussed further in this report.

Type C Particles

When the Special Virus Cancer Program was initiated, highest priority was given to the search for human leukemia viruses resembling the Type C viruses causing chicken and mouse leukemias. Since that time, many Type C viruses, the total is now about 85, have been found in a variety of tumors from many species of two vertebrate classes. All of these species continue to be studied intensively under the broader scope of the Special Virus Cancer Program. Several of the Type C viruses are established as the causative agents in leukemias, lymphomas, and sarcomas of chickens, mice, cats and hamsters. Many of these can infect and produce malignancies in other species (e.g. a sarcoma virus of the cat produces tumors in marmoset monkeys). Furthermore, some of these viruses can cause malignant transformation to occur in animal and human cells grown in the laboratory (e.g. cat leukemia and sarcoma viruses alter embryonic human cells). Type C virus particles have been found in association with malignancies of a spectrum of animal species including non-human primates, rats, cattle, woolley monkeys, gibbons, and man. Although electron microscopy is an important method in the search for Type C virus particles, certain newer biochemical, biophysical and immunological techniques, developed through intensive collaborative studies of animal cancer viruses, are now being directed toward finding viruses present overtly or covertly in the cells of human cancer.

1. Reactions between Type C viruses causing leukemias and sarcomas (solid tumors)

When inoculated into appropriate cell cultures, Type C sarcoma viruses of chickens, mice and cats produce foci of altered cells. This fundamental discovery provides a readily visible indicator reaction for the detection of sarcoma viruses. On the other hand, leukemia viruses grown in tissue culture do not cause foci or other detectable changes. The finding that leukemia viruses can either inhibit or enhance focus formation by sarcoma viruses of the same species has led to the development of methods for the detection and quantitation of leukemia viruses indirectly.

Certain of the chicken, cat and mouse sarcoma viruses are "defective" in that they do not produce foci in cell cultures or tumors in animals in the absence of a co-infecting, "helper" leukemia virus. Further, in the presence of a defective sarcoma virus the helper action of leukemia viruses can be used as a specific indicator for their detection and quantitation. It is now believed that defective sarcoma virus--leukemia virus interactions may be more widespread in nature than originally thought and that similar systems may be found in man. A mouse leukemia virus which

b. Progress Highlights

RNA Viruses

The availability of larger amounts of MMTV from cell cultures than possible from mouse milk and of antisera to purified virion polypeptides has provided increased opportunity to study the disease process in mice and to probe for related components expressed in mammary cancers in humans and in other animals.

A single dominant gene (Mtv-1) in the C3Hf mouse strain controls the release into milk of MMTV-L, the low oncogenic variant of mouse mammary tumor virus. Mtv-2, which is responsible for release of high levels MMTV-P and early hormones dependent mammary tumors, consists of about eight tandem copies of MMTV-DNA.

Vaccination of mice with gp52, a glycoprotein component of MMTV, resulted in a slight delay of mammary tumor development. Resistance to tumor transplants was obtained when low doses of vaccine were used with an adjuvant; higher doses of MMTV protein accelerated tumor growth and appeared to be associated with poor cellular immune reactivity to the virus.

Antisera to the major glycoprotein component of MMTV, gp52, were used to probe for related antigenic determinants in human milk specimens fractionated by zonal centrifugation. Some positive findings were obtained with pooled milks, but the negative results with individual specimens suggested very low concentrations or infrequent occurrence of the reactive component.

The detection in tumor tissue of reverse transcriptase (RT) activity suggests the presence of RNA tumor virus genetic information. Extracts of 20 human placentas examined contained RT activity. Electron microscope studies detected virus-like particles in the trophoblast layer of 7 of 19 human placentas. The enzyme activity was not neutralized by antisera against the RT of the known primate, feline, or murine type C viruses. The virus has not yet been propagated in cell cultures.

Human leukemic cells were probed for the presence of antigens related to simian sarcoma virus (SSV-1), previously isolated from a woolly monkey. No evidence of antigens related to the p30 or gp45 virus polypeptides was found. However, nucleic acid sequences related to the virus nucleic acid were reported to be present in specimens from some patients with acute myelogenous leukemia. A glycorpotein with m.w. of 55,000 daltons detected in membranes of human chronic granulocytic leukemia cells appears to be related to a minor antigenic determinant on the gp71 of the Friend murine leukemia virus.

Considerable work has been done to characterize the protein products of RNA tumor virus genetic expression. Many gp70 MuLV envelope glycoproteins were isolated from viruses purified from sera and excretions of several classes of related virus envelope proteins. More than one type of gp70 could be isolated from a single mouse strain, depending on the anatomical site or origin. Thus, virus-specific cell surface antigens have been identified which may be important in identifying preleukemic cells and in immunoprophylaxis.

are specific subunits of the internal structure of the Type C RNA tumor viruses. One type of group-specific antigen is common only to the viruses of a particular animal species (species specific) and has now been demonstrated in the embryonic tissues of most mammalian and avian species. This suggests the possible role of these viruses in normal cell growth and differentiation as well as in the induction of malignancy.

Very recently, a second group antigen associated with Type C viruses has been demonstrated. Preliminary evidence would indicate that it may be an interspecies-specific group antigen in that it is present and common among the Type C viruses of 3 mammalian species, the mouse, cat, and hamster, but not the chicken. Furthermore, this antigen has been detected in highly concentrated extracts of cells from two different human tumors, one of which has at times produced small amounts of a virus resembling animal Type C particles. If confirmed, the implication of this finding is obvious--a formidable probe for the presence of virus or viral genetic material in human cells has been developed.

Projecting that Type C viruses will be found to be as ubiquitous in man and other vertebrates as in mice, chickens, and the cat, the hypothesis is proposed as a unifying theory consistent with the phenomena of radiation and chemically induced cancer as well as the predictable occurrence of spontaneous cancers that develop in various populations of animals, including man. An exciting consequence of this theory, even if it is found to hold only for a limited number of cancer types in man, is the prediction that a new approach may become available for the control of these cancers, namely the delineation of factors responsible for both derepression and repression of virus expression.

A somewhat different approach has been based on the premise that tumor cells containing a common antigen may indicate a similar viral causation. In examining the tissues from various human sarcomas, particles resembling known animal Type C viruses were found in at least one liposarcoma. With continued culture of the tumor, particle production ceased, but immunologic tests using the serums of sarcoma patients revealed that the cells still contained a substance (an antigen) which appears to be present only in sarcoma cells (sarcoma-specific). A high incidence of serum antibody to sarcoma antigen was found in patients with various types of sarcomas and their close associates; a significantly lower incidence was found in normal serums. The data suggest that a viral agent may be the causative factor in this disease. Hopefully, continuing studies will establish a more definite relationship of the antigen to this disease.

3. New approaches to detection and control of Type C virus infections.

Increasing evidence has accumulated to substantiate that the genetic material of Type C RNA viruses can become integrated into the host-cell DNA (mammalian genetic material). A dramatic breakthrough in the investigation of the biochemical pathways of tumor virus infection and

replication has been the demonstration that RNA viruses contain enzymes called polymerases which can direct the synthesis of DNA by the host cell. In rapid succession many investigators found that one of these enzymes (RNA-dependent DNA polymerase) is associated with all of the known RNA Type C tumor viruses. Viruses that cause leukemias or sarcomas in chickens, mice, cats and hamsters, as well as breast cancers in mice, rats, and monkeys, have been shown to contain polymerase activity. By contrast, well-characterized, non-tumor producing viruses do not contain this activity. This research has resulted in the development of new, extremely sensitive methods for the detection of RNA tumor viruses in human cancer. Assays for the polymerase enzymes exceed by 100-fold the sensitivity of electron microscopy and are capable of detecting viruses which are present either in latent form or as whole virus particles.

Intensive investigations have now revealed polymerase activity in cells of patients with acute lymphoblastic leukemia; more preliminary evidence has shown the enzyme is in cells of sarcomas, Burkitt's lymphoma and breast cancer. Since the RNA-dependent DNA polymerase is apparently always present in the RNA tumor viruses of animals, its discovery in the human tumor cells offers good supportive evidence that viruses are associated with cancers in man. The RNA-dependent DNA polymerase of human leukemia cells is inhibited by a drug, n-demethyl rifampicin, which also inhibits the enzyme activity found in the Type C RNA tumor viruses of animals. Studies are underway to explore the action of this drug and the various modifications of it. These investigations could provide new approaches to the treatment of malignancies in man.

Type B Particles

"Type B" viruses causing breast cancer in mice possess certain properties in common with Type C viruses, such as having genetic material of the RNA type and being transmitted from parent to offspring along with the normal genetic inheritance. But they also differ in: (1) the manner in which the nucleoid is formed during virus reproduction; (2) the fine details of their ultrastructural appearance; and (3) transmission in infectious form, under natural conditions.

The potential existence in humans of an infectious breast cancer virus similar to that of mice, together with epidemiological evidence of "clustering" of breast cancer in some human families similar to that observed in the earliest studies of cancer in mice, led to systematic viral studies on this human disease. Particles resembling the Type B virus of mouse breast cancer have been observed in 40% or more of milk specimens from women with breast cancer, as well as from healthy women of high-risk populations (high-breast-cancer-families, inbred Parsi sect of Bombay, India), as compared with a frequency of only about 6% for specimens from healthy women of the general population. Similar particles have also been observed in two tissue culture lines of human breast cancer that have been successfully grown in the laboratory. More recent studies of one of

these cell lines have revealed additional evidence consistent with presence in the cells of an RNA tumor virus: the presence of the RNA-dependent DNA polymerase, the interspecies antigen.

Breast cancer occurs in about 4 to 5% of American women. It is the most prevalent and responsible for more deaths than any other type of cancer, not only in American women but also among women of several other countries. Because breast cancer occurs 2 or 3 times more frequently in some families than in others it is strikingly similar to observations made on breast cancer of different populations of mice. These animal studies led to unequivocal evidence of the association of a virus and the demonstration that an infectious form of it is transmitted through milk. One of the major objectives of the SVCP is now the determination whether an agent similar to that of mice is responsible for the unusually high incidence of breast cancer in certain human populations.

Herpes-type Viruses

A type of virus associated with some forms of chronic leukemia, lymphoma, and postnasal carcinoma is the herpes-type virus (HTV), similar in size and shape but not identical to other known herpesviruses. Unlike human Type C particles, HTV grows well in the laboratory and fairly large quantities of purified and concentrated HTV can be recovered for study. One of the most active areas of viral oncology is that concerned with definitive characterization of the HTV.

Considerable interest has developed in the herpes group of viruses as cancer-causing agents in animals and man. Herpes-type viruses have been shown to induce kidney carcinomas of the frog and to be causally related to lymphoproliferative diseases in chickens, monkeys, marmosets and rabbits. Projects within the Program have focused on the significance of the Epstein-Barr virus (EBV) from Burkitt's lymphoma and postnasal carcinoma and Herpesvirus hominus type 2 (HSV-2) from cervical carcinoma.

Seroepidemiological studies on the relationship of EBV infection to nasopharyngeal cancer are being conducted through the International Agency for Research on Cancer. A study in the West Nile District of Uganda to determine the feasibility of further studies on EBV in relation to Burkitt's tumor is nearing completion. Other studies also suggest an association between infection by HSV-2 and cervical carcinoma. Results of a study made in Texas showed the presence of serum antibodies to this virus in about 85% of cases of invasive cervical carcinoma in comparison to 22% in controls. Recent findings in Colombia showed a much higher incidence of antibody in the control population selected, approximating the incidence in the tumor-bearing group. At present, insufficient data is available to conclude that this virus is implicated in this cancer.

EBV infection has been associated with the development of infectious mononucleosis in young adults, a disease with the attributes of a self-limiting leukemia. The generally high levels of antibodies to EBV in patients

with Burkitt's tumor or with nasopharyngeal carcinoma also suggest that the virus might be causally-related to these diseases; high levels of antibody also occur in patients with the sarcomatous form of Hodgkins disease, chronic lymphocytic leukemia, and sarcoidosis. Present methods show no significant differences between EBV isolated from these different diseases.

Several facts make EBV suspect as a prime if not a sole factor in the induction of hematological disease in man. (1) The virus is ubiquitous; no human population has been found to be free of antibody to EBV. (2) The virus is consistently found in association with Burkitt's lymphoma. (3) Only lymphoid cells appear to be susceptible to infection. (4) Lymphocytes from negative donors have not been capable of continuous cultivation unless they are infected by the virus. (5) Infected continuous cell lines which do not releave virus contain soluable antigens that react with serums containing antibodies to EBV. (6) EBV has been strongly associated with infectious mononucleosis, cell lines of which grew as tumors in immunosuppressed hamsters. (7) Other herpesvirus of animals have been causally related to lymphoproliferative diseases. (8) The genetic material of EBV is related to that of similar viruses known to induce malignancies in animals. Thus, EBV has several features characteristic of known tumor viruses. Current data suggest that: genetic material of EBV may be incorporated into lymphoid cells; mature virus production may be a rare event; infected cells acquire the capacity for unlimited growth and produce tumors in the immunologically deprived host; infection by the virus may be a factor in tumor development under certain unknown conditions affecting the immunity of the patients.

D. Projections:

Based largely on the recent findings from work conducted within the Special Virus Cancer Program, the following broad areas of research will be developed and/or expanded: (1) Virus (or viral antigens) - tumor relationships, (2) Molecular studies, (3) Immunologic studies, (4) Test systems, and (5) Resources.

1. Virus (or viral antigen) - tumor relationships

a. Model Studies. Studies on animal, RNA and DNA, tumor viruses, known to cause malignancies in several mammalian species, will be continued. The results of these studies have already provided a broad base of knowledge on the characteristics of tumor viruses applicable to the isolation and identification of human agents. This work will remain an integral part of the Program.

b. Human Studies. Efforts to identify viruses or detect virus expression in human tumors are underway. The Program is now prepared to broaden its activities to search for candidate viruses or subviral products which induce human malignancies as follows:

(1) To identify and isolate candidate viruses or subviral products in leukemias, lymphomas and sarcomas.

(2) To identify and isolate candidate viruses or subviral products in breast, lung and other carcinomas.

(3) To develop methods for the detection of high cancer risk groups, i.e. individual susceptibility of predisposition to transformation by human viruses.

(4) To extend present and develop new methods for the successful propagation of significant amounts of human candidate viruses.

(5) To develop suitable reagents for mass diagnostic screening for candidate viruses.

(6) To characterize, biologically and biochemically, presumptive viral agents.

(7) To increase emphasis on understanding the relationship of environmental agents (e.g. chemical carcinogens) as co-factors in viral carcinogenesis. This represents a major expansion of effort requiring combined efforts of the Viral Oncology and Chemical Carcinogenesis Areas.

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Molecular Studies

In recent months rapid major advances have been made in the field of molecular biology. These findings have direct application to the study of the relationship of viruses to tumors. There is evidence that the genetic material (RNA) of the tumor viruses can direct the synthesis of new DNA. The demonstration that RNA tumor viruses contain enzymes (polymerase, ligase) which may be required for viral infection, interaction with host cell genome, and viral replication has provided the basis for the development of new, extremely sensitive methods for the detection of oncogenic viruses or their "fingerprints." Indeed, knowledge of the fundamental molecular events which occur during virus infection and subsequent cell transformation provides the first truly rational approach to therapy. Enzyme activities analogous to those of RNA tumor viruses have recently been found in cells of human leukemics. This offers strong supportive evidence that viruses are associated with cancers in man.

a. Basic studies

The Program is prepared to broaden its activities for identifying and characterizing the spectrum of enzymes (and other mediators) required by tumor viruses for replication and transformation.

b. Applied studies

As knowledge of the fundamental molecular events in virus-cell interactions is developed, the Program will apply this information to the study of human cancer as follows:

(1) To identify and characterize similar enzymes or enzymatic activities within normal and malignant human cells.

(2) To develop highly sensitive methods for the detection of virus or virus activity in human cells.

(3) To develop a rational basis for therapy or prevention by exploring various approaches to blocking of viral replication and/or tumorigenesis at the cellular and subcellular levels. The therapy could be directed at any or all of the stages of cell transformation beginning with cell infection by a tumor virus.

Ultimately these studies will require an exhaustive effort to develop drugs, anti-enzymes, gene repressors or inhibitors effective at the molecular level.

3. Immunological Studies

Immunologic research has provided extremely sensitive techniques for detection and characterization of tumor viruses, viral antigens, and changes in surface membranes of tumor cells. Indeed, such efforts have contributed to an understanding of the role of immunological mechanisms in host-tumor and host-virus interactions which provide an approach to the prevention and treatment of cancer.

a. Basic studies. Investigations of selected model systems, representing tumors induced by Type C, Type B, and Herpes-type viruses, will be extended to further identify, characterize and determine the viruses, viral antigens, and membrane antigens of tumor cells. This includes development and application of improved techniques with the sensitivity and specificity required to detect cellular alterations induced by tumor viruses alone or as the result of interaction with other environmental agents (e.g. chemicals, irradiation). Efforts will be increased to develop similar immunological methods and diagnostic reagents for application to human cancer. Research will be intensified and expanded:

(1) To study cellular and humoral immune mechanisms and to determine their relative significance in host recognition of and response to tumor and/or tumor viruses.

(2) To develop methods to enhance host response to tumor or virus antigens.

Increasing emphasis will be directed toward research on spontaneous or naturally occurring tumors in model systems relevant to human cancer. These studies would provide the basis for a rational approach to prevention (vaccines) and treatment (immunotherapy) of cancer.

b. Applied studies. Basic research will provide the framework for identification and characterization of viruses, viral antigens, and cell membrane alterations in human cancers. Immunological methods and reagents will be developed and applied:

(1) To relate candidate human viruses to known oncogenic agents.

(2) To identify and characterize interspecies viral antigens which are present in known mammalian tumors, and therefore, could provide the basis for a formidable probe to detect human tumor viruses or viral antigens.

(3) To launch large-scale seroepidemiological surveys which will define high risk populations.

(4) To determine the presence of cross-reacting antigens in various human tumors.

Clinical studies will be directed toward understanding and manipulation of immune mechanisms in human cancer as a basis for:

(1) Development of vaccines from identified and fully-characterized human tumor virus (es).

(2) Determination of the role of host immune responses in tumor recognition and rejection.

(3) Application of (1) and (2) in the prevention and control of human cancer.

As research progresses, increased emphasis on application will be as follows:

(1) Immunodiagnosis and seroepidemiology
(2) Clinical studies on the role of immune mechanisms in human cancer
(3) Immunotherapy
(4) Vaccines (conventional or other)

Ultimately, these studies would be organized to coordinate and integrate the application of appropriate biochemical, immunological, and genetic methods of detection, prevention, and control of various types of human cancer.

4. Test Systems

In vitro and in vivo (animal) test systems will be carefully selected to evaluate the work outlined in the previous research areas; specifically: (a) to determine the oncogenic potential of candidate human viruses; (b) to develop bioassay systems for testing viral, and viral/chemical carcinogens; (c) to begin vaccine (conventional or other) testing and immunization programs; (d) to begin therapy testing programs; and (e) to explore special animal tumor systems with particular relevance to human cancer.

Well-characterized cell culture lines and virus-defined animal stocks (small mammalian and primate species) will be developed and maintained.

5. Resources

Research efforts will undergo continual change in emphasis and scope as new leads emerge. A variety of resources will have to be developed, maintained, and coordinated within a flexible program which meets these needs. This requires: (a) Human Resources - collection and storage of serum and tissue specimens, integration of data on clinical records, storage and distribution; computerization, coordination of specimen collection, storage, and distribution; (b) Animal Models - maintenance of various mammalian animal colonies for basic research and special studies; (c) Reagent Production - large scale production of animal tumor viruses for basic research; production of standardized lots of purified viruses; and production of high quality diagnostic reagents; (d) Candidate Human Virus Production - intensive developmental research effort to isolate and produce human viruses; and (e) Biohazards Control and Containment - controlled environment facilities are required for research on known oncogenic viruses and candidate human tumor viruses as well as for maintaining animal colonies which are protected from extraneous infections. To fulfill this need, a substantial and costly effort is required to provide research, supportive services, and advice to the Program's research laboratories.

SUMMARY REPORT

Office of Biohazards and Environmental Control

The Office of Biohazards and Environmental Control conducts research pertaining to the physiological and environmental factors that alter host susceptibility and response to oncogenic and non-oncogenic viral infections. It also evaluates and develops techniques and equipment to minimize cross-infection and provide adequate environmental controls.

The Biohazards Section conducts research in an effort (1) to describe the role of immunocompetence in oncogenesis; (2) to define biochemical factors which might lead to the induction of malignancy and how best to detect and modify these inductive changes; and (3) to develop in vivo and in vitro systems to assess the host response to physiological imbalance.

A sensitive and reliable system to culture mouse lymphocytes, responsive to specific and non-specific antigenic stimulation, has been developed. Hormones associated with normal physiological functions have been shown to alter blastogenesis. It has further been established that several chemical and physical factors are required in the process of blastogenesis. Using Type C RNA viruses and myxo-viruses we have also demonstrated blastogenic repression associated with oncogenic virus infection in contrast to blastogenic enhancement with myxo-virus infection. We have further demonstrated that many hormones influence the derepression and possibly the repression of group-specific-antigens of the Type C RNA viruses.

Alteration of certain species of tRNA have been suggested during early stages of oncogenic virus infection. Thus far, we have observed at least one altered species of tRNA associated with tumor virus-infected mouse tissue.

The Environmental Control Section uses environmental monitoring as a method for identifying sources of contamination. To assure the integrity of primary and secondary biological barrier systems, they evaluate engineering and operational parameters and their effect on experimental and personnel contamination control. Similarly, they collect and evaluate information pertinent to biological safety and evaluate methods for handling potentially hazardous agents. This approach has resulted in improved performance of biological barriers and has served as a basis for developing more efficient and safer operating procedures. Thus, more flexible and safer laminar flow cabinetry is currently under fabrication and will undergo in-use testing. Physical tracer systems are being developed to aid in the evaluation of dynamic containment equipment to assure proper use of facilities and to maintain a high level of laboratory safety practice.

SUMMARY REPORT

Office of Program Analysis and Communications

Acquisition, management, analysis, and dissemination of Program data within the SVCP are the major activities in PAC. These efforts fall under the two general areas of: (1) data management and statistics; and (2) scientific information storage and retrieval.

Data management and statistics

1. Performs statistical consultant service for scientists in the SVCP program on specific problems of research design, acquisition and handling of experimental data, and its computer automation and analysis.

2. Plans and maintains automated inventories for NIH sponsored serum and tissue specimen collections.

3. Plans and consults with research contractors on automation of their specimen inventories and research data.

4. Promotes compatible automated systems and codes in a multi-institutional plan for a comprehensive clinical and laboratory information storage system for specimens and donors. The goal is a central specimen inventory at NIH to enhance the resource potential of the many large specimen repositories throughout the country.

5. Maintains a system of regular progress reporting from all SVCP research contracts; compiles and distributes summaries of reported progress to program scientists.

Scientific information management

The Information Unit of PAC continued to focus on scientific information retrieval and its dissemination to program scientists. Sources of data are current publications in the field of viral oncology, and other notifications and summaries of current research projects in the field. Major contributions of the Information Unit in FY 1971 were as follows:

1. Bibliographic Service: The automated systems for rapid search, identification, and reference printouts, covering any desired subject in the published literature on viral oncology was maintained.

2. Reading Guide to Cancer Virology Literature: The monthly publication featuring abstracts of current literature on viral oncology, from scientific journals throughout the world,was continued. These abstracts are now incorporated in Carcinogenesis Abstracts; hence, this service will be terminated.

3. Viral Tumorigenesis Report: Indexed summaries of current research projects in the field of viral oncology are compiled and released as semi-annual publications.

4. Report on NCI Support of Cancer Virology Grants: This quarterly report presents an organized summary of updated fiscal data on current NCI research grants in the cancer virology area. The basic information is received from the Program Analysis and Reports Section, NCI.

5. Viral Oncology Contractor Directory: This publication contains pertinent information on all contracts and principal investigators in the Viral Oncology program. Its purpose is to facilitate and expedite communications between staff members and contractors; directory is updated quarterly.

6. Compilation of Journal Instructions to Authors: This displays in one volume the instructions-to-authors from a majority of pertinent scientific journals. It is a reference aid for research investigators in writing papers and also for the secretaries who type them. Compilation will be updated and expanded periodically.

Other responsibilities of the Information Unit are: administration of library facility; collection and distribution of translations of foreign publications in viral oncology; maintenance and lending of recorded tapes on NIH seminars related to viral oncology; and continuous compilation of the SVCP bibliography containing citations to all papers published by viral oncology staff members and contractors.

SUMMARY REPORT

Office of the Coordinator for Ultrastructural Studies

The Virus Studies Section continues to study several aspects of the relationship between oncogenic viruses and their host cells. The problem is approached a) by using the ultramorphologic examination of a number of tumors for the search for viruses; b) by evaluation of different malignancies grown in vitro in respect to the presence or absence of viruses; and c) by studying early events of viral infection which may result in the "neoplastic transformation" of the treated cells. In addition, studies are under way to elucidate the transfer of genetic information from the nucleus to the cytoplasm.

It is important to determine the frequency of the natural occurrence of RNA and DNA containing viruses in human and animal tumors, since both types of viruses may possibly represent causative agents for a number of different malignancies. To our knowledge, to date no systematic attempts to make this determination have been published. Dr. A. J. Dalton in collaboration with Dr. L. Dmochowski, M. D. Anderson Hospital and Tumor Institute, Houston, Texas, and Dr. J. David-Ferreira, Institut Gulbenkian de Ciencia, Oeiras, Portugal, has examined 54 biopsies from human tumors (mostly leukemia, lymphoma and Hodgkins cases) with the electron microscope for the presence of virus particles. All tumors were found to be negative.

Dr. Dalton in collaboration with Dr. J. Szakacs, St. Joseph's Hospital, Tampa, Florida, Dr. U. Heine, Dr. D. Ablashi, and Mrs. J. Kondratick and T. Ben (all NCI) has studied 18 tissue culture isolates from biopsies of human solid tumors for the presence of virus particles. Seventeen cell lines were obtained from these isolates; most lines are fibroblastic in appearance, a few are epithelial-like, and two are suspension cultures resembling the Burkitt lymphoma and human leukemia cultures. One lymphoid cell line, upon co-cultivation with WI-38 cells yielded a cytomegalo virus isolate. The virus was characterized by ultramorphological, biophysical and immunological studies. The other cell lines were negative for virus particles.

Dr. A. Bader, in collaboration with Dr. H. C. Orr, DBS, has examined tissue cultures from a naturally occurring fibrosarcoma in a rattlesnake with the electron microscope for the presence of virus particles. Neither the cells nor tissue culture fluids contained any virus particles.

Dr. Heine in collaboration with Dr. D. Ablashi (NCI) has succeeded in growing Herpes virus saimiri, originally observed in subhuman primates, in different cell lines of human origin (WI-38, primary HEF, WHE). The presence of the virus and its mode of multiplication were demonstrated by virologic and electron microscopic methods.

The study of the early events in viral infection were approached in two separate ways:

Dr. A. Bader in collaboration with Dr. J. Bader (NCI) initiated an investigation to determine the mode of entry of RNA containing tumor viruses into the cell. In addition, by studying the influence of bromo-deoxyuridine of chick embryo cells, controls and RSV-infected ones, they contribute to the understanding of the relationship between viral DNA and viral RNA during early infection with an RNA tumor virus. It appears that a viral DNA is made early in the course of infection by avian leuko-sarcoma viruses and that this DNA serves as a genome from which progeny viral RNA molecules are copied.

Dr. Suskind developed quantitative autoradiographic techniques, using tritiated RNA, DNA and Actinomycin D, at the level of light and electron microscopy in combination with ultrastructural examination to study and relate functional and morphologic changes within the nucleus of the intact cell during early stages of infection with the Rous sarcoma virus, Schmidt-Ruppin strain. The results demonstrate differences in both morphology and function in transformed cells with respect to their recovery from inhibition by Actinomycin D. The time interval during which these changes arise has been shown to be about 80 minutes after infection. The findings suggest a causal relationship between the activity of the viral genome and the interval between infection and induction of transformation.

Dr.U. Heine continued studies already in progress correlating cellular particulate material, i.e., particulate nuclear and cytoplasmic structures, with cellular function. Morphologic observations combined with biochemical findings provided evidence that a) 45 S RNA is contained in the fibrillar nucleolar material, b) perichromatin granules may represent the morphologic entities containing messenger RNA and c) the arrest of m RNA transfer from the nucleus into the cytoplasm results in the breakdown of polysomes and their replacement by monosomes.

Dr. Dalton has been responsible for the organization of two monographs in the series "Ultrastructure in Biological Systems" and an Atlas on the ultrastructure of viruses. One hundred twenty-two applications for duty free entry transmission electron microscopes, scanning electron microscopes and vacuum evaporates have been reviewed with recommendations of approval or disapproval to the Florence Agreement Committee, N.I.H.

As a resource for ultramorphologic studies the tissue culture unit established new cell lines of human origin, chick embryo cells for membrane studies and for autoradiographic and ultramorphologic examinations.

In addition to the support of the electron microscope activities, special photographic work was done for the office of the Director (Dr. Baker), the office of the Associate Scientific Director (Dr. Moloney), the office of the Scientific Coordinator for Viral Oncology (Dr. Bryan), and for other sections and branches within NIH (Dr. Manaker, Dr. S. Stewart, Dr. Nomura, Dr. Nirenberg).

The Office of the Coordinator for Ultrastructural Studies has published four papers and presented by invitation six lectures or seminars.

SUMMARY REPORT

VIRAL LEUKEMIA AND LYMPHOMA BRANCH
July 1, 1970 - June 30, 1971

The Viral Leukemia and Lymphoma Branch conducts research designed to elucidate the role of viruses in the etiology of human neoplasms, particular leukemias, lymphomas and sarcomas. A variety of scientific approaches are used which provide a broad base of knowledge applicable to the identificatio and isolation of human oncogenic agents and the prevention or control of the disease as it occurs in man. More specifically, the Branch encompasses a range of scientific disciplines including molecular biology, genetics, immunology, biochemistry, pathology and cell culture techniques.

The Section of Molecular Biology seeks to obtain comprehensive knowledge of the biology and biochemistry of sarcoma and leukemia viruses and conducts quantitative studies on the interaction of oncogenic viruses and cells to determine the mechanisms of viral replication and cellular transformation at the molecular level. The Section of Viral Pathology studies the biology of the RNA-containing oncogenic viruses using a combination of in vitro and in vivo techniques; thus, animal species and tissue culture systems are infected with leukemogenic and sarcomagenic viruses to elucidate the role of the host in viral propagation and tumor development. The Section of Immunology examines the antigenic nature of oncogenic viruses and the induced tumors as well as the immune response of the host to viral infection and tumor development. The Section of Tumor Viruses is concerned with defining in detail the biological and biochemical properties of tumor viruses in order to understand how they may be applied to the search for human tumor viruses. A "helper" assay to "rescue" oncogenic virus information is currently being applied to human cell systems. The Section of Genetics is concerned with genetic factors of both the tumor virus and the host it infects that are involved in the oncogenic process. Particular emphasis is placed on viral genes involved in oncogenesis and cellular "susceptibility genes, particularly those genes of man that predispose individuals to the development of cancer. The Office of the Chief coordinates the research of the various sections by recognizing the scientific freedom of the individual investigators. The office is responsible for establishing collaborative efforts with investigators in other areas of NIH and elsewhere such that information derived from studies within the Branch is constantly being applied in investigations leading to a better understanding of the etiology of human neoplasia.

One of the most important findings in the tumor virus field in the past year has been the discovery that certain RNA tumor viruses carry, in the virus particle itself, enzymes that are capable of transcribing the viral RNA back into DNA. These observations lead to the possibility of using extremely sensitive biochemical probes to search for evidence of viral

etiology of cancers, and especially, cancers in man. Some of the potential applications to the etiology and control of human cancer are:

1. The use of synthetic DNAs produced from the viral RNA to search for complementary RNA in human tumors by DNA-RNA hybridization techniques.

2. The use of highly effective synthetic templates and optimal enzymatic conditions to search for tumor virus specific enzymes in human tumor cells.

3. The use of the tumor virus polymerase and human tumor cell polymerase to screen for specific inhibitors that may be used as possible chemotherapeutic agents (e.g., rifampicin derivatives prevent the synthesis of DNA from RNA by the viral polymerase).

4. The use of specific antiserum prepared against the purified viral enzymes to identify individuals that have been exposed to the viral enzyme. It is reasonable to expect that the antibodies to viral specific proteins may persist for much longer periods than the virus itself would persist.

All of the above approaches are being actively followed by members of the Viral Leukemia and Lymphoma Branch. One of the major concerns was to answer whether the enzyme is specific for tumor viruses and whether it is specific for tumor cells. All "C" type and "B" type oncogenic viruses tested have been found to have DNA polymerase as detected both by an endogenous reaction and by a synthetic polymer-stimulated reaction, using such templates as poly rA·rU, poly rI·rC and poly rA·dT. The species that have polymerase-containing virions include the mouse, rat, hamster, cat, chicken and monkey. A variety of non-oncogenic viruses that, like the oncogenic viruses, develop by budding from the plasma membrane, have shown no evidence of this enzyme activity; these include influenza, Newcastle Disease Viruses, reovirus, Vesicular Stomatitis Virus, poliovirus, respiratory syncytial virus, Sendai virus and such common infectious viruses of man as measles, mumps, and rubella. This survey indicated a very strong association between presence of the enzyme and oncogenic potential of the virus. However, two exceptions were found. The first is visna virus, a virus of sheep which produces a chronic, progressive, neurologic disease but has, heretofore, not been associated with malignancies in sheep. The second major exception are the group of "foamy" viruses. These can be commonly isolated from monkeys, cattle, and cats and have also been isolated from hamsters. These viruses, though latent, have not yet been associated with any disease. Studies in this Branch by Drs. Edward Scolnick and Stuart Aaronson in conjunction with Dr. Wade Parks of the Viral Carcinogenesis Branch have established that the foamy viruses are RNA containing viruses that, like the tumor viruses, appear to replicate via a DNA intermediate. RNA dependent DNA polymerase has been found in the simian "foamy" viruses and also the bovine, hamster and feline "foamy" viruses. The finding of this enzyme in this long neglected class of viruses raises the possibility that they may be involved in the oncogenic process. Since they are extremely common in primates, a

study of their biology, biochemistry and epidemiology appears to be indicated. Visna and "foamy" viruses at the moment, then, appear to be exceptions to the rule that only tumor viruses contain this enzyme; however, since neither has been seriously tested for its oncogenic capacity, this question remains open.

Work in the Viral Leukemia and Lymphoma Branch established that the enzyme is located in the nucleoid of the virion and the antiserum directed against the polymerase could inactivate the enzymatic activity. The use of purified gamma globulin to inhibit the rate of the enzymatic reaction appears to offer an extremely sensitive test for detecting antibody and the combined disciplines of immunology and biochemistry are being utilized to see if this approach can be extended to detection of antibodies to tumor virus enzymes in human serum.

Other Research Developments in the Branch

The development of continuous, contact-inhibited mouse cell lines from Balb/c and NIH/Swiss embryo cells has provided excellent model systems for study of the effects of tumorigenic viruses both in vitro and in vivo. These cell lines are supplied to numerous investigators throughout the world, and are becoming the standard cell lines for biochemical and biological investigations of cellular growth control mechanisms.

A new type of MSV-transformed cell, the nonproducer cell, has been discovered. These cells are morphologically transformed and are highly tumorigenic, yet they lack all the known antigens of the murine sarcoma-leukemia complex. The sarcoma genome can be readily rescued by the addition of "helper" leukemia virus. These nonproducer cells provide a very good model for cancer in man. Methods for the detection of virus specific information in these cells are in progress. Those methods that are most sensitive and most specific will be applied to the study of human sarcoma cells.

The isolation of cloned cell lines infected with MSV in the absence of detectable MuLV (S^+L^- cells) has permitted the study of the defectiveness of MSV. Superinfection of S^+L^- cells with MuLV resulted in a logarithmic rise in titer of both MSV and MuLV in the supernatant, beginning 12 hours after infection and becoming maximal at 72 hours. While MSV was not produced without the simultaneous release of progeny MuLV, an excess of MSV was produced early after superinfection. This MSV excess permitted the isolation of MSV from MuLV. Application of these preparations of MSV to 3T3 cells resulted in the production of S^+L^- cells. Inhibition of DNA synthesis during the period of eclipse between superinfection and the release of MSV and MuLV resulted in a decrease in production of both virus types.

A potent murine sarcoma virus (MSV) isolated from a spontaneously arising mouse tumor was found to have properties similar to those other well

so induced produce a virus that is antigenically related to the mouse virus but is apparently non-infectious. The sarcoma genome can be rescued from the hamster tumor cells. The hamster derived virus contains 70S RNA and has a density of 1.16 but is deficient in RNA-dependent DNA polymerase, and does not contain gs-3. Preliminary data suggests that the virus is a sarcoma virus produced in the absence of detectable leukemia virus of either murine or hamster origin.

Poly I · Poly C, a potent interferon inducer, has two actions in MSV tumor induction. Pretreatment with small doses greatly enhances MSV induced tumors, while repeated injections of large toxic doses suppress tumor formation. Poly I·C and MLV enhance the appearance of antibodies to RNA in New Zealand mice. Pre-immunization with Poly I·C retards the appearance of natural and MLV induced antibodies. The data indicates that the mechanism of production of antibodies to RNA following inoculation of MLV is similar to that operative in the natural state. DEAE-dextran enhances MSV both in vivo and in vitro. In both cases the enhancement is approximately tenfold. The in vivo effect is seen both after local and systematic administration of DEAE-dextran.

The relationship between age, autoimmune status and susceptibility to MSV has been studied in New Zealand mice of various ages. While old and young NZ mice are equally susceptible, old mice have considerable difficulty in regressing their tumors. This effect is seen prior to the appearance of autoimmune disease, indicating that immune depression precedes the appearance of autoimmunity. Weanling New Zealand mice can regress MSV induced tumors at a considerably earlier age than other strains, indicating early appearance of immunologic competence.

Improved biochemical methods for fractionation and separation of murine leukemia virus antigens have been developed. Both major and minor antigens have been found. Methods have also been improved to purify and separate the antiviral antibodies from other serum antibodies and proteins. The goal of these studies are to produce monospecific antibodies to each of the viral structural proteins and each of the viral enzymes.

Efforts continue toward the isolation of viruses or antigens specific to human and feline breast cancer using the murine MTV model system. Two human and two feline cell lines are under study. Serologic tests (HA, HAI, ID and neutralization) were adapted this year to the identification and quantitation of feline leukemia virus and antibodies. Tests to monitor cytotoxic antibody and cellular immunity were also developed. Experiments are in progress to develop cellular hyperimmunization protocols against oncogenic viruses and tumor antigens. Tests of 375 patient sera for HAI antibodies against FeLV, MTV and RLV showed that about 20% of the patients react with these viruses. There was no pattern of association of antibodies to any virus and a given diagnosis. Yet, certain human sera appeared to

significance of these findings is being actively studied.

Methods have been developed which greatly increase the sensitivity of detection of the viral RNA dependent DNA polymerase using synthetic templates with manganese as the divalent cation. It has been possible to disrupt the tumor virus and to partially solubilize it. Polymerase activity is associate with the internal core of the virus. Certain antisera to RNA tumor viruses inhibit the polymerase and studies to date indicate that these sera contain an antibody which inhibits the viral polymerase of several mammalian C-type RNA viruses but do not inhibit the polymerase of avian C-type RNA viruses or the polymerase of the mammary tumor virus.

Syncytium forming ("foamy") viruses of several species including the primate, bovine, hamster and feline have been found to contain an RNA dependent DNA polymerase. Studies to define their pathologic, antigenic and biochemical characteristics are in progress. Attempts to find isolates of such viruses from human tissues are also under way; since they are so common in other species it is reasonable to suspect that there are human polymerase-containing "foamy" viruses.

Recently developed biochemical methods to study the viral RNA dependent DNA polymerase in virions have been extended to search for evidence of such enzymatic activity in cells using the model system of MSV transformed and normal Balb/3T3 cells. It has been possible to detect enzyme activity in normal as well as transformed Balb/3T3. In addition, normal human fibroblasts and normal human lymphocytes contain polymerase activity. The relationship between the enzymes in normal cells, tumor cells and tumor viruses provides the major focus for present studies.

Inhibitors of the tumor virus polymerase are being tested for their ability to inhibit virus replication and transformation. Certain rifampicin derivatives have been found to be 50-100 times more active than N-demethyl rifampicin in in vitro assays using the purified mouse leukemia virus polymerase.

Eight patients with acute leukemia and their identical twins were evaluated by a variety of techniques. Lymphocyte cytotoxicity, mixed lymphocyte culture and skin testing were used to determine whether cellular immunity factors could identify a tumor specific antigen, and whether there was evidence that this antigen caused immune reactivity in family members and controls. Cells from the patient and his identical twin were used as sources of antigens directly and materials were also placed in tissue culture. Skin fibroblasts from family members were tested to determine whether any genetic factors could be associated with acute leukemia. Electron microscopy and immunofluorescence, using antisera against Rauscher and feline leukemia virus, would be utilized to determine whether any of the antigens detected by cellular immunity tests were related to any known animal leukemia viruses. An antigen which appears to be a leukemia associated antigen was detected in five of seven sets of identical twins.

The study of identical twins will continue in an attempt to more carefully define the specificity of the leukemia associated antigens and the immune response of humans to these antigens. New tests will be applied to the study of twins as they are developed. Since these families are ideal for the study of genetic and immunological susceptibility, more identical twins will be sought for study.

A study of the effect of UV irradiation of the DNA tumor virus, SV40, on the survival of its viral functions in mouse and human cells has revealed that the viral functions for T antigen and transformation show comparable UV sensitivities but that the virion or V antigen is much more UV sensitive. The UV survival of SV40 functions in normal cells and cells from patients with xeroderma pigmentosum (XP) have been compared. In this disease, cells lack the ability to repair UV damage to cellular DNA. The results show that normal cells have the ability to repair UV damaged virus while XP cells do not.

A large scale screening program is underway to test human fibroblast cells from patients with a variety of diseases associated with a high incidence of tumors for their susceptibility to transformation by SV40.

Serological studies in American Burkitt's patients indicate that their antibody levels to EBV are much less than African patients with Burkitt's lymphoma. The titers in American Burkitt's patients were linked to prognosis, however, with patients having low levels of EBV antibody doing poorly. The higher immunoglobulin levels and the high incidence of EBV in normal Africans compared to normal Americans suggest that the differences in EBV titers may be related to factors other than etiology.

Herpes saimiri is a DNA containing virus indigenous to the squirrel monkey. Recent studies have shown that this virus will induce acute lymphocytic leukemias as well as lymphomas and reticulum cell sarcomas in owl monkeys and marmosets. This is the first demonstration that a virus produces leukemia and lymphomas in primates and may serve as an important model for human leukemia and lymphoma.

A very sensitive assay for the serum factor which induces cellular DNA synthesis in mouse cells in much the same way that the tumor viruses induce cellular DNA synthesis has been developed using 3T3 and Balb/3T3 cells. The characterization of the serum protein involved in the initiation of cell division will be important in understanding the normal mechanism by which cell division is controlled and how tumor viruses are able to overcome this control.

Other Activities of the Branch

During this reporting period, senior investigators of the Branch published a total of 74 papers which covered various aspects of viral oncology.

groups in this country and abroad, and over 30 abstracts were presented at various scientific meetings. The Branch entertained approximately 70 visitors for discussions in various aspects of viral oncology; this included representatives from every major research institution in this country and from 9 foreign countries. The Branch also provided training for periods up to six months in a variety of experimental procedures to outside visitors This training consisted of orientation in immunological, biological and biochemical procedures as well as virus handling techniques.

Many of the investigations described in the Viral Oncology portion of this report depend on the availability of clinical and laboratory materials of optimal purity, viability, potency, etc. Indeed, comparative studies in an integrated program of international scope, as encompassed in the SVCP, can make more meaningful and rapid progress when adequate quantities of standardized reagents, cell cultures and test animals are available.

The Resources and Logistics Activity provides viruses and antisera, human tissues, special laboratory animals (including infectious leukosis virus-free eggs), all produced, characterized, stored and distributed under various contract operations. Diagnostic services for the detection of murine, non-human primate, and cat viruses, and the viral reagents for these tests, are also provided.

An annually revised catalog lists and describes all resources available through the Resources and Logistics Activity. Usually the information on each item includes origin, processing procedure, degree of purity (freedom of contamination), infectious titer, or other biological activity. Human materials, including sera, tumors, cell outgrowths from tumors and normal tissues, are obtained for distribution to qualified investigators. A central inventory of information on each specimen is being developed which should greatly facilitate matching requests for human resource material with specimens, regardless of the geographic location of the laboratory in which it is stored.

About one and one-half years ago, a nationwide effort was initiated to obtain cancerous cats (primarily leukemias and sarcomas) from cat breeders and owners. Research laboratories local specimen supply has been supplemented by the nationwide contacts for materials and cancerous pure-bred cats with pedigrees. Letters are being sent to cat owners soliciting cats with veterinarian diagnosed cancers. The procurement effort not only obtains valuable research material but reassures the cat owners and other concerned citizens of NCI's active program for the evaluation of the unlikely hazard of this disease to human health.

In addition, several of the senior investigators within the Branch spent a portion of their time in support of the Special Virus Cancer Program. The members serve as Chairmen, Vice Chairmen, Work Group members and Secretaries of the major segments of the Program. They provide scientific

guidance as Project and Assistant Project Officers on research contracts supported by the Special Virus Cancer Program.

The activities of the SVCP and the direction of the internal research program of the Branch are aimed at the common goal of the determination of the viral etiology of human cancer. It is apparent that the efforts of the Branch members have played a significant role in the progress of the SVCP to date. The broad scientific perspective developed by these investigators in their SVCP activities has also contributed significantly to the direction of the Branch program for the attainment of research goals.

The effective functioning of senior personnel in dual capacities, i.e., in-house research and program administration requires a delicate balance of effort. It must be realized through constant monitoring, that such a balance does exist and over-emphasis in either direction would be to the detriment of both programs. It has become clearer during the past year that an understanding of the suspected relationship between tumor viruses and human neoplasia requires an interaction between, among others, highly skilled molecular biologists, epidemiologists, cell biologists and physicians, along with sound and constructive administrative support; the answers will come from no one discipline alone.

SUMMARY REPORT

VIRAL BIOLOGY BRANCH

July 1, 1970 - June 30, 1971

The Viral Biology Branch conducts research to develop and apply new conceptual and technical approaches to the study of the viral etiology and control of neoplastic diseases. More specifically, the Viral Biology Branch is active in: The isolation and characterization of various constituents of tissues which are involved in or related to the carcinogenic process; the study of the biological interactions of viruses with host cells in vitro and in vivo; the investigation of viruses in relation to human neoplasia by virological, immunological and biochemical methods; the study of interrelationships between viruses in the neoplastic process; the study of cells and viruses at the ultrastructural level; the investigation of host factors in relation to development of neoplastic diseases; and the control of neoplasia in experimental animals.

The Cell Biology Section studies methods for increasing the antigenicity of weak tumor transplantation antigens. Vaccination of mice with a homogenate of tumor cells previously infected with influenza virus gave significant protection of the animals against challenge with viable cells of that tumor. No protective effect was observed in control animals vaccinated with homogenates of the tumor cells not infected with influenza virus or with homogenates of normal cells. The presence of the influenza virus antigen on tumor cells increased the immunogenicity of tumor cell antigens.

Methods for quantitatively measuring contact inhibition of locomotion were developed and successfully applied to normal and SV40 transformed cells.

Evidence was obtained demonstrating that the so-called membrane antigen detected in EB virus-infected cells was not integrated into cell membranes. This antigen is loosely adsorbed to either the inner or outer aspect of cell plasma membranes, and may represent non-specifically adsorbed viral components.

The Head of the Cell Biology Section serves as Project Officer on six extramural research contracts within the Special Virus Cancer Program and is a working member of the Immunology Segment.

The Virus and Disease Modification Section conducts a multi-disciplinary approach to the detection, prevention, treatment and/or control of leukemia and sarcoma. Virus infection and chemical treatments were used in attempts to modify tumor cell surface antigens. For this prupose, an AKR mouse

line carrying Gross virus, was infected with several non-oncogenic viruses. Newcastle disease virus infection induced loss of the Gross leukemia virus antigen complex in half of the cells, while measles and Germiston virus infection resulted in the loss of G+ cellular antigens and gs-1 antigen from over 90 percent of the cells. AKR cells infected with Germiston virus lost their capability to grow as tumors.

In animals previously vaccinated with BCG, a mixture of BCG and Moloney pseudotype mouse sarcoma virus (MSV-M) induced no tumors after inoculation although a similar inoculum of MSV-M in non-BCG-treated mice resulted in tumors in all infected animals. The protective effect of adoptive immunotherapy was demonstrated in mice bearing the transplantable leukemia, LSTRA. Tumor-bearing mice were placed in remission with bis-chloronitrourea (BCNU), and then inoculated with tumor-immune mouse spleen cells. After 70 days observation 94 percent of the experimental animals survived in comparison with 40 percent survivors among animals treated with BCNU alone. Untreated mice inoculated with LSTRA cells succumb in 14 days.

Interferon therapy was effectively applied in mice infected with the Moloney sarcoma virus plasma variant (MSV-PV). The survival rate of virus-infected mice treated with the interferon-inducing poly Ir:Cr-poly-D-lysine complex was nearly twice that achieved with poly Ir:Cr alone. Mice immunized with formalinized MSV-PV were also refractory to challenge with MSV-M and Friend leukemia virus as well as with MSV-PV.

A micro assay developed for delta-aminolevulinic acid (ALA) synthetase, an important enzyme in the biosynthesis of red cell porphyrins, was applied to detect enzyme activity in leukemic white cells. ALA synthetase was found in leukocytes from patients with acute and chronic myelogenous leukemia and acute monocytic leukemia, but not in leukocytes from donors with chronic lymphocytic leukemia nor from normal donors. These observations may have diagnostic value. Further studies were made with IM-1 cells, a line of cultured lymphoblasts which have a completely functional heme biosynthetic pathway. ALA synthetase was detected in these cells and found to be sensitive to puromycin but not to actinomycin D. This suggests a shorter half-life for the enzyme than for its messenger RNA. Polyphoric fibroblasts were sensitive to rotenone, an inhibitor which reduces oxygen consumption by blocking transfer of reducing equivalents between NADH and cytochrome B. These investigations on heme synthesis in leukocytes may reveal the importance of porphyrin synthesis in the leukemic state.

The Head of the Virus and Disease Modification Section serves as Chairman of the Special Animal Leukemia Ecology Segment, SVCP, and as Project or Assistant Project Officer on eight research contracts. Other investigators within the Section also have Project Officer duties.

The Human Tumor Studies Section conducted studies on EB virus. In an attempt to select a lower primate susceptible to infection by EB virus, different species of monkeys were first screened for antibodies reactive with EB virus. Immunofluorescence tests showed a high incidence of

World monkeys obtained from the wild but not in New World monkeys. Monkey sera reacted with the Beta antigen in EB virus-infected cells and gave a line of identity with human sera in immunodiffusion tests. Seronegative monkeys were inoculated with infectious EB virus. Attempts to establish lymphoblastoid cell cultures from infected animals were not successful.

Specimens of tissue from various organs of Burkitt tumor patients taken post-mortem were tested for the presence of EB virus antigens. In one case immunodiffusion tests showed viral antigen in the spleen and tumor tissue but not in other organs. Other case material was negative.

The Mason-Pfizer monkey virus (M-PMV), recovered from a spontaneous rhesus monkey mammary tumor, banded at a density of 1.166 gr/cc on density gradient centrifugation. The RNA of the virus analyzed by polyacrylamide gel electrophoresis appeared to be of the same size as other RNA tumor viruses. Its calculated molecular weight was 7.4×10^6 daltons. Applying the Spirin formula yielded an estimated sedimentation value of 56 S, which does not agree with reported S_{20w} values of 65-70 S which were calculated from sedimentation velocity data. The basis for this variation is under investigation.

Hemadsorption tests made on M-PMV infected cells showed no evidence of the presence of SV5 virus. Preliminary serology yielded no reaction between antiserum to M-PMV and simian foamy virus types 1,2,3,4,5,6, or 7.

The Head of the Human Tumor Studies Section is Project or Associate Project Officer on six research contracts within the Special Virus Cancer Program.

Within the Microbiology Section, a study was completed in vitro on a viral inhibitor reported to be present in the JLS-V5 cell line chronically infected with Rauscher virus. Silicotungstic acid, a protein precipitant applied in the preparation of this inhibitor, was found to be the active factor inhibiting replication of murine tumor viruses in vitro. No activity was found by another laboratory testing the inhibitor in vivo.

The lactic dehydrogenase virus (LDV) was found to potentiate oncogenesis by murine sarcoma viruses of the Harvey (MSV-H) and Moloney (MSV-M) strains. Co-infection of mice by LDV and MSV-H gave a 90 percent increase in tumor incidence and mortality. The influence of an inocuous agent such as LDV on the incidence of disease induced by a tumor virus is particularly significant.

A complex of poly-D-lysine and poly Ir:Cr induces higher levels of interferon in vivo than does poly Ir:Cr alone. When serum interferon responses were determined in different strains of mice, activity varied by a factor of about 100 from the most to the least responsive mouse strain.

The Electron Microscopy Section is newly established. Collaborative studies with other investigators were begun and support services for the Viral Oncology Area were instituted. A considerable amount of routine examination

of tissues and cell cultures to detect virus was completed. Several of the more recent murine sarcoma and lymphoma virus isolates were studied in the organs of infected animals of different species. A simian cytomegalovirus isolate was tentatively identified on the basis of morphological criteria, and identity was later confirmed serologically. Studies with Herpesvirus saimiri to determine its host range were monitored.

The possibility that M-PMV might be a simian foamy virus was investigated by comparison with seven different types of known foamy viruses in human kidney cell cultures. Unlike M-PMV, all the foamy viruses induced cellular degenerative changes. Although foamy viruses form intracytoplasmic A-type particles which bud from the cell surface as does M-PMV, the mature extracellular particles differ in that they form spiked particles. Further, antiserum to M-PMV failed to neutralize the foamy agents.

Biopsies of a spontaneous mammary carcinoma of a Siamese cat contained 100 μm diameter particles resembling virus. The viral nature of these particles is still uncertain.

Research conducted within the Virus Studies Section was discontinued upon the retirement of the principal investigator, Dr. Stewart. Under this project, four human tumor cell lines spontaneously transformed without morphological evidence of the presence of virus. Transmission studies with clarified and with filtered fluids from these cultures were suggestive of the presence of a factor inducing transformation of other cells.

The Office of the Chief coordinates the research efforts of investigators in each of the above sections and collaborative studies with investigators in other laboratories. The Chief serves as Chairman of the Developmental Research Segment of the Special Virus Cancer Program and is responsible for the administration and management of the extramural research contracts within the Segment. He is supported by the Vice-Chairman of the Segment, secretarial assistance, and investigators within and outside the Branch who serve as Project Officers and/or members of the working contract review group.

Proposed Course of Effort Within the Viral Biology Branch:

Investigations within the newly reorganized Branch have been largely directed to the characterization of specific viruses immunologically and biochemically, to the evaluation of measures for control of virus-induced neoplasia, to the study of normal and malignant cell *in vitro*, and to the initiation of intensive investigations on cell-mediated immune mechanisms. These studies will continue.

A susceptible lower primate for studies on the EB virus will be sought. Present evidence indicated that a number of species of monkey are naturally infected with a virus antigenically related to EB virus. Attempts will be

The tumor promoting effect of non-oncogenic viruses interacting with oncogenic strains is highly significant in relation to human neoplasia. Investigations will continue to determine the mechanism for such potentiatir activity.

Immunological control measures effective against virus tumor development will be further evaluated. Methods to increase the antigenicity of weakly antigenic tumors will be sought. Studies will be conducted to ascertain the nature of factors effectively transmitting specific cellular immunity within and across species.

Biochemical characterization of specific viruses will be continued.

Publications and Other Activities:

During the reporting period a total of 15 papers were published or accepted for publication. Members of the Branch presented by invitation more than twelve lectures to research groups in this country and abroad.

In addition to the intramural research efforts of members of this Branch, most also contribute substantial amounts of their time to support research contract projects within the Special Virus Cancer Program. Specifically, Dr. Michael Chirigos, Associate Chief of the Viral Biology Branch, serves as Chairman of the Special Animal Leukemia Ecology Segment and Dr. George Burton is Executive Secretary of this Segment. Dr. Jack Gruber is the Vice Chairman and acts as Executive Secretary for the Developmental Research Segment, Dr. Timothy O'Connor serves as advisor in Molecular Virology to the Associate Scientific Director for Viral Oncology and as a working member of the Developmental Research Segment Contract Review Group. Dr. Charles Boone is a working member of the Immunology Segment Contract Review Group. In addition, these investigators, as well as other investigators within the Branch, act as Project Officers on research contracts within the SVCP. These duties require direction and consultation with contractors, site visits, preparation of regular reports, and attendance and presentation of data during contract reviews.

VIRAL CARCINOGENESIS BRANCH

July 1, 1970 June 30, 1971

During the past year the comprehensive and highly integrated intramural and contract research programs of the VCB were reoriented to test the validity of the new oncogene theory of cancer. This unitary theory (first proposed by VCB investigators in 1969) postulated that most, if not all, types of cancer are specified by inherited RNA tumor virus oncogenes which like all other gene operons are controlled by other regulatory genes.

Cancer which in natural (feral) species generally occurs after the reproductive period is viewed as the result of breakdowns in appropriate regulation brought on by (1) endogenous factors such as mutant defective genes, hormonal imbalances, immunological deficiencies, and simply senescence; and (2) exogenous factors such as environmental carcinogens. One of the chief objectives, then, is to discover the molecular and biochemical mechanisms by which cells regulate oncogenes in the hope of finding ways to postpone neoplastic change.

Numerous isolates of the C-type RNA viruses are now established in laboratory systems from three classes of vertebrates (snakes, birds and mammals), and from three orders and four species of mammals. Since current evidence indicates that RNA viruses in these vertebrates do not depend on horizontal transmission, but on genome inheritance, we postulate further that the arrangement (integration) of the RNA viral genomes with cell genomes must have been already well established in the common ancestors of these different vertebrates, thus an arrangement that probably existed more than 300 million years ago. The biochemical similarities exhibited by the major polypeptide group-specific (gs) antigens of the C-type RNA viruses of the three classes of vertebrates, which when assembled constitute 30% of the mass of the C-type particle, provide additional contemporary evidence of common phylogenetic relationships and attest further to a common ancestry and a profound evolutionary linkage to the species in which they are found. It is very unlikely that man, who experiences cancers very similar to those of the other mammals, should have escaped involvement with these "built in" RNA oncogenes despite his long span of life and the longer periods during which the oncogenes must be maintained in repressed state.

More specific recent support for the oncogene theory.

In 1971 the heuristic value of the oncogene theory was proved in terms of the many newly launched research ventures stimulated by it, particularly in molecular biology, in vitro carcinogenesis, and immunogenetics. Although the theory still requires definitive proof, considerable confirmatory evidence in support of it was brought forth in FY 1971 by investigators outside the VCB as well as those in it. The landmark discovery of the reverse RNA transcriptase by Temin and Baltimore supplied a clearcut mechanism by which RNA genomes could be integrated with host

cell DNA; indeed vertical transmission and inheritance of the RNA genomes are implicit in the new protovirus theory of Temin. Similarly, recent reports by Vogt and Hanafusa describe endogenous RNA virus "helper" factor in normal embryonic cells of chickens; others, including Meier, Lilly, Pincus and Hartley, Payne and Chubb, working with inbred mice and chickens elucidated both dominant and autosomal recessive host genes which clearly regulate and control the various antigenic, infectious and oncogenic expressions of the endogenous RNA tumor virus genomes. Recently, also, the Russian scientist Abelev and his associates in a series of reports confirmed previous findings in VCB that gs antigen subunits of the RNA virus are prevalent in embryonic and tumorous tissues of all strains of mice, even those having maximum genotypic resistance to cancer and RNA virus expressions. Finally, intramural and contract supported investigators working on highly targeted VCB programs have contributed significantly in elucidating (1) the natural behavior of the RNA tumor virus genomes in mice, chickens, hamsters, cats and rats; (2) the profound effects of host genotypes on cancer and virus activity; and (3) the biochemical and immunological specificities of the independent virogene and oncogene expressions specified by the RNA virus genomes, including those which occur "spontaneously" and those which are induced by various carcinogenic agents.

The most significant contributions of the VCB program in Fiscal Year 1971 can be briefly highlighted as follows:

1.0 Properties and natural prevalence of the group-specific (gs) antigens of the C-type RNA virus genome.

1.1 The gs antigens of the RNA myxoviruses represent perhaps the best studied of viral products; they provide the basic marker used for group classifications of the numerous influenza and parainfluenza viruses of man and animals. Similar gs antigens, first reported in 1964 by VCB scientists, have been demonstrated in the RNA viruses of chickens, mice, cats, hamsters, rats, and snakes. These antigens which have been studied intensively by some of the top biochemists and immunologists in the world, provide a diagnostic marker (handle) for the RNA tumor viruses which not only makes it possible to demonstrate the presence of the genome in cells and tumors free of infectious virus, but has increased at least 100-fold our knowledge of the natural distribution of the RNA tumor virus genome.

1.2 Isolation, purification of gs antigen and preparation of specific antibody to it: In FY 1971 VCB investigators Drs. Gilden, Oroszlan and Kelloff succeeded in isolating and highly purifying the gs antigens of mouse, cat, hamster and rat, following which monospecific antisera to the major gs-1 component were prepared in guinea pigs and rats. When purified by isoelectric focusing, the gs antigens from all 4 mammals were found to be stable homogenous molecules with molecular weights of 20,000 to 30,000; however, when assembled they found that the gs antigen molecules make up at least 30% of the total virus particle. Gilden et al. confirmed the

separate reactivities of the major gs-1 (species specific) and gs-3 (interspecies) determinants; however he subsequently showed that both determinants were located on the same molecule.

1.3 Universal prevalence of gs antigens: In the meantime, Huebner, Sarma, Kelloff, Gilden, Meier, Whitmire, Peters, and Gardner, with the use of rat, guinea pig and hamster antisera to gs antigens, reported that gs antigens were commonly detectable in virtually all embryonic tissues of mice, certain rapidly replicating postnatal tissues, and many if not most spontaneous and chemically induced cancers of mice. Sarma and associates, using hamster sera and rabbit sera (from Dr. David Allen) specific for avian RNA virus gs antigens, similarly demonstrated expressions of gs antigens in all avian embryos from 4 to 20 days in ovo including those derived from RIF-free flocks known to be free of infectious RNA virus. Similar but less extensive studies have indicated widespread distributions of gs antigen expressions in embryos and tumors of cats and hamsters as well.

1.4 Immunological tolerance to gs antigens: These direct demonstrations of RNA C-type virus expressions in prenatal tissues were followed up by studies which revealed that all mice, hamsters, cats, and nearly all chickens were inherently incapable of producing antibody to the gs antigens of their isologous species. The fact that most members of these species could produce antibodies to gs antigens in heterologous species and to envelope antigens of their own species showed not only that the antigens were antigenic but that the animals were immunologically competent. This classic manifestation of immunological tolerance provided solid support for our hypothesis that the universally distributed embryonic gs antigen expressions probably play important roles in the development and differentiation of embryonic tissues. This concept leads logically to the speculation that the RNA genome activities may for the most part be beneficial perhaps by helping to regulate cell replication; viewed in this way, the development of cancer then results from an unfortunate breakdown in normal regulating mechanisms induced by endogenous or exogenous factors. Cancers therefore would appear as abortive efforts to produce a genetically identical twin at an inappropriate time and place, thus accounting for the fact that the body generally recognizes and accepts the tumors as "self."

1.5 Activation of gs antigens concomitantly with induction of chemically induced tumors in mice: Previous reports by VCB investigators of the activation of complement-fixing gs antigens in subcutaneous fibrosarcomas induced in mice by 3MC were confirmed in FY 1971 by Whitmire and associates in 16 strains of inbred and outbred laboratory mice. Complement-fixing antigens reactive with anti-gs rat antisera were demonstrated in most of the fibrosarcomas when tested with broadly reactive antisera. When tested with specific antisera to gs-1, the distribution of positives in the inbred mice varied from 0% to 100% depending on the genotypes of the different strains. The k, s and q alleles were associated with high incidences of gs-1 antigen expression, while the b and d alleles were associated with

lower incidences. Since adjacent normal mesenchymal tissues were negative for antigen, the switch on of gs antigen in tumors was attributed to de-repression of the RNA virogenes concomitant with tumor development.

1.6 In vitro activation of gs antigen (and infectious virus) in mouse, hamster and rat fibroblasts: That individual mouse cells (C3H fibroblasts grown in culture can switch on oncogenes was first observed by Earle and his associates; subsequently Evans, Hartley et al. reported that most such cells were "switched on" for gs antigen and infectious expressions of RNA virus, and in 1970 Aaronson, Hartley and Todaro made similar observations in additional cultured mouse cells.

In FY 1971, Freeman, Hartley, Gardner, Officer and Kelloff observed spontaneous and chemically induced switch on of murine RNA viruses in wild (feral) mouse cells; recently, also, of hamster RNA viruses in 5 of 6 hamster cell lines transformed by 3MC and fractions of tobacco smoke.

Finally, Rhim and his associates discovered a new antigen having the properties of gs antigens in rat cells transformed in early subcultures by polyoma virus and in the normal rat cell lines when carried beyond 60 subcultures.

We concluded from these findings that mouse, hamster and rat cells grown in vitro contain "switched off" RNA virus genomes that are subject to de-repression not only by many different chemical and DNA virus carcinogens, but also as a consequence of long term culture. Such observations not only make less likely the contention that mesenchymal tumor cells in vivo depend for their viral expressions on the migration of virus information from other areas in the body such as the hematopoietic tissues, but furnish cogent reasons for assuming that the oncogenic and virogenic events were indeed induced in situ.

2.0 Oncogenes of C-type RNA infectious viruses as determinants of accelerated cell transformation by carcinogenic chemicals and DNA tumor viruses: In a series of recent reports Freeman, Rhim and Huebner and their associates described greatly accelerated transformations induced by various carcinogenic chemicals in rat, mouse and hamster cells when such cells were superinfected with various mouse and hamster non-transforming C-type RNA viruses. The normal cells treated with chemicals alone or virus alone failed to transform and could not be transplanted in isologous newborn hosts; however the infected cells treated with carcinogens readily trans-formed and in most instances were transplanted successfully in newborn isologous hosts.

Thus cells of 3 species cultured in vitro, carrying one of four strains of RNA tumor viruses (RLV, CF-1, AKR, HaLV) and treated with at least 8 different carcinogenic chemical preparations, in every case led to the development of tranformed foci, continuous neoplastic cell lines and, with only one exception, tumors in isologous hosts. The transformations

occurred rapidly within 9 to 45 days after 7 to 14 days of exposure to the chemicals in the media. Dosages as low as 0.01 ugm of MC and DMBA produced rapid transformation; thus providing at least 100-fold range for carcinogenic assay below the toxicity levels of the chemicals. The high predictability achieved with many different combinations of viruses, cells and chemicals clearly suggested that the added RNA viral genomes provided numerous additional oncogene determinants which, when derepressed by the chemicals, accounted for the accelerated neoplastic changes observed.

3.0 RNA dependent DNA polymerase; enzymatic and antigenic properties: VCB contract supported scientists were among the first to extend Temin's and Baltimore's observations on the reverse transcriptase. Green and associates showed that the entire RNA genome of avian, mouse and cat viruses was copied by the polymerase as small DNA molecules and that the DNA dependent as well as the RNA dependent polymerases were inhibited independently by various rifampicin derivatives. The latter also inhibited RNA-DNA hybrids while duplex DNA's were inhibited by actinomycin derivatives. These observations were interpreted as providing potential leads to a rational approach to chemotherapy of cancer.

Current studies in cooperation with Dr. Green will explore the effects of rifampicin specifically on endogenous oncogene expressions in vitro. Inhibitors specifically directed against the RNA polymerases responsible for assembly of the virus particle may not have specific activities in relation to transformation or tumor induction since the oncogene expressions are independent of the virogene expressions. Since most cancer must be due to endogenous rather than exogenous viruses, answers to the question of specificity inhibitor action are critically needed.

3.1 Specific antibody to RNA dependent DNA polymerases: Equally as exciting were the findings of Gilden and Hatanaka (Flow Laboratories, Inc.) who purified the cat virus polymerase and with it produced monovalent antibody which inhibited the activity of the mouse, cat, hamster and rat virus polymerases but not that of the avian viruses. Thus the polymerase represents an additional group-specific interspecies antigen protein of the mammalian viruses; and the ability to specifically inhibit polymerase activity should make it possible to determine its role in spontaneous and carcinogen-induced cancers, both of which we postulate are the product of endogenous oncogenes.

4.0 Host gene controls of RNA tumor virus expressions: One of the spin-offs of the inherited oncogene theory was increased interest in studies of genetic and moelcular mechanisms by which host genes influence expressions of the postulated endogenous virogenes and oncogenes. Meier et al., who in FY 1971 provided much of the natural history data on the universality of the C-type RNA genomes and lack of horizontal spread of virus in inbred mice at The Jackson Laboratory, also observed large and well defined influences of strain genotypes on predisposition or resistance to the development of spontaneous cancer, to susceptibility to environmental carcinogens, and to various independent expressions of the RNA tumor viruses.

Cross-breeding and other experiments have revealed the importance of a variety of dominant and recessive alleles as determinants of RNA virus and tumor expression. Breeding programs are well underway which indicate that by genetic manipulation, high cancer (lymphoma) strains can be converted to resistant strains and vice versa. Current experiments are designed to determine the number, mode of inheritance, linkage relationships and chromosome location of the host genes which regulate oncogene and virogene expressions.

4.1 Studies of host gene controls in vitro: Similarly, Hartley, Rowe and Pincus in characterizing embryo cells derived from 21 strains of mice according to sensitivity to N- or B-tropic murine leukemia viruses, found that all cells were similar to either the N-type or B-type. The patterns of sensitivity of N-type x B-type F1 hybrids indicated dominance of resistance to both types of virus, and embryos from back-cross experiments suggested that a single locus (N-B) is the primary determinant of the host cell response patterns; subsequently the N-B locus was found to be identical with the FV1 locus which controls sensitivity to Friend leukemia virus.

The numerous host genes that have been found to control RNA virus expressions and the discovery that the "resistance" allele on the N-B locus controlling wild type infectious RNA virus replication not only supports the inherited oncogene theory, but provides evidence of built-in natural host gene systems designed through long evolutionary association to control the RNA viral genome. This is also part of the natural inheritance of mice. Similar host controls of cancer and RNA virus have been described for chickens. It would seem inevitable therefore that similar host genes and endogenous RNA genomes will be found in the cells of most or all other vertebrates.

5.0 Cat leukemia (FeLV) and cat sarcoma (FSV) viruses: detection and natural history: FeLV and FSV have special importance because infectious virus is rather prevalent in tumored cats; the virus is found in cat salivary secretions and is known to grow well in human cells in culture. Consequently VCB investigators (Sarma, Gardner, Riggs, Oshiro, Schneider and Arnstein) have been much involved in developing and applying sensitive detection methods such as the new COCAL test for FeLV, focus-forming tests for FSV, fluorescent antibody (FA), complement-fixation (CF), neutralization and other serological tests for antigen and antibodies. Serological studies by Riggs, Oshiro and Sarma have shown that sera from 600 veterinarians show no evidence of specific FA or neutralizing antibodies to FeLV; thus despite maximum exposure to sick and cancerous cats, veterinarians apparently are not susceptible to infection with cat viruses.

5.1 Epidemiological studies of cats as a cancer risk factor: Controlled epidemiological studies designed to examine human exposure to cats as a factor in human cancer cases in Los Angeles by Gardner and Hanes of the University of Southern California showed no excess exposures to cats in the households of several hundreds of leukemia, sarcoma and carcinoma

cases as compared to comparable control households. In fact 80% of the cancer and control households had no household exposure to cats during the previous 10 years.

A similar survey in Northern California by Schneider failed also to show that household exposure to leukemic cats increased the incidence of cancer in the exposed persons as compared to control households without such exposure.

These studies of course do not eliminate the possibility that human cancer might occasionally result from a cat virus source, but they suggest that cat virus is unlikely to account for detectable amounts of human cancer. The study in Northern California will be carried on as a prospective study in order to increase numbers and more conclusive interpretation.

6.0 Etiological and epidemiological studies of human cancer.

6.1 Search for evidence of C-type RNA virus expressions: The search for C-type RNA viruses in human cancers at the University of Southern California were no more successful in FY 1971 than in previous years. Electron micrographic examinations of hundreds of human tumors including all available types of leukemia and solid cancers, and of embryo and tumor cell cultures, were entirely negative for C- and B-type particles. The tumors were acquired and examined at USC and the cultures were produced at the Naval Biological Laboratory under Dr. Nelson-Rees.

Since there are no currently extant human cell cultures carrying C-type particles, and since infectious virus has proved equally elusive, the VCB's future efforts will be focused on attempts to demonstrate antigens in human tumor and embryo cells which might be expected to cross react with one or more of the interspecies antigens demonstrated in mouse, hamster, cat and rat C-type viruses; we plan to look for evidence of all types of antigens specified by the known RNA viruses including: group-specific antigens 1, 3 and other postulated interspecies antigens; the cell surface antigens specified by the RNA genome, variously described as GSA (Aoki) and TSTA (Sjogren and Pasternak); and viral envelope antigens.

6.2 Serological data: Recent attempts to demonstrate cross-reacting antigens by complement-fixation in extracts of human tumors, utilizing broadly reactive anti-gs antisera produced in Fischer rats carrying MSV-induced tumors or in rats hyperimmunized with extracts of the MSV rat tumors, yielded many positive human tumor and embryo tissue preparations; however the reactive antigenic moieties so far have been found to be ether resistant and associated with sedimentable cell membranes. Surveys of human cancer patient sera have yielded a few which react at low serum dilutions with the sedimentable antigens and the patterns of serum reactivity are almost exactly similar to that provided by the rat sera. Although the antigens detected are clearly not related to the gs antigens found in the mammalian RNA tumor viruses, they appear to be shared widely and thus are interesting enough to be characterized and identified. It

is possible that they will prove to be similar to the carcino-embryonic antigens described by Gold, Uriel, Grabar and others.

New and more sensitive autoradiographic serological test systems developed by Gilden's group, in addition to CF, FA and gel diffusion, will be combined with absorption studies and utilized in an exhaustive study of human tumors for antigens related to those now demonstrable in the RNA viruses and tumors of mice, cats, hamsters and rats.

In order to adequately prepare for such studies, numerous human tumors and sera from cancer patients have been collected and frozen for future studies; cell lines have been derived from many tumors and some of these are being subjected to intensive studies as described below.

6.3 Studies of human tumor cells in vitro: Having established several human sarcoma cell lines which have retained their sarcomatous characteristics, McAllister and his associates at Children's Hospital of Los Angeles have attempted to rescue the sarcoma genome by superinfecting them with murine and cat viruses; these efforts have been unsuccessful despite the fact that the viruses replicated in the human cells. In cooperation with Klement, Nicolson and Gardner, McAllister is seeking evidence of phenotypic modifications in the mouse and cat viruses replicating in the human tumor cells. Current tests suggest modification of the envelope and host range but not of viral gs antigen and RNA moieties; tests of the specificities of the viral RNA polymerases are underway. Serial subcultures of the tumor cells, both infected and non-infected, are being continued indefinitely in anticipation that, like mouse, rat and hamster cells, human cells that grow continuously in vitro will eventually derepress and yield expressions of the postulated human RNA virus genome. At various subcultures the human sarcoma cells were transplanted into cat fetuses in which tumors developed having histological appearances identical with that of the original tumors seen years before in the patients; and chromosome histograms confirmed the human characteristics of the tumor cells. A similar in vivo human tumor cell growth system in the mouse developed recently by Stanbridge and Hayflick which utilizes ALS to block rejection of the human tumor cells provides a much more available and simpler system for studies of genome rescue of these and other human tumors.

6.4 Transformations of human cells with polyoma virus: In preliminary attempts it appears that Dr. Rhim in collaboration with Dr. Takemoto (NIAID) has succeeded in transforming human cells (WI-38) with large doses of polyoma virus. The cells contain polyoma T antigen but no polyoma viral antigen. Since rat and hamster cells transformed by polyoma virus develop new antigens which are distinct from known polyoma specified antigens, but which react with MSV rat antisera, related antigens will also be sought in the human polyoma transformed cells as well.

6.5 Epidemiological and ecological studies of cancer distributions in Los Angeles: A comprehensive cancer surveillance program designed to provide a "now" registry of all cancer patients in Los Angeles County was planned in FY 1971; as this is funded in 1972, Drs. Henderson, Hanes and Gardner, in cooperation with NCI's demography and epidemiology groups, and with Shimkin (University of California, San Diego) and Winkelstein (University of California, Berkeley), plan to utilize information eventually to be made available on 20,000+ annual cancer cases in Los Angeles in order to mount field studies designed to answer specific questions concerning the etiology of cancer. Examples include questions about effects of maternal age on cancer in offspring and of course the effects of industrial, food-borne and air-borne carcinogens. In support of the latter program, Bryan and Gordon have underway long term studies characterizing smog constituents in 4 different ecologic areas in Los Angeles. Freeman, Rhim, Rasheed and Gardner have already initiated studies of smog residue and tobacco smoke fractions in cell culture systems; preliminary results now in progress and in press indicate that such materials have relatively strong carcinogenic activities (vide supra). Future studies will be focused on tests of chemically defined fractions of known environmental residues and on such additional assays as may be indicated by the results of epidemiological and enviromental studies in the Los Angeles ecology.

RESEARCH LOGIC

FOR

SPECIAL VIRUS CANCER PROGRAM

VIRAL ONCOLOGY, NCI

JULY 1971

TABLE I

ANALYSIS OF CONTRACTS BY SEGMENTS IN VIRAL ONCOLOGY

SEGMENT	NO. OF CONTRACTS	ANNUAL LEVEL [1]	(PERCENT)
TOTAL	120 [2]	$31,590,401	(100)
Developmental Research	24	10,119,281	(32)
Immunology Group	8	956,709	(4)
Special Animal Ecology	17	4,068,618	(13)
Program Resources & Logistics	24	2,354,772	(7)
Biohazards Control & Containment	5	388,796	(1)
Program Management	11	2,979,100	(9)
Solid Tumor Virus	23	9,603,320	(30)
Breast Cancer Virus	8	1,119,805	(4)

1/ Funds obligated by Viral Oncology during Fiscal Year 1971.

2/ The total includes non-recurring contracts funded during FY 1971.
See TABLE III for individual contracts involved.

TABLE II Analysis of Contracts by Activity[1/]

Phase I: SELECTION OF SPECIMENS AND DETECTION OF VIRUS OR VIRUS EXPRESSION
Step 1: SELECTION OF VIRUS SOURCES

Contractor	Cont. No.	Description of Work
Aichi Cancer Center	69-96	Seroepidemiological studies of BL, NPC in Southeast Asia
Baylor University	68-678	Seroepidemiology of cervical carcinoma
California SDPH	69-87	Human-feline retrospective epidemiological studies
Children's Hosp (Phila)	66-477	Immunological studies of EBV-associated cancers
CDC	VCL-42	Epidemiological studies of selected leukemi cases (clusters)
Georgetown University	65-53	Collaborative studies on populations at high risk to breast cancer
IARC	70-2076	Seroepidemiological study of BL and NPC in Southeast Asia and Africa
Inst. for Med. Res.	68-1000	Collaborative studies on populations at high risk to breast cancer
Johns Hopkins University	71-2121	Seroepidemiology of cervical carcinoma
Karolinska Institute	69-2005	Immunological studies of EBV-associated cancers
Maimonides Hospital	71-2046	Genetic analysis of human cancer associated with chromosomal abnormalities
Makerere University	67-47	Epidemiological studies of Burkitt Lymphoma in Uganda

1/ For description of research phase and step, refer to the SVCP convergence chart (fold-out: Research Logic for Special Virus Cancer Program, Viral Oncology, NCI, July, 1971)

TABLE II Analysis of Contracts by Activity

Phase I: SELECTION OF SPECIMENS AND DETECTION OF VIRUS OR VIRUS EXPRESSION
Step 1: SELECTION OF VIRUS SOURCES (continued)

Contractor	Cont. No.	Description of Work
Michigan Cancer Foundation	71-2421	Collaborative studies on populations at high risk to breast cancer
Minnesota, University of	71-2261	Immunological Studies of high risk groups with immuno-deficiency diseases
Natl. Ctr. for Health Stat.	FS-35	Information on incidence and causes of childhood deaths
Southern Calif., Univ. of	68-1030	Cancer surveillance and epidemiologic studie in Los Angeles County
Texas, University of	65-604	Serological studies of human leukemia, lymphoma, and solid tumors
Texas, University of	71-2135	Gather information on laboratory-acquired infections

TABLE II Analysis of Contracts by Activity

Phase I: SELECTION OF SPECIMENS AND DETECTION OF VIRUS OR VIRUS EXPRESSION
Step 2: SOURCES OF VIRUS OR SUBVIRAL MATERIAL

Contractor	Cont. No.	Description of Work
Aichi Cancer Center	69-96	Acquire normal fetal and tumor tissue
Baylor University	68-678	Acquire clinical data and specimens of human neoplastic tissues
California, University of	70-2048	Acquire clinical data and specimens of normal and neoplastic tissue
CDC	VCL-42	Collection of clinical data and specimens from human leukemias
Colorado University	69-2080	Pediatric and adult tumor specimens
Georgetown University	65-53	Acquire clinical data and human breast cancer specimens
Hospital for Sick Children	65-97	Human leukemia and normal tissue collecti
Howard University	70-2178	Acquire clinical data and human breast cancer specimens
IARC	70-2076	Acquire clinical data and specimens from BL and NPC
Johns Hopkins University	71-2109	Acquire clinical data and specimens from human leukemias and lymphomas
Karolinska Institute	69-2005	Acquire clinical data and specimens from BL, NPC, and leukemias
Maimonides Hospital	71-2046	Supply normal and neoplastic tissues from patients with various chromosomal abnormalities

TABLE II Analysis of Contracts by Activity

Phase I: SELECTION OF SPECIMENS AND DETECTION OF VIRUS OR VIRUS EXPRESSION
Step 2: SOURCES OF VIRUS OR SUBVIRAL MATERIAL (continued)

Contractor	Cont. No.	Description of Work
Makerere University	67-47	Collection of Burkitt Lymphoma specimens
Memorial Hospital (N.Y.)	71-2116	Acquire clinical data and specimens of human neoplastic tissue
Memorial Hospital (N.Y.)	71-2194	Supply clinical data and specimens for human breast cancer studies
Michigan Cancer Foundation	71-2421	Acquire clinical data and specimens for human breast cancer studies
Michigan, University of	65-639	Collection of adult leukemia/lymphoma specimens
Minnesota, University of	71-2261	Acquire clinical data and specimens from patients with immunological deficiency diseases
Montreal Children's Hosp.	65-1020	Collection of human leukemia and normal bloo specimens and normal tissue
Naval Biomedical Res. Labs.	FS-8	Cell cultures of normal and neoplastic tissues
Padua, University of	68-1389	Collection of untreated human tumor and normal tissue specimens
Southern California, Univ. of	68-1030	Acquire clinical data and specimens of human neoplastic tissues
St. Joseph's Hospital	69-2074	Acquire clinical data and human sarcoma and breast cancer specimens
Texas, University of	65-604	Acquire clinical data and specimens of human neoplastic tissue
Texas, University of	71-2178	Acquire clinical data and specimens of human neoplastic tissues

TABLE II Analysis of Contracts by Activity

Phase I: SELECTION OF SPECIMENS AND DETECTION OF VIRUS OR VIRUS EXPRESSION
Step 3: DETECTION OF VIRUS OR VIRUS EXPRESSION

Contractor	Cont. No.	Description of Work
Aichi Cancer Center	69-96	Detection using cell culture techniques-human tumors
Atomic Energy Commission	FS-7	Developmental research on immunological detection of tumor antigens--fetal antiger
Atomic Energy Commission	FS-13	Detection using immunological techniques
Baylor University	68-678	Detection using immunological and cell culture techniques
Bionetics Research Labs.	71-2025	Screening of human/primate neoplastic tissue with biochemical techniques
Bionetics Research Labs.	69-2160	Detection using immunological techniques
California, Univ. of (Davis)	70-2048	EM, biochemical, immunological techniques in comparative leukemia/sarcoma virus studies
California SDPH	68-997	Studies on the role of oncogenic viruses in cancer of man and domestic animals
California, Univ. of (also NBL - FS-8)	63-13	Tissue culture studies of normal and neoplastic human tissues
CDC	VCL-42	Immunological and virological studies of human leukemia and lymphoma
Children's Hospital (Phila)	66-477	Immunological detection of EBV-associated antigens in human cancer
Columbia University	70-2049	Screening human leukemia/lymphoma specimens with biochemical techniques
Cornell University	71-2508	Isolation, characterization of cat leukemia viruses

TABLE II Analysis of Contracts by Activity

Phase I: SELECTION OF SPECIMENS AND DETECTION OF VIRUS OR VIRUS EXPRESSION
Step 3: DETECTION OF VIRUS OR VIRUS EXPRESSION (continued)

Contractor	Cont. No.	Description of Work
Cornell University	70-2224	Service - feline virus diagnostic laboratory
Einstein Medical College	65-612	Genetic studies on tumor/virus susceptibility
Flow Laboratory	71-2097	Immunological studies of mammalian Type C viruses
Georgetown University	65-53	Human breast cancer detection - EM, tissue culture techniques
Hazelton Labs.	69-2079	Immunological/biochemical detection of virus in canine tumors
Howard University	70-2178	Immunological studies of human breast cancer
Huntington Research Ctr.	69-54	Immunological reagent production
IARC	70-2076	Immunological studies of BL, NPC
Indiana University	69-2048	Immunological characterization of avian RE tumor virus
Instit.for Med. Res.	68-1000	Screening human and animal breast cancer specimens by EM, immunological techniques
Jackson Labs.	67-744	Genetics of susceptibility to cancer in mice
Johns Hopkins University	71-2121	Immunological studies on herpesvirus antigens in cervical carcinoma
Johns Hopkins University	71-2109	Immunological studies of human leukemia and lymphoma
Karolinska Institute	69-2005	Immunological studies of EBV-associated human neoplasia

TABLE II Analysis of Contracts by Activity

Phase I: SELECTION OF SPECIMENS AND DETECTION OF VIRUS OR VIRUS EXPRESSION
Step 3: DETECTION OF VIRUS OR VIRUS EXPRESSION (continued)

Contractor	Cont. No.	Description of Work
Maimonides Hospital	71-2046	Genetic analysis of human tumors
Mt. Sinai School of Med.	69-2078	Immunological study of HTV antigens
Mason Research Institute	70-2204	Development of primate test systems for breast cancer virus detection
Meloy Labs.	66-458	Immunological studies of murine mammary tumor virus
Meloy Labs.	70-2047	Detection using immunological, biochemical and tissue culture techniques
Microbiological Assoc., Inc.	70-2068	Detection using immunological and cell culture techniques
Microbiological Assoc., Inc.	67-697	Bioassay of murine leukemia/sarcoma viruses
Microbiological Assoc., Inc.	67-700	Service - murine viral diagnostic reagents and testing
Minnesota, University of	69-2061	Development of immunological tests for tumor antigens and antibodies
Minnesota, University of	71-2261	Immunological and virological studies of immunodeficiency disease
Ohio State University	69-2233	Immunological testing (PRILAT) for human tumor antigens and antibodies
Pennsylvania, University of	65-1013	Studies of viruses associated with bovine leukemia
Pennsylvania State	70-2024	Biochemical, genetic studies of Herpes-type viruses
Pfizer, Chas., and Co.	70-2080	Test systems for quality control of virus production
Pfizer, Chas., and Co.	67-1176	Detection of virus by EM-human and animal breast cancer
Princeton University	71-2372	Detection of cell membrane antigens by agglutination techniques

TABLE II Analysis of Contracts by Activity

Phase I: SELECTION OF SPECIMENS AND DETECTION OF VIRUS OR VIRUS EXPRESSION
Step 3: DETECTION OF VIRUS OR VIRUS EXPRESSION (continued)

Contractor	Cont. No.	Description of Work
Public Health Res. Inst.	71-2129	Development of methods for isolation of virus from human neoplasia
Robert Brigham Hospital	71-2172	Detection using immunological methods
Rush-Presbyterian	71-2032	Immunological, biological, tissue culture studies of tumor viruses in non-humun primates
Salk Institute	67-1147	Genetic, biochemical studies of viral-induced transformation
Southern California, Univ. of	68-1030	Immunological studies of human fetal and tumor tissues
Southwest Foundation	69-93	Service - indigenous simian virus diagnostic reagents
Stanford University	69-2053	Development of cell culture methods for human tissue
St. Joseph's Hospital	69-2074	Screening of human sarcomas by EM
St. Louis University	67-692	Detection using tissue culture and biochemical techniques
Texas, University of	65-604	EM tissue culture and immunological studies of human neoplastic tissues
Texas, University of	71-2178	Immunological methods for detection of human tumor antigens and antibodies
TRW	70-2200	Immunological reagent production
Washington, University of	71-2171	Development of immunological tests for tumor antigens and antibodies

TABLE II Analysis of Contracts by Activity

Phase I: SELECTION OF SPECIMENS AND DETECTION OF VIRUS OR VIRUS EXPRESSION
Step 3: DETECTION OF VIRUS OR VIRUS EXPRESSION (continued)

Contractor	Cont. No.	Description of Work
Weizmann Institute	69-2014	Detection of tumor cell surface antigens plant agglutinins
Wistar Institute	71-2092	Techniques for isolation and characterization of viral-induced tumor antigens
Worcester Foundation	69-2007	Techniques for isolation and characterization of viral-induced tumor antigens

TABLE II Analysis of Contracts by Activity

Phase II-A: ESTABLISHMENT OF REPLICATION AND INITIAL CHARACTERIZATION
Step 1: ESTABLISH REPLICATION OF VIRUSES

Contractor	Cont. No.	Description of Work
Baylor University	68-678	Human leukemia transmission studies in non-human primates
Bionetics Research Labs.	71-2025	Inoculation of nonhuman primates with various animal virus and human materials
California SDPH	68-997	Isolation and characterization of feline tumor viruses
California, Univ. of	63-13	Development and evaluation of cell cultures for viral oncology research
California, Univ. of	70-2048	In vitro and in vivo studies of simian and feline virus infectivity and replication
Children's Hospital (Phila)	66-477	Developmental research on growth and replication of EBV in human cell lines
Cornell University	71-2508	Isolation and characterization of feline tumor viruses
Duke University	71-2132	Production and characterization of avian leukosis viruses
Electronucleonics	71-2253	Production and characterization of selected mammalian oncogenic viruses
Germfree Life Research Ctr.	65-95	Studies on host range effect of chemical cocarcinogens, and infectivity of RSV strains
Health Research, Inc.	63-593	Infectivity assays for EBV
IARC	70-2076	Growth and replication of Herpes-type virus in nasopharyngeal carcinoma and Burkitt's lymphoma specimens
Life Sciences	69-63	Production and infectivity studies of Marek's disease virus
Marquette University	68-1000	Stimulation of Type C virus production in human breast cancer cell line by various hormones

TABLE II Analysis of Contracts by Activity

Phase II-A: ESTABLISHMENT OF REPLICATION AND INITIAL CHARACTERIZATION
Step 1: ESTABLISH REPLICATION OF VIRUSES (continued)

Contractor	Cont. No.	Description of Work
Mason Research Institute	70-2204	Studies on infectivity and effect of hormones on monkey mammary tumor virus replication
Meloy Labs.	66-458	In vitro and in vivo production of murine mammary tumor virus
Meloy Labs.	70-2047	Growth and replication of mammalian Type C and syncytial viruses for tissue culture, biochemical studies of mammalian and avian tumor viruses
Miami University	70-2211	In vitro production of rat mammary tumor derived virus
Microbiological Assoc., Inc.	67-697	Cell-free transmission of murine mammary tumors with extracts from spontaneous tumor
Microbiological Assoc., Inc.	70-2068	Isolation, characterization, and transmission studies of mammalian and avian tumor viruses
Naval Biological Laboratory	FS-57	Studies of environmental factors influencing virus-host interactions--research on laboratory biohazards
North Dakota University	66-8	Role of vectors in transmission, host range of tumor viruses
Ohio State University	65-1001	Studies on factors affecting horizontal transmission of tumor viruses
Pennsylvania, University of	65-1013	Experimental and natural transmission of bovine leukemia
Pennsylvania State	70-2024	Herpes-type virus replication in human cells
Pfizer, Chas., and Co.	70-2080	Tissue culture production of Type C and Herpes type viruses
Rush Presbyterian	71-2032	Mammalian tumor virus infectivity in nonhuman primates
Southwest Foundation	71-2348	Study of latent virus infection and transmission--research on laboratory [illegible]

TABLE II. Analysis of Contracts by Activity

Phase II-A: ESTABLISHMENT OF REPLICATION AND INITIAL CHARACTERIZATION
Step 1: ESTABLISH REPLICATION OF VIRUSES

Contractor	Cont. No.	Description of Work
St. Jude's Hospital	71-2134	Isolation and characterization of oncogenic Herpes-type viruses
St. Louis University	67-692	In vitro cultivation of various mammalian Type C tumor viruses for biochemical studies
Texas, University of	65-604	Infectivity, oncogenicity and host range studies of hamster sarcoma virus; isolation and characterization of candidate human Type C oncogenic virus
University Labs.	66-1133	In vitro and in vivo production of murine and avian tumor viruses

TABLE II Analysis of Contracts by Activity

Phase II-A: ESTABLISHMENT OF REPLICATION AND INITIAL CHARACTERIZATION
Step 2: INITIAL CHARACTERIZATION

Contractor	Cont. No.	Description of Work
Baylor University	68-678	Comparative characterization of human Herpes-type viruses
California, University of	71-2173	Studies on the structure and replication of RNA tumor viruses
California, Univ. of (Davis)	70-2048	Comparative studies on simian leukemia/sarcoma viruses
Children's Hospital (Phila)	66-477	Immunological, tissue culture characterization of EBV
Columbia University	70-2049	Biochemical characterization of mammalian Type C viruses
Cornell University	71-2508	Immunological characterization of feline tumor virus isolates
Flow Labs.	71-2097	Immunological, tissue culture studies of mammalian DNA and RNA oncogenic viruses
IARC	70-2076	Isolation and characterization of Herpes-type virus in culture of Burkitt's lymphoma and nasopharyngeal carcinoma
Indiana University	69-2048	Immunological, biochemical characterization of avian RE virus
Karolinska Institute	69-2005	Immunological and biochemical characterization of EBV
Life Sciences	69-63	In vitro and in vivo studies of Marek's disease virus
Meloy Labs.	70-2047	Biochemical characterization of mammalian Type C viruses
Miami University	70-2211	EM, biological, biochemical characterization of rat mammary tumor derived virus

TABLE II Analysis of Contracts by Activity

Phase II-A: ESTABLISHMENT OF REPLICATION AND INITIAL CHARACTERIZATION
Step 2: INITIAL CHARACTERIZATION (continued)

Contractor	Cont. No.	Description of Work
Microbiological Assoc., Inc.	70-2068	Immunological characterization of mammalian Type C tumor viruses
Nebraska University	71-2076	Biochemical characterization of temperature-sensitive mutant viruses
Pennsylvania, University of	65-1013	Characterization of Type C virus associated with bovine leukemia
Pennsylvania State	70-2024	Genetic, biochemical studies of cells "transformed" by viral (Herpes-simplex)--chemical cocarcinogenesis
Pfizer, Chas., and Co.	70-2080	Immunological, EM, tissue culture characterization of Type C and Herpes-type viruses
Rush Presbyterian	71-2032	Immunological, biological characterization of Herpes viruses of nonhuman primates
Rutgers University	71-2077	Studies on the oncogenic potential of "non-oncogenic" animal RNA viruses
Southern California, Univ of	68-1030	Immunological characterization of mammalian tumor viruses
St. Louis University	67-692	Biochemical characterization of oncogenic RNA and DNA viruses
Texas, University of	65-604	Studies on the relationship of animal tumor viruses to human leukemia and lymphomas; characterization of candidate human Type C oncogenic virus
Wistar Institute	71-2092	Isolation and characterization of oncogenic DNA and RNA virus-induced tumor antigens
Worcester Foundation	69-2007	Biochemical characterization of DNA virus induced tumor antigens

TABLE II Analysis of Contracts by Activity

Phase II-B: REPLICATION AND CHARACTERIZATION OF VIRUS EXPRESSION
Step 1: INDUCE VIRAL REPLICATION OF WHOLE VIRUS OR TRANSMISSION OF EXPRESSION

Contractor	Cont. No.	Description of Work
Aichi Cancer Center	69-96	Tissue culture methods to induce virus replication in human tumor cells
California, Univ. of	63-13	Tissue culture of human neoplastic tissue for induction of virus replication
California SDPH	68-997	Establish cultures from tumors of domestic animals and attempt to rescue defective viral genome
Flow Labs.	71-2097	Cell hybridization technique to rescue "defective" virus
Hazelton Laboratories, Inc.	69-2079	Attempts to rescue virus from canine neoplasms
Marquette University	68-1010	Co-cultivation of human breast cancer and hormone-secreting cell lines
Meloy Labs.	70-2047	Characterization of non-producer transformed cells; virus rescue techniques
Microbiological Assoc.	67-697	Effect of hormones on virus expression
Microbiological Assoc.	70-2068	Studies on Type C viral genome expression--effect of chemical carcinogens
Salk Institute	67-1147	Studies on the activation of Type C virus genome by polyoma virus
Southern California, Univ	68-1030	Studies of virus expression in human fetal and tumor tissue
Stanford University	69-2053	Tissue culture, biochemical methods to induce virus replication in human tumor cells
Texas, University of	65-604	Attempts to induce viral replication in human cell lines

TABLE II Analysis of Contracts by Activity

Phase II-B: REPLICATION AND CHARACTERIZATION OF VIRUS EXPRESSION
Step 2: INITIAL CHARACTERIZATION

Contractor	Cont. No.	Description of Work
Atomic Energy Commission	FS-13	Biochemical studies on regulation of gene expression
Bionetics Research Lab.	71-2025	Biochemical characterization of viral-specific enzymes
California, University of	71-2147	Molecular studies of avian tumor-virus-associated enzymes
California, University of	71-2173	Molecular studies of the structure of oncogenic viruses and characterization of viral-specific-enzymes
Columbia University	70-2049	Biochemical characterization of viral-specific enzymes
Einstein College of Med.	71-2251	Biochemical characterization of viral-specific enzymes and other proteins
Flow Labs.	71-2097	Characterization of tumor virus expression in mammalian systems
Massachusetts Gen. Hos.	71-2174	Characterization of nucleic acids and proteins of AMV
Mass. Instit. of Technol.	71-2149	Biochemical characterization of viral-specific enzymes
Meloy Labs.	70-2047	Multidisciplinary approaches to characterization of virus-expression and mediators of replication
Microbiological Assoc.	70-2068	Immunological identification of antigens related to known tumor viruses
Nebraska University	71-2076	Biochemical characterization of temperature-sensitive mutant viruses
Oregon State University	71-2175	Correlation of ultrastructural and biochemical changes associated with transformation by viruses

TABLE II Analysis of Contracts by Activity

Phase II-B: REPLICATION AND CHARACTERIZATION OF VIRUS EXPRESSION *
Step 2: INITIAL CHARACTERIZATION (continued)

Contractor	Cont. No.	Description of Work
Southern California, Univ	68-1030	Immunological identification of antigens relate to known tumor viruses
St. Louis University	67-692	Multidisciplinary approaches to characterization of oncogenic virus expression and mediators of replication

TABLE II Analysis of Contracts by Activity

Phase III-A: COMPLETE CHARACTERIZATION AND DEFINITION OF PRESUMPTIVE DISEASE RELATIONSHIPS

Contractor	Cont. No.	Description of Work
Atomic Energy Commission	FS-13	Interaction of RNA tumor viruses and host immune mechanism; studies on relationship of embryogenesis and carcinogenesis
Baylor University	68-678	Studies on presumptive disease relationships of HTV
Bionetics Research Labs.	71-2025	Biochemical studies on relationship of Type C viruses to human leukemia
Children's Hosp (Phila)	66-477	Relationship of EBV to human lymphoma
Columbia University	70-2049	Biochemical studies on relationship of Type C viruses to human leukemia and sarcoma
Einstein Medical College	65-612	Genetic studies on tumor/virus susceptibility
Flow Laboratories	71-2097	Complete characterization of RNA and DNA viruses and viral antigens in mammalian tumors
Georgetown University	65-53	Studies on Type B particles associated with human breast cancer
IARC	70-2076	Seroepidemiological studies of Burkitt's lymphoma, NPC
Instit. for Med. Res.	68-1000	Studies on Type B particles associated with human breast cancer
Jackson Labs.	67-744	Natural occurrence of RNA tumor viruses and host gene control of virus expression
Johns Hopkins Univ.	71-2121	Studies on the relationships of herpes simplex type II to cervical carcinoma
Karolinska Institute	69-2005	Immunological studies on the etiology of EBV-associated diseases

TABLE II Analysis of Contracts by Activity

Phase III-A: COMPLETE CHARACTERIZATION AND DEFINITION OF PRESUMPTIVE DISEASE RELATIONSHIPS (continued)

Contractor	Cont. No.	Description of Work
Life Sciences	69-63	Studies on Marek's disease Herpes virus
Makerere University	67-47	Epidemiological studies on role of EBV in Burkitt's lymphoma
Meloy Labs.	70-2047	Biochemical studies on relationship of Type C viruses to human leukemia and sarcoma
Miami University	67-1187	Immunological responses in avian tumor virus infection
Microbiological Assoc.	70-2068	Evaluation of cocarcinogenic factors in viral oncogenesis
Microbiological Assoc.	67-697	Type C virus antigen expression during embryogenesis and in spontaneous cancers
Mt. Sinai Sch. Medicine	69-2078	Immunological responses to EBV in human lymphoma
Naples, University of	71-2056	Isolation and characterization of Herpes simplex virus-induced antigens
Pennsylvania State	70-2024	Effect of cocarcinogens on oncogenic potential of human viruses
Rutgers, The State Univ	68-1025	Immunological responses to inactivated MuLV in mice
Southern California Univ	68-1030	Possible role of animal tumor viruses, environmental cocarcinogens

TABLE II Analysis of Contracts by Activity

Phase IV-A: IMMUNOLOGICAL CONTROL
Step 1:

Contractor	Cont. No.	Description of Work
Merck and Co.	71-2059	Developmental research for virus vaccine production
Res. Fdn. of State Univ. of New York	71-2137	Clinical studies on enhancement of tumor immunity

TABLE II Analysis of Contracts by Activity

Phase IV-B: BIOCHEMICAL CONTROL

Contractor	Cont. No.	Description of Work
Bionetics Research Lab.	71-2025	Screening of various chemicals as inhibitors of polymerases
California, Univ. of	63-13	Screening of various chemicals as inhibitors of viral induced transformation
Columbia University	70-2049	Screening of various chemicals as inhibitors of polymerases
Meloy Labs.	70-2047	Screening of various chemical and immunological reagents as inhibitors of polymerases
St. Louis University	67-692	Screening of various chemicals as inhibitors of polymerases

RESOURCES

Contractor	Number	Type	Species
Aichi Cancer Center	69-96	tissue	human
Bionetics Research Lab.	69-2160	sera	human
Bionetics Research Lab.	71-2025	animal	primates
California, University of	70-2202	animal	feline
California, University of	63-13	tissues	human
California, SDPH	68-997	animal	feline, canine
Chicago Park District	65-1017	animal	primates
Colorado, University of	69-2080	tissues, sera	human
Commun. Dis. Ctr.	VCL-42	tissues	human
Connecticut, University of	69-52	animal	SPF chickens
Cornell University	70-2224	service	feline
Duke University	71-2132	virus	avian
Electronucleonics	71-2253	virus	animal
Emory University	71-2256	animal	primate
Flow Laboratories	71-2341	animal	rodent
Flow Labs.	65-1012	repository	murine
Germfree Life Research Ctr.	65-95	animal	avian, rodent
Hosp. for Sick Children	65-97	tissues	human
Howard University	70-2178	tissues	human
Huntingdon Research Ctr.	69-54	service	
Johns Hopkins University	69-2008	animal	chicken
Life Sciences	68-711	animal	chicken
Louisville, University of	66-902	virus	primate
Maimonides Hospital	71-2046	tissue	human
Meloy Labs.	66-458	virus	murine
Memorial Hospital	71-2116	tissues, sera	human
Memorial Hospital	71-2194	sera	human
Michigan Cancer Fdn.	71-2421	tissues	human
Michigan, University of	65-639	tissues	human

RESOURCES (continued)

Contractor	Number	Type	Species
Microbiological Assoc. Inc.	69-914	animal	murine
Microbiological Assoc. Inc.	67-700	service	murine
Montreal Children's Hospital	65-1020	tissues, sera	human
Padua, University of	68-1389	tissues	human
Pfizer, Chas. and Co.	70-2080	virus	animal, huma
Rush-Presbyterian	71-2032	animal	primate
Southern California	68-1030	tissues	human
Southwest Foundation	69-93	service	simian
Southwest Foundation	69-2011	animal	primate
Stanford University	69-2053	PPLO testing; tissue	human
St. Joseph's Hospital	69-2074	tumor, sera	human
University Labs.	66-1133	virus	animal
Zoological Society of San Diego	63-56	animal	primate

TABLE III
ALPHABETICAL LISTING OF CONTRACTS
AND INDEX TO CONTRACT NARRATIVES

CONTRACTOR	FUNCTIONS[1/]	PAGE
Aichi Cancer Center (69-96)	I(1,2,3); II-B(1)	100
Atomic Energy Commission (FS-13)	I(3); III-A; II-B(2)	180
Atomic Energy Commission (FS-7)	I(3)	185
Baylor University (68-678)	I(1,2,3); II-A(1,2); III-A	101
Baylor College of Medicine (71-733)	Scientific meeting	186
Bionetics Research Labs. (70-2025)	I(3); II-A(1); III-A; II-B(2); IV-B	104
Bionetics Research Labs. (69-2160)	I(3)	187
California SDPH (68-997)	I(3); II-A(1); II-B(1)	227

1/ For description of research activities, see SVCP Convergence Chart and TABLE II.
2/ For five months

TABLE III (continued)

CONTRACTOR	FUNCTION	PAGE
California SDPH (69-87)	I(1)	229
California, University of (70-2048)	I(2,3); II-A(1,2)	145
California, University of (63-13)	I(3); II-A(1); II-B(1); IV-B	230
California, University of (70-2202)	Resources	196
California, University of (71-2147)	II-B(2)	232
California, University of (71-2173)	II-A(2); II-B(2)	233
Chicago Park District (65-1017)	Resources	106
Children's Hosp. of Phila. (66-477)	I(1,3); II-A(1,2); III-A	130
Colorado, University of (69-2080)	I(2)	
Columbia University (70-2049)	I(3); II-A(2); III-A; II-B(2); IV-B	122

TABLE III (continued)

CONTRACTOR	FUNCTION	PAGE
Communicable Disease Center (VCL-42)	I(1,2,3)	
Connecticut, University of (69-52)	Resources	198
Cornell University (70-2224)	I(3)	213
Cornell University (71-2508)	I(3); II-A(1,2)	138
Cornell University (65-620)	See Cornell Univ (71-2508)	
Dow Chemical Co. (65-1045)	Service	222
Duke University (71-2132)	II-A(1)	176
Einstein Medical College (65-612)	I(3); III-A	158
Einstein Medical College (71-2251)	II-B(2)	128
Electronucleonics, Inc. (71-2253)	II-A (1)	199
Emory University (71-2256)	Resources	199

TABLE III (continued)

CONTRACTOR	FUNCTION	PAGE
Flow Laboratories (69-97)	See Flow Labs. (71-2097)	
Flow Laboratories (70-2015)	See Flow Labs. (71-2097)	
Flow Laboratories (65-1012)	Resources	200
Flow Laboratories (71-2097)	I(3); II-A(2); III-A; II-B(1,2)	233
Flow Laboratories (71-2341)	Resources	178
Georgetown University (65-53)	I(1,2,3); III-A	264
Germfree Life Res. Center (65-95)	II-A(1)	161
Hazelton Laboratories, Inc. (69-2079)	I(3); II-B(1)	148
Health Research, Inc. (63-593)	II-A(1)	106
Hospital for Sick Children (65-97)	I(2)	201

TABLE III (continued)

CONTRACTOR	FUNCTION	PAGE
Howard University (70-2178)	I(2,3)	155
Huntingdon Research Center (69-54)	I(3)	202
Indiana University (69-2048)	I(3); II-A(2)	141
Institute for Med. Res. (68-1000)	I(1,3); III-A	265
Int'l. Agency for Res. Cancer (70-2076)	I(1,2,3); II-A(1,2); III-A	131
Jackson Laboratories (67-744)	I(3); III-A	236
Johns Hopkins University (69-2008)	Resources	203
Johns Hopkins University (71-2121)	I(1,3); III-A	124
Johns Hopkins University (71-2109)	I(2,3)	132
Karolinska Institute (69-2005)	I(1,2,3); II-A(2); III-A	109

TABLE III (continued)

CONTRACTOR	FUNCTION	PAGE
Life Sciences, Inc. (69-63)	II-A(1,2); III-A	163
Life Sciences, Inc. (68-711)	Resources	204
Louisville, University of (66-902)	Resources	206
Maimonides Hospital (71-2046)	I(1,2,3)	235
Makerere University of (67-47)	I(1,2); III-A	207
Marquette University (68-1010)	II-A(1); II-B(1)	207
Mason Research Instit. (70-2204)	I(3); II-A(1)	266
Mass. General Hospital (71-2174)	II-B(2)	124
Massachusetts Instit. Tech. (71-2149)	II-B(2)	188

TABLE III (continued)

CONTRACTOR	FUNCTION	PAGE
Meloy Laboratories (70-2047)	I(3); II-A(1,2); III-A; II-B(1,2); IV-B	190
Meloy Laboratories (66-458)	I(3); II-A(1)	270
Memorial Hospital (N.Y.) (71-2116)	I(2)	125
Memorial Hospital (N.Y.) (71-2194)	I(2)	269
Merck and Co. (64-55)	See Merck and Co. (71-2059)	111
Merck and Co. (71-2059)	IV-A(1)	269
Miami, University of (67-1187)	III-A	151
Miami, Universit of (70-2211)	II-A(1,2)	153
Michigan Cancer Fdn. (71-2421)	I(1,2)	268
Michigan, University of (65-639)	I(2)	208

TABLE III (continued)

CONTRACTOR	FUNCTION	PAGE
Microbiological Assoc., Inc (67-697)	I(3); II-A(1); III-A; II-B(1)	238
Microbiological Assoc., Inc (66-914)	Resources	211
Microbiological Assoc., Inc (70-2068)	I(3); II-A(1,2); III-A; II-B (1,2)	240
Microbiological Assoc., Inc (67-700)	I(3)	209
Minnesota, University of (69-2061)	I(3)	133
Minnesota, University of (71-2261)	I(1,2,3)	211
Montreal Children's Hosp. (65-1020)	I(2)	212
Mt. Sinai Sch. of Medicine (69-2078)	I(3); III-A	112
Naples, University of (71-2056)	III-A	126
Nat'l. Ctr. for Health Stat. (FS-35)	I(1)	167

TABLE III (continued)

CONTRACTOR	FUNCTION	PAGE
Naval Biomedical Labs. (FS-8)	I(2); See Calif., Univ of (63-13)	
Naval Biomedical Labs. (FS-57)	II-A(1)	224
Nebraska, University of (71-2076)	II-A(2); II-B(2)	193
Nederlands Kankerinstitut (71-678)	Scientific meeting	193
North Dakota, University of (66-8)	II-A(1)	170
Ohio State University (69-2233)	I(3)	108
Ohio State University (65-1001)	II-A(1)	220
Oregon State University (71-2175)	II-B(2)	126
Padua, University of (68-1389)	I(2)	214
Pennsylvania State Univ (70-2024)	I(3); II-A(1,2); III-A	113
Pennsylvania, University of (65-1013)	I(3); II-A(1,2)	165

TABLE III (continued)

CONTRACTOR	FUNCTION	PAGE
Pfizer, Chas. and Co. (70-2080)	I(3); II-A(1,2)	114
Pfizer, Chas. and Co. (67-1176)	I(3)	262
Presbyterian-St. Luke's Hos (62-179)	See Rush-Presbyterian (71-2032)	
Princeton University (71-2372)	I(3)	245
Public Health Res Instit. (71-2129)	I(3)	127
Res. Fdn., St. Univ. of N.Y. (71-2137)	IV-A(1)	133
Robert B. Brigham Hospital (71-2172)	I(3)	134
Rush-Presbyterian Hospital (71-2032)	I(3); II-A(1,2)	117
Rutgers The State Univ (71-2077)	II-A(2)	174

TABLE III (continued)

CONTRACTOR	FUNCTION	PAGE
Rutgers The State Univ (68-1025)	III-A	194
Salk Institute (67-1147)	I (3); II-B(1)	248
Southern California, Univ. (68-1030)	I(1,2,3); II-A(2); III-A; II-B(1,2)	249
Southwest Foundation (69-93)	I(3)	215
Southwest Foundation (69-2011)	Resources	216
Southwest Foundation (71-2348)	II-A(1)	225
Stanford University (69-2053)	I(3); II-B(1)	255
St. Joseph's Hospital (69-2074)	I(2,3)	116
St. Jude's Hospital (71-2134)	II-A(1)	173
St. Louis University (67-692)	I(3); II-A(1,2); II-B(2); IV-B	246

TABLE III (continued)

CONTRACTOR	FUNCTION	PAGE
Texas, University of (65-604)	I(1,2,3); II-A(1,2); II-B(1)	119
Texas, University of (71-2178)	I(2,3)	135
Texas, University of (71-2135)	I(1)	226
TRW, Inc. (70-2200)	I(3)	136
University Laboratories (66-1133)	II-A(1)	217
Washington, University of (71-2171)	I(3)	257
Weizmann Institute (69-2014)	I(3)	258
Wistar Institute (62-157)	See Wistar Instit. (71-2092)	
Wistar Institute (71-2092)	I(3); II-A(2)	259
Worcester Foundation (69-2007)	I(3); II-A(2)	260

TABLE III (continued)

CONTRACT	FUNCTION	PAGE
World Comm. for Comparative Leukemia Research (71-1033)	Scientific meeting	195
Zoological Society of San Diego (63-56)	Resources	218

SPECIAL VIRUS CANCER PROGRAM
ETIOLOGY AREA, NCI
Fiscal Year 1971

DEVELOPMENTAL RESEARCH PROGRAM SEGMENT

Dr. Robert A. Manaker, Chief, VBB, Etiology Area, Chairman
Dr. Roy F. Kinard, VBB, Etiology Area, Vice Chairman
Dr. Jack Gruber, VBB, Etiology Area, Executive Secretary[1]

AICHI CANCER CENTER (NIH-69-96)

Title: Virus Rescue Studies in Human Leukemia/Lymphoma Cell Lines

Contractor's Project Officer: Dr. Yohei Ito

Project Officers (NCI): Dr. Jack Gruber
Dr. Virginia C. Dunkel

Objectives: (1) To establish cell lines in vitro from human neoplasms and examine these for virus or antigens by electron microscopy, immunology and transformation experiments. (2) To supply human embryonic cell cultures and lymphoma-type tumor tissues available in the Far East.

Major Findings: Efforts to establish continuously growing cell lines from human neoplastic tissues were resumed as one of the main lines of study in the second year of the contract. In addition to the cultures from neoplasia of the hematopoetic system, cell cultures from solid tumors such as nasopharyngeal carcinoma (NCP) were also attempted. This was done because of the well established fact of high herpes-type virus (HTV) antibody titer in the sera of patients with the disease. Among 13 NPC specimens cultured, 8 gave rise to monolayer culture, of which half showed morphological alteration. From these cultures, one free-floating cell line was established. The presence of HTV antigen in these cells was demonstrated by direct immunofluorescence test.

To obtain an established cell line which grew in a floating state with less or hopefully no HTV antigen, cultures from hyperplastic tonsils of children were carried out. Of 136 specimens, 12 cell lines were established as free-floating cells. The ratio of cells containing HTV antigen was relatively small but HTV antigen was detected in all the cell lines.

Some 50 strains of cells were maintained in the laboratory and they served as a procurement center for the supply of the cells for research workers in the area. The procurement of human embryonic cultures was also continued into the second year. About 40 human embryos in total were processed for such culture. Human sera of high HTV antibody titer were also supplied to colleagues of the SVCP.

[1] Replaced Dr. Roy Kinard as Vice Chairman on March 2, 1971.

continued to accumulate more data on the HTV antibody titer of individuals with various neoplastic diseases and normal subjects. However, a hope to reveal a new disease with high HTV antibody titer turned out to be fruitless so far.

Contacts have been strengthened with the institutes of the Asiatic area to provide access to the human tumor material and serum specimens which might be useful to the SVCP.

Significance to Biomedical Research and the Program of the Institute: This project will supply supporting data from Far Eastern sources to supplement information obtained in the U.S. on the association of viruses with specific neoplastic diseases.

Proposed Course: In general, studies initiated previously will be continued. A new aspect of the work scope is the introduction of biochemical techniques to search for the presence of RNA-dependent DNA polymerase among the approximately 90 cell lines established from human neoplastic tissue during the past two years. Furthermore, fresh human cell materials from leukemic and lymphoma patients at the Aichi Cancer Center Hospital will be tested for polymerase activity. Such studies would provide new data on neoplastic cells from patients of oriental origin. Additionally, plans are to study the in vitro effect of various chemical carcinogens on established lymphoblastoid cell lines, and to initiate new investigations on other human neoplasms where virus activity is suspected.

Date Contract Initiated: May 2, 1969.

BAYLOR COLLEGE OF MEDICINE (PH43-68-678)

Title: Studies on Viruses as Related to Cancer with Emphasis on Leukemia

Contractor's Project Director: Dr. Joseph Melnick

Project Officers (NCI): Dr. Jack Gruber
Dr. Roy Kinard

Objectives: (1) To isolate, propagate and identify viral agents to provide evidence of association with human neoplasia and (2) to continue to hold and observe primates inoculated with suspected cancer viruses or cancer tissues.

Major Findings:

A. Viral etiology of leukemia and mononucleosis.

Propagation of selected lymphoblastoid (Ly) cell lines from patients with leukemia or mononucleosis and normal individuals is continuing, for use in immunological and biophysical studies.

Comparative chromosomal analysis of 16 lymphoblastoid cell lines cultive up to 61 months revealed no specific association between the presence of virus and any of the chromosomal anomalies observed. Regardless of the source of cells or the presence of EB virus, cells with marker chromosom or trisomies appear to have a selective advantage of growth in vitro, as documented by the increase in frequency of clones with these anomalies with progressive passage.

Studies were continued to search for antibody to possible tumor antigens in acute leukemia of childhood. Peripheral blood leukocytes are being reacted with serial autologous serum samples by indirect membrane immuno- fluorescence (IMIF). In autologous tests, cells from 20 of 25 children in remission have yielded negative results and 5 questionable. Cells obtained from 6 children during relapse were also tested for autoantibody The IMIF assay has been uniformly negative for all sera and autologous relapse phase cells tested so far.

A 78 A1 cell line of rat embryo fibroblasts that had been transformed by the murine sarcoma-leukemia virus (MSV-MLV) complex is being used as a mo for characterization of RNA species obtained from various human Ly cell lines. The 68S RNA of MSV-MLV was found to dissociate after heating or dimethylsulfoxide treatment into 37S, 18S and 4S subunits, differing in b composition and buoyant densities in cesium sulfate. A double-stranded D with a sedimentation coefficient of 7S density was isolated from highly purified MSV-MLV. This DNA was complementary to the 18S subunit, but not the 37S or 4S subunits of the viral RNA. Work is being initiated to foll infectious virus synthesis and macromolecular synthesis during de novo infection and transformation of cells by MSV-MLV.

B. Comparative studies on herpesviruses.

Studies continue on the distribution of complement-fixing (CF) antibody to EB virus-induced S antigen in groups with various disease entities and in normal individuals. The results are compared with those obtained in the immunofluorescence (IF) test with fixed EB3 cell preparations. Sera from 21 patients with sarcoidosis, kindly supplied by Dr. Phillip Glade, reveal both IF and S antibody to EB virus, a result similar to that reported earlier for patients with post-nasal carcinoma. A longitudinal study of the development of IF and S antibody starting with newborn children is being conducted. The results to date indicate that: (1) IF antibody to EB virus appears within several months after maternal antibody has disappeared, indicating an early infection with this agent. (2) There is a lag of several months between the appearance of IF and S antibodies. (3) Infection with the other three herpesviruses (herpes simplex, cytomega virus and zoster virus) occurs much later than infection with EB virus.

Work continues to purify and characterize the soluble CF antigens extracted from two EB virus-positve (EB3 and P3J) and from one EB virus-negative (NC37) Ly cell lines. Ly cell line RPMI 8226, free of both EB virus and soluble CF antigen, is used as negative control. Studies on the non-serum-requiring complement-fixation (NSR-FCF) by EB virus-positive Ly cells

indicate that the reaction may be measuring an EB virus directed antigen-antibody. Attempts are being made to identify antibody to EB virus in extracts of Ly cells.

The biochemical and biophysical properties of the DNA of HSV type 1 and type 2, infectious bovine rhinotracheitis (IBR) and Pseudorabies (Pr) viruses were compared. DNA-DNA hybridization was employed to investigate genetic relatedness between these four members of the herpesvirus group. Saturation and competition hybridizations demonstrated greater than 90% homology between three strains of HSV-1 and less than 5% homology between HSV-1, Pr virus and IBR virus. Saturation hybridization indicated at least 90% homology between two strains of HSV-2, but no homology between HSV-2 Pr virus and IBR virus. Preliminary experiments indicate that there is less than 50% homology between the DNAs of type 1 and type 2 HSV.

Further attempts to obtain additional BUDR-induced ts mutants of HSV have resulted in the isolation of 50 potential mutants. The 22 original ts mutants of HSV have been tentatively assigned to 8 complementation groups. Groups so far contain from 1-4 mutants. Before detailed characterization of representative members of complementation groups is undertaken, repeat tests are planned using both the 22 original mutants and the newly isolated mutants mentioned above.

The protein and glycoprotein synthesis by a HSV temperature-sensitive mutant (ts 343) and the wild-type virus has continued to be compared by polyacrylamide gel electrophoresis. Evidence was obtained that mutant ts 343 does not synthesize glycoprotein C5, a major envelope protein, at the nonpermissive temperature (40°). Studies are in progress on the glycoprotein synthesis of additional HSV ts mutants. Preliminary findings indicate that the glycoprotein profiles of mutants belonging to separate complementation groups may be significantly different.

C. Role of herpes virus type 2 in cervical carcinoma.

Seroepidemiologic studies of women with invasive cervical cancer and controls from Uganda and Israel failed to reveal the same strength of association between antibodies to herpesvirus type 2 and malignancy, as previously observed in Houston, Atlanta, Baltimore and Brussels. Studies in different populations are continuing. Recently, 118 cervical cancer patients and 83 controls from Muslim and Christian women in Yugoslavia were studied. No difference in type 2 herpes antibodies was found between Muslim cases and controls, but the occurrence of antibodies among Christian women was twice that of control women. In New Zealand, the occurrence of antibodies was only slightly higher in the patients than in the control women. In the USA most of the studies that yielded a high incidence of antibodies were carried out among women of the lower socioeconomic group. Thus, in populations of different composition and different life styles, differences are found in the association between type 2 herpesvirus and cervical carcinoma.

Type 1 strains are susceptible to cytoside arabinoside and IUDR (iododeoxy uridine), inhibitors of replication of DNA-containing viruses. However, type 2 strains are resistant to these DNA antagonists. This is apparently due to the low levels of thymidine kinase which are characteristic of type 2 strains in contrast to the high levels of this DNA-synthesizing enzyme found with type 1 strains.

D. Immunofluorescent cell surface antigens in human tumors.

Cell lines have been established from human melanoma and sarcoma tissue. Attempts are being made to separate tumor cells from the normal cells by (1) density gradient centrifugation, (2) by cloning out tumor cells, and (3) by injecting the mixture into immunosuppressed mice. With one of the two melanoma cell lines tested, the patient's serum reacted in the membrane fluorescence test. Sera from other melanoma patients reacted positively for antigen in the cytoplasm of the tumor cells. Lymphosarcoma cells react in the membrane fluorescence test with autologous sera, and cross reactions were seen with sera from other lymphosarcoma patients.

Significance to Biomedical Research and to the Program of the Institute: This contract provides a progressive comprehensive research program to determine the significance of viruses in human neoplasia. Techniques used are tissue culture, immunology, electron microscopy, primate inoculation, cytogenetics and nucleic acid homology.

Proposed Course: Investigations will continue to detect the presence of nucleic acid characteristic of the RNA tumor viruses in human tumor cells. Immunological studies with antigens associated with EB virus will be continued. Further serologic data will be acquired to aid in determining the relationship between the venereal herpes hominis type 2 virus and cervical carcinoma.

Date Contract Initiated: June 27, 1963

BIONETICS RESEARCH LABORATORIES, INC. (NIH-71-2025)

Title: Investigations of Viral Carcinogenesis in Primates

Contractor's Project Directors: Dr. John Landon
Dr. David Valerio
Dr. Robert Ting

Project Officers (NCI): Dr. Roy Kinard
Dr. Jack Gruber
Dr. Robert Gallo

Objectives: (1) Evaluation of long-term oncogenic effects of human and animal viral inocula in primates of various species, especially newborn macaques; (2) maintenance of monkey breeding colonies and laboratories necessary for inoculation, care and monitoring of monkeys; and (3) biochemical studies of transfer RNA under conditions of neoplastic transformation and studies on the significance of RNA-dependent DNA polymerase in human leukemic tissues.

Major Findings: This contractor continues to produce over 300 excellent newborn monkeys per year. This is made possible by diligent attention to reproductive physiological states of female and male breeders. Semen evaluation, artifical insemination, vaginal cytology and ovulatory drugs are used or tried as needed.

Inoculated and control infants are hand-fed and kept in modified germ-free isolators. They are removed from isolators at about 8 weeks of age and placed in filtered air cages for months or years of observation. The holding area now contains approximately 1200 animals up to 5 years old. Approximately 300 are culled every year at a rate of about 25 per month. This is necessary to make room for young animals inoculated with new or improved virus preparations.

During the past year macaques were inoculated at birth or in utero with the Mason-Pfizer monkey mammary virus, Epstein-Barr virus, Herpesvirus saimiri, and Marek's disease virus. EB virus was given with immunostimulation and immunosuppression (ALS, prednisone, imuran). Australia antigen was given to newborn African green monkeys.

The breeding and holding colonies were surveyed for antibody to EBV. All breeders were positive and their offspring contain maternal antibody for several months. Colony-born offspring that have lost maternal antibody and are sero-negative will be surveyed periodically for conversion to the EB positive state.

An RNA-dependent DNA polymerase similar to that associated with RNA tumor viruses was detected in human leukemic cells but not in normal cells stimulate by phytohemagglutinin. The enzyme was isolated, purified and concentrated 200-fold, making possible its further characterization and study in relation to the leukemic process in man.

Significance to Biomedical Research and to the Program of the Institute: Inasmuch as tests for the biological activity of candidate human viruses will not be tested in the human species, it is imperative that another system be developed for these determinations and, subsequently for the evaluation of vaccines or other measures of control. The close phylogenetic relationship of the lower primates to man justifies utilization of these animals for these purposes. Further study of altered transfer RNA and polymerase enzymes would determine their significance in neoplastic change and provide a basis for selection of therapeutic agents.

Proposed Course: Continuation with increased emphasis on monitoring and intensive care of inoculated animals to determine if active infection occ effects of infection, and degree of immunosuppression when used. Further studies of human neoplasms at a molecular level will continue.

Date Contract Initiated: February 12, 1962.

CHICAGO PARK DISTRICT, LINCOLN PARK ZOO (PH43-65-1017).

Title: Marmoset Breeding Colony

Contractor's Project Director: Dr. Lester E. Fisher

Project Officer (NCI): Dr. Roy Kinard

Objectives: To provide marmosets in a quantity and quality sufficient for the needs of the research on tumor viruses conducted under Contract NIH-71 2032 with Rush-Presbyterian-St. Luke's Hospital.

Major Findings: The marmoset breeding colony is regularly providing newbo animals at the rate of 100 per year.

Significance to Biomedical Research and the Program of the Institute: This contract is part of a program utilizing lower primates for testing selected laboratory specimens for oncogenic activity. The marmoset, a small, inexpensive primate is under investigation to determine its value fc cancer virus research.

Proposed Course: The project will be continued to insure the availability of experimental animals of quality.

Date Contract Initiated: June 28, 1965.

HEALTH RESEARCH, INC. (PH43-63-593)

Title: Biological and Electron Microscopic Studies of Viruses from Leukemia Cells, Tissues and Plasma.

Contractor's Project Directors: Dr. Edwin Mirand
Dr. Julius Horoszewicz

Project Officer (NCI): Dr. Jack Gruber
Dr. Roy F. Kinard

Objectives: To study the EB virus associated with cells cultured from Burkitt's tumor.

Major Findings:

A. Animal studies with EB virus.

Hematologic studies were done on a total of 34 EBV-injected and uninjected adult male Long-Evans rats; approximately one half of the animals were subjected to partial hepatectomy within 24 hours prior to virus injection intravenously; three different virus preparations were used. There is no indication that injection of EBV induces a sustained leukocytosis, or that hepatectomy has any effect per se in the response of the rat to EBV injection

Cultures of the buffy coat from heart blood have been made from all experimental and control rats. It appears that the buffy coats from EBV-injected rats grow somewhat faster and better than those from uninjected rats; there is no significant difference between cultures from hepatectomized and intact animals. There appear to be cytological changes in some of the cells of the buffy coats from virus-injected rats suggestive of host response to the virus and/or the foreign human 64-10 cells used as virus-carriers in the preparations injected; further study is necessary before any conclusions can be drawn.

B. Quantitative infectivity assay for EBV.

The practical range of EB virus titration was increased and extends now from 2.5×10^2 to 1×10^6 infectious units per ml. This was accomplished by introducing total counts of immunofluorescent cells in place of estimates based on percentages only. The previously reported procedures to concentrate and store EB virus were found to preserve quantitatively the infectious properties of viral inocula.

During time studies on localization of viral antigens and virus formation in EBV infected cells, Early Antigen (EA) first appeared 6 hours post-infection and was localized in the cell nucleus. Nuclear Capsid Antigen (CA) could be detected 9 to 10 hours post-infection. Soluble Antigen"Beta" (SAB) accumulates in the cytoplasm at 16 hours. Assembly of viral nucleocapsids begins at 14 to 16 hours and "coating" reaction specific for new membrane antigen (MA) becomes positive at 20 to 24 hours post-infection.

Significance to Biomedical Research and the Program of the Institute: The development of a more sensitive quantitative assay is important for further characterization of the EB virus and for assessing the quality of infectious virus preparations. It is hoped that purified non-virion antigen may be useful in the control of infection by EBV.

Proposed Course: This contract will terminate on June 30, 1971.

Date Contract Initiated: April 18, 1963.

OHIO STATE UNIVERSITY (NIH-69-2233)

Title: Application of Radioiodine Labeled Antibody Technique to Studies of Virus-Induced Tumors.

Contractor's Project Director: Dr. David S. Yohn

Project Officers (NCI): Dr. Jack Gruber
Dr. Virginia C. Dunkel

Objectives: To apply the paired radioiodine labeled antibody technique (PRILAT) to the detection of virus induced tumor antigens in human tumor cells.

Major Findings: Various forms of the paired radioiodine labeled antibody technique (PRILAT) have been evaluated for sensitivity and quantitative applicability to viral oncology using Adenovirus-12, Rous sarcoma virus and feline leukemia virus model systems. Direct, indirect and inhibition (blocking) procedures have been found applicable. Direct PRILAT yields highly accurate quantitative estimates of the antigen content of test samples or individual cells. Indirect and inhibition of direct PRILAT are highly sensitive methods to detect low levels of antibody. Antibodies have been detected to Ad-12 T-antigen and to avian leukosis group specific antigens(s) in sera which are negative by other serologic procedures.

Significance to Biomedical Research and the Program of the Institute: The project is designed to develop and apply the paired radioiodine labeled antibody method as a very sensitive tool for the detection of specific reactions between humoral antibodies and antigens on the surfaces of tumor cells. Because the method permits discrimination of specific and non-specific binding of serum globulins, it has considerable promise in the study of human neoplasms.

Proposed Course: The main thrust of this project for the next year will be to apply the various PRILAT procedures to the search for tumor specific antigens in human tumors and to detect antibodies in human sera. Liaison is being established with the Departments of Surgery and of Hematology of the Ohio State University Hospital for this purpose. Collaboration is also being established with the M. D. Anderson Hospital in Houston which will also be providing sera and cell lines from human tumor conditions for testing.

Date Contract Initiated: June 27, 1969.

KAROLINSKA INSTITUTE (NIH-69-2005)

Title: Studies on the Significance of Herpes-Type Virus in the Etiology of Some Human Cancers.

Contractor's Project Director: Dr. George Klein

Project Officers (NCI): Dr. Virginia C. Dunkel
Dr. Jack Gruber

Objectives: To study the surface membrane antigens of lymphoblastoid cells infected with Epstein-Barr virus (EBV).

Major Findings: Studies were continued on EBV-associated reactions in relation to Burkitt's lymphoma, nasopharyngeal carcinoma and Hodgkin's disease, in comparison with other malignancies. Antigenic studies on carrier cultures revealed that (1) VCA-positive cells are always membrane antigen positive, (2) VCA-early antigen positive cells exist with a variable frequency in different cell lines, (3) VCA-early antigen positive cells can be either positive or negative for membrane antigen, and (4) membrane antigen positive-early antigen and VCA negative cells occur with variable frequency, in different EBV-carrier cell lines. The number of membrane positive-VCA negative cells can be increased by small doses of mitomycin, actinomycin or X-irradiation. In lines with a high frequency of membrane antigen-positive cells, the antigen expression was maximal during the G1 phase of the cell cycle and lymphoblastoid cell lines derived from Burkitt's lymphoma and NPC show a similar spectrum of membrane antigen reactivity.

Antigenic studies on virus infected cells showed that (1) no VCA is made in the presence of DNA inhibitors; (2) membrane antigen is an early product of the viral genome; (3) early antigen starts to appear at the same time or slightly later than membrane antigen; and (4) VCA when observed appeared 3-4 days after infection and at a low level (1-2%).

In two Burkitt's lymphoma patients studied in detail, anti-early antigen antibody titer increased and anti-membrane antigen antibody titer decreased in parallel with tumor recurrence. Anti-VCA titers, and titers against five unrelated viruses remained unchanged in both patients. In patients treated with BCG there is a temporary increase in anti-membrane antigen titers. With repeated treatment there was often a tendency toward diminishing efficiency. Local radio-therapy to tumors in Burkitt's lymphoma or NPC patients results in an increase in anti-membrane antigen titer. The same increase in titer does not take place in patients with "control" tumors, that is, anti-VCA positive donors with malignant tumors other than Burkitt's lymphoma or NPC.

Serological analysis of EBV-associated reactivity in chronic lymphocytic leukemia (CLL) and lymphocyte-lymphoblast lymphoma (LL) has been completed. The mean anti-VCA and MA values in the CLL group did not differ significantly from the control group. The LL group showed a significantly higher reactivity

in both tests. High reactive sera and their mean antibody titers approache the levels characteristic for Burkitt's lymphom (BL), nasopharyngeal carci oma (NPC) and Hodgkin's sarcoma.

Using isozyme markers, a number of African solid tumors were identified as uniclonal. Six of 20 lymphoblastoid culture lines established from BL patients were found to differ from the host from which the lines were suppo to have been derived.

A membrane associated antigen induced by the FLV-FSV virus was detected by immunofluorescence. Antibodies against this antigen in FSV-inoculated cats showed an inverse correlation with the development of progressively growing tumors. Passively transmitted antibodies protected newborn kittens from tumor induction by FSV.

Developmental and exploratory work is being carried out with (1) cell-mediated immunity; (2) osteogenic and other sarcomas: (3) characterization of the hybrid human-mouse cell line, and (4) fusion of A9 cells with other mouse tumor lines and studies on the release of C-type particles.

Significance to Biomedical Research and the Program of the Institute: The membrane antigens present on tumor cells are important since new membran antigens have been demonstrated on all virally induced experimental tumors investigated whether the interaction with the causative virus was productive or non-productive. This project was undertaken to provide data on the cell membrane antigens and reactive antibodies in specimens from patients with different diseases with which EBV has been associated. A compilation of all data relating to the virus will help to ascertain whether the virus has etiologic significance.

Proposed Course: Current studies will be continued. Attention will be directed to the serological patterns in Burkitt's tumor and other lympho-reticular malignancies, the relationship of membrane antigens to the viral envelope, antibody spectra in relation to disease category and tumor status, levels of membrane reactive antibody in relation to tumor progression, regression, and recurrence, and to the possible existence of fine antigenic differences between the membrane antigen complexes on Burkitt's tumor and nasopharyngeal carcinoma derived cells. Further efforts will be made to measure cell mediated immunity in Burkitt's lymphoma. Collaborative investigations on patient immune responses to sarcoma antigens will be continued.

Date Contract Initiated: April 9, 1969.

Merck and Company, Inc. (NIH-71-2059)

Title: Study of Viruses in Human and Animal Neoplasia.

Contractor's Project Director: Dr. Maurice R. Hilleman

Project Officers (NCI): Dr. Robert A. Manaker
Dr. Jack Gruber

Objectives: To perform investigations designed to develop vaccines or other agents effective for the prophylaxis and therapy of human neoplasia of suspected viral etiology.

Major Findings: This is a new contract.

Significance to Biomedical Research and the Program of the Institute: Current data support the concept that a virus or viruses are the essential element in most animal tumors studied and that viruses are probably the necessary etiological component in human neoplasia, though expression may be greatly influenced and modified by host and environmental factors. If viruses are the essential element in human cancer, then prophylaxis by vaccines to prevent or minimize infection should provide a rational approach to cancer prevention. This could be accomplished by utilization of live or killed virus vaccines or possibly by vaccines of purified virion subunits.

Vaccines would obviously provide their greatest benefit in preventing infection with oncogenic viruses transmitted horizontally after birth. However, even the possible vertical transmission of hypothetical neoplastic agents does not rule out a potential benefit from vaccines. Nononcogenic viruses may function as essential cofactors in expression of neoplasia, and immunity against such secondary agents might prevent expression of the neoplastic state. Additionally, antibody or cellular immunity may be enhanced by vaccination with homologous virus in virus-dependent cancer. Obviously this research investigation is of fundamental importance to the goals of SVCP and can make unique contributions to the total program.

Proposed Course: The investigators will devote initial efforts to developing methods for propagation, purification, concentration and specific quantitation of candidate viruses suspected or shown to cause cancer in man. At the present time, investigations will be focused upon herpes-type (DNA) viruses and "B" and "C" type (RNA) particles. Parallel studies to evolve live attenuated and killed virus vaccines in appropriate animal model systems will be conducted. Particular attention will be given to developing and applying optimal methods for viral attenuation, viral inactivation, viral quantitation, vaccine safety assessment, and vaccine potency assay.

Date Contract Initiated: March 1, 1971

Title: Studies of EBV and Lymphoproliferative Diseases.

Contractor's Project Directors: Dr. Kurt Hirschhorn
Dr. Philip Glade

Project Officers (NCI): Dr. Virginia C. Dunkel
Dr. Jack Gruber

Objectives: To investigate the relationship of herpes-type virus (EBV) to different diseases with a lymphoproliferative phase in order to determine whether EBV is a specific etiologic agent with multiple modes of biologic expression or an adventitious entity which emerges under the stimulus of the lymphoproliferation resulting from the underlying disorder.

Major Findings: Vaccinia immunization appears to increase the potential of circulating lymphoid cells for long term proliferation *in vitro* and the lymphoproliferative effects consequent to vaccination may stimulate HLV antibody synthesis *in vivo*. In addition, high antibody levels to HLV in sarcoidosis may reflect a specific event in this disorder, since similar alterations were not found for poliovirus antibody. High antibody titers to HLV may correlate with the degree of impairment of cell-mediated immunologic responses in patients with sarcoidosis. Similar increased levels of anti-HLV reactivity were not found in patients with granulomatous colitis. Sarcoidosis does not support the thesis of Dennis Burkitt that chronic R.E. stimulation influences the host response to HLV with an increased incidence of lymphoreticular neoplasms.

Studies in patients with sarcoidosis and lepromatous and tuberculoid forms of leprosy indicate that cell-mediated immunity has a direct bearing on the degree of the humoral antibody response of the host to infection with the herpes-like virus (HLV Epstein-Barr). While anti-HLV antibody was found in all of these patients, the high anti-HLV antibody titers usually occurred in individuals who had significantly impaired delayed-type hypersensitivity as monitored by skin testing. These findings indicate that it may no longer be tenable to assign an etiologic role to HLV based upon high antibody titers in patients without an assessment of their cell-mediated immune responses. It is quite possible that high anti-HLV antibody results secondarily from the impairment of cellular immunity associated with disease rather than primary overwhelming infection with the HLV.

Studies utilizing the newly developed lymphoid cell line migration technique suggest that high circulating levels of anti-HLV antibody have effects on lymphoid cells which may alter their functional capacities to react. Further studies demonstrated a significant alteration in the P3J clone of Jijoye Burkitt lymphoma cells in terms of their ability to respond to their own inhibitory products. These studies may provide further information about the persistence of HLV in lymphoid cells and perhaps a specific lymphoid defect in Burkitt's lymphoma.

Significance to Biomedical Research and the Program of the Institute: The association of EBV with all cases of sarcoidosis studied and the high antibody levels detected suggest a specific relationship of the virus to this disease and raises the possibility that the agent may be a necessary co-factor in several disease conditions including Burkitt's lymphoma nasopharyngeal carcinoma in addition to a probably direct etiological relationship to infectious mononucleosis.

Proposed Course: This study will terminate October 31, 1971.

Date Contract Initiated: June 17, 1969.

THE PENNSYLVANIA STATE UNIVERSITY (NIH-70-2024).

Title: Studies on the Oncogenic Potential of Defective Human Viruses.

Contractor's Project Director: Dr. Fred Rapp

Project Officers (NCI): Dr. Virginia C. Dunkel
Dr. Jack Gruber

Objectives: To conduct a systematic study of the oncogenic potential of defective human viruses.

Major Findings: Early events concerning the effect of herpes simplex viruses (HSV) on human chromosomes were analyzed. These experiments revealed that cytosine arabinoside (ara^{-C}) and HSV act synergistically to produce multiple chromosome breaks. These breaks could be prevented by human interferon, actinomycin D, and cycloheximide suggesting that the expression of the virus genome was necessary and that both mRNA and protein synthesis were required to produce the breaks. Latent infection of HSV in human cells maintained in the presence of ara^{-C} was demonstrated following removal of the inhibitor. Failure of the cells to replicate the virus was shown not to be due to inability of the cells to support virus replication.

Hamster cells were morphologically transformed following treatment with ultraviolet irradiated HSV-2. These cells induced tumors when inoculated into newborn hamsters and the tumor cells were then readily transplantable to weanling animals. Virus antigens were identified both in the in vitro transformed cells and in the resulting tumor cells by the indirect immunofluorescence technique. Tumor-bearing animals were found to have neutralizing antibodies against HSV-2 but not aginst HSV-1. Numerous attempts have failed to induce HSV from these transformed cells. Cells from the hamster tumors were extremely oncogenic when transplanted into weanling inbred hamsters. Tumors began developing within one week after injection and only approximately 10 cells were required for a TPD_{50}. Hamsters immunized with irradiated HSV-2 developed tumors at a slower rate than hamsters that had not been immunized or hamsters that had been immunized with irradiated HSV-1.

However, all immunized animals ultimately developed tumors. Some of the tumors metastasized following subcutaneous injection. These tumors resem the original subcutaneous tumors histologically. In addition, these expe ments have been repeated with the original HSV-2 isolate. Additional transformed clones have been observed and these have been isolated. Thes cells also contain virus antigens and are oncogenic. Other cells were treated with HSV-2 inactivated with DMBA. A cell lines missing 1 acrocent chromosome was developed. This line proved to be resistant to superinfec with HSV-2 but not with HSV-1. Attempts to isolate a "repressor" of HSV-2 replication have failed thus far.

Significance to Biomedical Research and the Program of the Institute: The neoplastic response to viral infection is a slow process in contrast t the rapid response to viruses associated with common diseases in man. The abortive infection of cells with some common viruses which are rendered defective by specific treatment raises the question whether such cells in time may undergo neoplastic change. This project is designed to investiga this possibility.

Proposed Course: The transformed hamster cells will be further characteri: for virus antigens and virus-specific nucleic acids. Attempts to relate t antigens to thoseinduced in lytic infection will be made. Other HSV-1 and HSV-2 isolates will be surveyed for transforming potential in hamster and human cells. Similar experiments will be carried out with cytomegalovirus and possibly with EB virus and with H. saimiri. Finally, it is hoped to begin experiments with human materials (especially from cervical carcinoma) later in the year when the systems under investigation have been better established.

Date Contract Initiated: October 27, 1969.

CHARLES PFIZER AND COMPANY, INC., (NIH-70-2080)

Title: Facility for Tumor Virus Research and Related Service Activities.

Contractor's Project Director: Dr. J. J. Oleson

Project Officers (NCI): Dr. Jack Gruber
Dr. W. Ray Bryan
Dr. Roy F. Kinard

Objectives: Provide research and services related to the isolation, production, purification, assay, and control of tumor viruses, including electron microscopy, tissue culture, and immunology applied to the study of animal and potentially oncogenic human viruses.

Major Findings: Large quantities of a variety of viruses were produced for studies by different investigators of the SVCP. Epstein-Barr virus, feline leukemia virus, Mason-Pfizer monkey mammary virus, rat mammary tumor virus (R-35), and Rauscher virus grown in human cells were the principal viruses produced. Considerable amounts of EBV were produced and assayed for infectivity. Some progress has been made in the study of early and late antigen synthesis following infection with EBV. Large amounts of lymphoblastoid cells originating from different donors were regularly supplied for investigators on human cell membrane antigens, cell enzymes, and soluble EB virus-mediated antigens.

Biological and serological studies of the feline leukemia virus and the Rauscher virus grown in human cells were carried out.

Specific rat antisera to the GS-3 antigen were prepared. These antisera would react with cell antigens from Hodgkin's disease, Burkitt's lymphoma, and chronic myelogenous leukemia but not with normal cells, lymphoblastic lymphoma or MPMV infected monkey cells.

The monkey mammary virus was produced for specific antisera production in monkeys, determination of the antigenic composition of the virus, studies on virus replication, and its biochemical characteristics including polymerase assays. The assay for infectivity of this virus has been increased 10 to 100-fold in sensitivity using immunofluorescence. The virus would not cross-react with the group specific antigen of the Moloney sarcoma virus, the Rauscher leukemia virus, the Rous sarcoma virus or Marek's disease virus. Excellent yields of this virus can be obtained from suspension cultures using infected human lymphoblastoid cells (NC37). The NC37 grown virus has the antigens of the monkey cell grown material and has excellent polymerase activity. Virus is more conveniently produced in this cell line.

Production of purified and concentrated ESP-1 virus supplied by the M. D. Anderson Hospital of the University of Texas has been initiated. This culture, derived from the pleural effusion of a boy with American-type Burkitt's lymphoma, continues to produce type-C virus particles. DMSO treatment results in a substantial increase in virus yield from the culture. Teramycin was found to inhibit the PPLO contaminant although it was not eradicated.

A summary of the patient survey case studies completed to date has been made. Two human cell cultures, subjected to arginine depletion or activation with ultraviolet light, X-irradiation and treatment with Mitomycin C, become moderately reactive with the serum of the original patient and with EBV positive control serum, this genome is not detectable prior to activation. Cell cycle and cell synchrony studies have been initiated with the objective of facilitating the activation of hidden genomes.

Immunoferritin tests using electron microscopy have been developed for several viruses and are in use to determine the localization of cell and viral antigens and in cross-reaction studies.

Electron microscope services were provided for outside investigators and supported the contractor's activities.

Significance to Biomedical Research and the Program of the Institute: Since its inception, this contract has provided support to individual investigators thereby making possible research which could not otherwise have been undertaken. The research conducted in the contractor's laborato has largely been directed to improving the quality of material support to different research activities.

Proposed Course: The production of virus and cell materials in support of pertinent research will continue.

Date Contract Initiated: November 6, 1961.

ST. JOSEPH'S HOSPITAL (NIH-69-2074).

Title: Study of Human Sarcomas and Possible Viral Etiology.

Contractor's Project Director: Dr. Jeno E. Szakacs

Project Officers (NCI): Dr. Albert J. Dalton
Dr. Roy F. Kinard

Objectives: To find and supply fresh human sarcomas or other tumors which contain EM evidence of virus particles, and to attempt to establish cell cultures from some of these tumors.

Major Findings: From the Tampa Bay area hospitals, 234 sarcomas were collected for study. Survey of the tumor tissue and screening for viral particles by electron microscopy reveals the presence of C type particles and filamentous, reticulated material (provirus?) in one case of malignant lymphoma, reticulum cell type, and the presence of virus particles in one case of liposarcoma. Cultures of the tumors are started now routinely and are being screened for particles by EM.

Study of surface characteristics of sarcoma cells is initiated by freeze etching technique.

Sera for the Serum Bank was collected on a total of 226 patients, 39 from patients with breast cancer.

Significance to Biomedical Research and the Program of the Institute: This is one of the projects for the primary serach for viruses in human tumors. As many fresh tumors as possible must be examined by EM if viral etiology is to be determined in reasonable time.

Proposed Course: Continue with collection and screening of sarcomas, and of tumor cultures by EM. Summarize the experience gained in ultrastructure of the tumors studies for publication. Utilize two new techniques now available to gather further information: Freeze etching and fluorescent antibody techniques.

Date Contract Initiated: June 24, 1969.

RUSH-PRESBYTERIAN-ST. LUKE's HOSPITAL (NIH-71-2032)

Title: Studies of Tumor Viruses in Small Primates.

Contractor's Project Director: Dr. Friedrich Deinhardt

Project Officers (NCI): Dr. Roy F. Kinard
Dr. Jack Gruber

Objectives: To induce viral neoplasia in small primate species, especially marmosets, and to continue to develop the marmoset as a laboratory animal for viral oncology.

Major Findings:

I. Marmosets.

Marmosets were inoculated with a variety of oncogenic or suspected oncogenic viruses, including: Feline sarcoma and leukemia, Rous sarcoma virus, Marek's and turkey virus, Herpesvirus saimiri, and human leukemia and sarcoma material.

II. Feline Sarcoma Virus.

Tumor transplantation: Both the Snyder-Theilen (ST) and Gardner (G) strains of feline fibrosarcoma virus (FSV) induced sarcomas in newborn white-lipped marmosets (WL). C-type virus particles were produced when cells were grown in culture, and virus from such cell cultures was oncogenic for kittens and marmosets. A focus assay *in vitro* for ST-FSV was developed.

Biochemical Studies: Three classes of RNA (15S, 38S and 60-70S) were recovered from FSV-induced marmoset tumor tissue and from marmoset tumor cells grown in culture.

III. Feline Leukemia Virus.

Gs antigen: Semi-purified antigen of FLV was produced and FSV gs-antigen is being produced by the same technique. Guinea pig antisera against FLV-gs antigen with titers of 160 to 320, and reacting in gel diffusion with 2 specific lines, was produced. The possibility that the second line

represents interspecies gs-antigen is under evaluation.

Immunoelectroosmophoresis (IEOP), was used for screening for gs-antigen an gs-antibodies and was found to be more sensitive than other gel diffusion methods, and particularly good for screening anti-complementary animal sera for gs-antibodies.

IV. Marek's Disease.

Studies of Marek's disease virus (MDV) and turkey herpesvirus (HTV) in vit and in vivo were undertaken to determine the infective and oncogenic potential in human and marmoset cells. No CPE, inclusion bodies, or viral antigen was detected in the marmoset or human cells after co-cultivation with primate cells. Twelve adult, twelve juvenile, and eight newborn marmosets have been inoculated either IP or IC with HVT and MDV. To date, no abnormal findings were observed and no virus was reisolated.

V. Herpesvirus saimiri (HVS).

A pathogenesis study was completed. Two papers are in preparation describi the characteristics of in vitro infection and disease in vivo. Disease in WL marmosets was prolonged with diffuse cell infiltrations and peripheral leukocytosis of up to 200,000 cells per mm^3.

VI. Immunological Studies.

Preliminary data were obtained for induction of delayed hypersensitivity tc DNCB in marmosets and for immunosuppression with ALG. Significant depression of lymphocyte counts were obtained with anti-patas and anti-human ALG.

Mixed lymphocyte reactions by microtechnique were used to demonstrate stimulation between lymphocytes of unrelated marmosets and lack of stimulation between lymphocytes of twins.

VII. STAS Inhibitor.

A 80-90% inhibition of focus formation in vitro with RSV was observed in al experiments with all materials prepared with silicotungstic acid, including mock inhibitors prepared both by the French group and Dr. Chirigo's group with saline instead of leukemia virus. No inhibition was observed with any materials in in vivo inoculation of quails.

Significance to Biomedical Research and the Program of the Institute:
As one of the small primates, the marmoset is an excellent animal for laboratory research. The contractor has developed a breeding colony of these animals and has overcome many of the problems associated with their breeding, care and nutrition. The marmoset has been shown to be susceptibl to oncogenesis by several known tumor viruses. Sufficient numbers of newborn animals now are becoming available to expand studies on selected human neoplasms suspected of having a virus etiology.

Proposed Course: Continue as described with emphasis on inoculation of newborn marmosets with human tumor specimens or cell lines and viruses derived from them.

Date Contract Initiated: March 15, 1962.

UNIVERSITY OF TEXAS, M. D. ANDERSON HOSPITAL AND TUMOR INSTITUTE (PH43-65-604)

Title: Studies of Relationship of Viruses to Human Neoplasms.

Contractor's Project Director: Dr. Leon Dmochowski.

Project Officers (NCI): Dr. Jack Gruber
Dr. Roy F. Kinard

Objectives: To institute and pursue a systematic study of selected human patients with neoplastic diseases to detect the presence of viruses or virus-mediated antigens.

Major Findings: Further immunofluorescence studies have extended observations that sera of some patients with osteosarcoma contain antibodies against antigens in cells from osteosarcomas. Antibodies in sera of patients with osteosarcoma apparently detect internal and not cell surface antigens of osteosarcoma cells. Cells of a tissue culture derived from a patient with osteosarcoma gave cytoplasmic fluorescence with 6 of 9 sera from patients with osteosarcoma. Anti-feline leukemia virus serum also gave cytoplasmic fluorescence with cells of this culture.

Soehner-Dmochowski virus (SD-MSV) derived from hamster bone tumor could be adapted to induce bone tumors in mice by passage in mouse embryo cells in tissue culture with no loss of bone tumor-inducing activity in hamsters. Infant New Zealand Black rats are almost 100% susceptible to the SD-MSV. SD-MSV induces three types of tumors in these rats: Soft tissue tumors, osteolytic and osteogenic bone lesions. Comparable tumors were also induced in Fisher, Sprague-Dawley, ACI, and (ACI-x NB) F1-hybrid rats by NC rat-adapted SD-MSV.

Electron microscope studies utilizing the ruthenium red staining technique have shown that acid mucopolysaccaride (AMS) plays a role in attachment of type C and type B virus from the cells of various tumors of animals of different species.

The majority of sera from patients with breast cancer reacted in the fixed immunofluorescence (FIF) test to give cytoplasmic and nucleolar fluorescence with cells derived from breast cancer and osteosarcoma.

A good correlation has been demonstrated between mixed hemadsorption (MHA) and fixed immunofluorescence (FIF) tests for detection of antigens of type B and type C particles. The MHA test was found to be 100 times more sensitive than the FIF test.

Sera from some melanoma patients gave nucleolar FIF with their own and/or homologous melanoma cells, but not with a variety of normal human embryonic cells, normal human skin cells, HeLa, KB, or HEp-2 cells. The presence of nucleolar antigen is associated with rapidly growing tumors. The nucleolar FIF reaction may provide a means of detecting progression in tumor growth and help in determining the type of treatment.

The use of the colony inhibition test of Hellstrom showed lymph node cell-mediated immunity and possible blocking serum factors in kittens and cats apparently immunologically tolerant to feline leukemia and sarcoma viruses.

A monolayer culture producing type C virus particles has been established from cells of a pleural effusion of a child with Burkitt's lymphoma (American type). No virus particles were found by electron microscope examination in the cells of the original pleural effusion nor in cells of passage 1. Type C virus particles were observed in cells from passage 10 and budding, immature and mature type C particles have been consistently observed through 40 tissue culture passages. The cell line has been designated ESP-1. Mixed hemadsorption tests with anti HeLa cell serum indicate human origin of the cell line. Preliminary karyotyping also indicates a human culture with 90 to 300 chromosomes. DMSO treatment of the ESP-1 culture produced a 2- to 3-fold increase in virus production. The culture also contains a PPLO contaminant which is not sensitive to the antibiotics employed to date. The results of immunodiffusion tests carried out in cooperation with Drs. Old, Geering, Hardy, and Nowinski, suggest that the ESP-1 virus has a unique gs-1 antigen, non-reactive with antisera to gs-1 antigens from all known mammalian type C viruses. Immunoferritin labeling studies of ESP-1 using anti-gs-1 and anti-gs-3 sera, have demonstrated labeling of ESP-1 virus particles with anti-gs-3 sera but no labeling with anti-MuLV-gs-1 serum. Anti-gs-1 serum labeled type C virus particles present in Rauscher leukemia virus infected human embryo cell line (HEK1-HRLV). Collaborative studies on various aspects of ESP-1 virus have been initiated with participants of the SVCP.

The ESP-1 cell line is the first culture of human origin shown to produce continuously type C virus particles, morphologically similar to those found in mice, rats, hamsters, cats, and monkeys.

Significance to Biomedical Research and the Program of the Institute:
This well organized program for the systematic study of selected cancer cases was instituted to apply existing and newer methodology to an in-depth study on the association of viruses or viral antigens with human cancers. The project should also expand current observations to determine their significance, if any. The data acquired should permit decisions regarding the effectiveness of the procedures applied, and develop areas for intensive investigation. The difficulty in determining the etiological significance

of any virus or tumor-associated antigens with oncogenicity in man requires assimilation of considerable supportive information. This project is designed to meet this need.

Proposed Course: Continuation of a systematic study of human tumor cases with emphasis on osteosarcoma and soft tissue sarcomas, melanoma, and mammary carcinoma.

Date Initiated: March 19, 1965.

COLUMBIA UNIVERSITY (NIH-70-2049)

Title: RNA and RNA Replicases in Tumor Cells Associated with RNA Oncogenic Viruses.

Contractor's Project Director: Dr. Sol Spiegelman

Project Officer (NCI): Dr. Timothy O'Connor

Objectives: To explore the mode of replication of RNA-containing oncogenic viruses and the molecular mechanism(s) underlying viral carcinogenesis. Studies are concerned with the characterization of both viral and host enzymes involved in oncogenesis and, in particular, with the enzymes present in purified virions.

Major Findings: An RNA-dependent DNA polymerase (RIDP) has been demonstrated in a variety of purified Type C (RSV, Twiehous Agent, AMV, RLV, MSV, FeLV, FSV) and Type B (Mouse MTV) oncogenic virions, as well as in the Mason-Pfizer Virus, but not in a variety of typical non-oncogenic RNA-containing viruses (NDV, A and W influenza, Reo, VSV and Polio). Polymerase was found in the RNA containing Type C Visna Virus which is the causative agent of a neurological disease in sheep. Visna Virus was found to resemble the Type C oncogenic virions in that synthesis of DNA is mandatory for productive infection of cells.

The presence of RIDP in purified virions was found to be invariably accompanied by a second enzymatic activity-DNA dependent DNA polymerase (DIDP). DIDP, which has the same substrate requirements as RIDP, was clearly demonstrated by enzymatic destruction of the RNA content of the ruptured virion followed by synthesis of specific DNA in response to addition of exogenous DNA template. DIDP, unlike RIDP, is inhibited by actinomycin D. Unlike the previously known cellular DNA polymerases, DIDP shows a preference for double-stranded rather than single-stranded DNA as template, and it is postulated that the enzyme is probably involved in conversion of the DNA-RNA hybrid product of RIDP to couble-stranded DNA product.

Using synthetic oligonucleotides as templates, it was established that oncogenic viruses contain DNA polymerase activities directed by single-stranded RNA, double-stranded RNA, double-stranded DNA, and DNA-RNA hybrids. Certain synthetic templates were found to be superior to natural templates by almost two orders of magnitude in stimulating polymerization. The number of different kinds of protein molecules required to handle this variety of templates is under investigation. Enzyme(s) from AMV and virus-infected avian myeloblasts has been solubilized and purified by several orders of magnitude using a sequence of velocity gradient centrifugation and column chromatography on DEAE-cellulose and CM-Sephadex. The apparently homogenous product which migrates as a single entity on acrylamide gels is dissociated into components on SDS-acrylamide gels. The enzymatic properties of each component is under study.

The buffy coats of 120 leukemic patients (ALL, CLL, AML, CML, monocytic, chronic granulocytic and leukosarcomas) and 70 normal patients as well as white blood cells from patients with leukoproliferative diseases other than leukemia (polycythemia vera, myeloid metaplasia and various leukocytoses) were tested for the presence of a DNA polymerase that responds to the synthetic templates dC:dG and dT:rA. Except for two monocytic leukemias, all enzyme preparations from leukemics were positive, whereas all preparations from control normal or "blood discrasia" patients were negative. When enzyme levels were followed through periods of chemotherapy, dramatic drops in enzyme activity were observed when patients entered remission. The enzyme(s) from human leukemic cells is now being characterized.

DNA polymerase activities that respond to the synthetic templates dC:dG and dT:rA have been found in embryonic tissues of chickens, mice, rats, and humans. These activities are highest during early periods of development and fall as embryogenesis progresses. The experience accumulated to date with cell lines suggests that every proliferating cell has an elevated response to dC:dG. Not all, however, show responses to dT:rA and rA:rU. The proteins responsible for enzymatic activity in embryonic, leukemic, and cancer cells are now being purified and compared.

Examination of the Mason-Pfizer monkey virus shows that it has all the biochemical and biophysical features of the RNA tumor viruses of other species. The virus has a density of 1.16 g/cm^3 and contains a 70S nucleic acid that bands in as RNA in a Cs_2SO_4 gradient. The virus contains some low molecular weight RNA as well as some RNA-DNA complex. The virion can be ruptured to yield a 1.23 g/cm^3 density nucleoid. Both RNA instructed DNA polymerase and DNA polymerase activities are associated with the virion.

All human milks positive for type-B particles by electron microscopy contain particles that band at densities characteristic of known RNA oncogenic viruses and contain RNA-dependent DNA polymerase.

Significance to Biomedical Research and the Program of the Institute: Elucidation of the molecular mechanisms involved in oncogenic viral replication and in cellular transformation may ultimately provide a rationale for effective control of neoplasia. Research by contractor and other investigators on "reverse transcriptase" during the current period has added a new chapter in the knowledge of genetic processes in mammalian cells. The polymerase activity of extracts of human leukemic cells as compared to normal lymphocytes may provide a useful diagnostic tool for human leukemia.

Proposed Course: Studies on the characterization of viral and host enzymes involved in viral replication and oncogenesis will actively continue.

Date Contract Initiated: October 29, 1969

Title: Studies of Herpesvirus Antigens and Virions in Neoplastic Cells fr Carcinoma of Human Cervix

Contractor's Project Director: Dr. Laure Aurelian

Project Officer (NCI): Dr. Jack Gruber

Objectives: To study the possible relationship between Herpes simplex virı type 2 and the etiology of squamous carcinoma of the human cervix.

Major Findings: This is a new contract. The following studies will be conducted: (1) A further evaluation of immunofluorescence tests as a means of studying the association between HSV-2 and cervical neoplastic cells. (2) An inquiry into the nature of the HSV-2 antigens detected in neoplastic cells. (3) Correlation between the presence of antigens and that of virion with herpetic morphology. (4) Comparison of the biologic and biochemical properties of such virions, if detected, with those of HSV-2 isolated from genital blisters.

Significance to Biomedical Research and the Program of the Institute: This contract will expand the studies on cervical carcinoma by investigatio to determine whether specific viral antigens can be detected in primary tumor cells or in cultured tumor tissue. The work will complement the sero- epidemiological efforts and may be expected to help lead to an early resolu- tion of the question whether this virus is a chance contaminant or a significant factor in the development of the neoplasm. Although sero- epidemiological evidence suggests this virus may have a role in oncogenesis and provides the supportive data necessary for such a role, it cannot provide conclusive information. If herpes type 2 infection is clearly implicated in the etiology of cervical carcinoma a basis for preventive measures against this disease will be provided.

Proposed Course: The studies outlined above have been initiated and will be continued.

Date Contract Initiated: May 5, 1971

MASSACHUSETTS GENERAL HOSPITAL (NIH-71-2174)

Title: Characterization of Nucleic Acids and Proteins of Avian Myeloblasto- sis Virus

Contractor's Project Director: Dr. Paul C. Zamecnik

Project Officer (NCI): Dr. Timothy O'Connor

Objectives: To characterize biochemically oncogenic viruses and the neoplastic state.

Major Findings: This is a new contract. Studies will be carried out on the Avian Myeloblastosis Virus and will include analysis and characterization of the viral nucleic acid, the transfer RNA, and the group specific antigens.

Significance to Biomedical Research and the Program of the Institute: This investigation should supply information concerning the group-specific antigens which are major components of all known type C oncogenic viruses, the nucleotide sequences of the 70S viral RNA and knowledge of the transcription process, and further definition of the translation processes in virus-infected cells. These three areas of investigation should provide understanding of the molecular basis of viral replication and oncogenic conversion of cells, and may provide a rationale for the control of viral-induced neoplasia.

Proposed Course: The studies described above have been initiated and will be continued.

Date Contract Initiated: June , 1971

MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES (NIH-71-2116)

Title: Acquisition of Human Materials for Use in the Search for Transmissible Agents in Human Tumors

Contractor's Project Director: Dr. Yashar Hirshaut

Project Officer (NCI): Dr. Jack Gruber

Objectives: To gather sera and tissues from patients with tumors to be used in the search for tumor-specific antigens and human oncogenic viruses.

Major Findings: This is a new contract. Emphasis will be placed on the collection of specimens from individuals with specific kinds of tumors as designated by the Project Officer. Tumors to be selected for special attention will be those for which evidence has accumulated suggesting that they are likely to be of viral origin. In an effort to improve the chances for detection and recovery of viruses or their antigens, specimens will be taken at various stages in the course of disease and from both patients and their immediate relatives. For each specimen careful records will be kept of all clinical information which may be of assistance to the investigative virologist or immunologist in the interpretation of his data.

Significance to Biomedical Research and the Program of the Institute: In the last ten years, rapid progress has been made in the study of oncogenic animal viruses. Unfortunately, human studies have frequently been limited by the lack of suitable materials to be used in virus isolation and

detection attempts. The proposed procurement program at Memorial Hospital for Cancer and Allied Diseases in New York City will provide cooperating investigators with a sufficient number of specimens from tumor-bearing patients to permit them to undertake intensive studies of the possible viral etiology of human cancer.

Proposed Course: The studies outlined above have been initiated and will be continued.

Date Contract Initiated: March 1, 1971

UNIVERSITY OF NAPLES, ITALY (NIH-71-2056)

Title: Studies of Non-virion Antigens

Contractor's Project Director: Dr. Giulio Tarro

Project Officer (NCI): Dr. Virginia C. Dunkel

Objectives: To obtain virus-specific, non-virion antigens from Herpes simplex virus types 1 and 2 for use in tests on the possible etiological role of these viruses in selected types of human malignancies.

Major Findings: This is a new contract.

Significance to Biomedical Research and the Program of the Institute: This contract will study "early" non-virion antigen which appears in cells infected with herpes simplex virus type 1 and the possibility that such antigen may appear in cells infected with herpes simplex virus type 2. The detection of such antigens in tumor cells would provide evidence for the incorporation of the virus genome in the absence of viral replication or mor complete antigenic expression detectable by available serological reagents. The determination that these antigens are specific markers for the presence of virus genomes within tumor cells would provide additional data supporting an etiological role in selected human malignancies.

Proposed Course: The studies indicated above have been initiated and will l continued.

Date Contract Initiated: April 9, 1971

OREGON STATE UNIVERSITY (NIH-71-2175)

Title: Study of the Replication and Function of Nucleic Acids from Oncoger Viruses

Contractor's Project Director: Dr. George S. Beaudreau

Project Officer (NCI): Dr. Albert J. Dalton

Objectives: To study the enzymatic and biochemical changes occurring during transformation by oncogenic viruses and to relate these biochemical modifications to observable ultrastructural events and alterations.

Major Findings: This is a new contract. The work scope involves an extensive enzymatic and biochemical investigation of the early events following infection and transformation of chick embryo tissue culture by the MC29 strain of avian leukosis virus.

Significance to Biomedical Research and the Program of the Institute: Viruses are known to be the essential elements in many animal tumors. They are thought to be an imperative factor in a number of malignant human diseases. A study that might elucidate the early changes in cells after infection (up to 10 hours) by a known tumor virus is of extreme importance for the understanding of the mechanisms leading to uncontrolled growth. Studies of such kind make valuable contributions to providing a rationale for control measures.

Proposed Course: The studies described above have been initiated and will be continued.

Date Contract Initiated: June , 1971

PUBLIC HEALTH RESEARCH INSTITUTE OF THE CITY OF NEW YORK (NIH-71-2129)

Title: Evaluation of Methods for Isolation of Virus from Human Neoplasia

Contractor's Project Director: Dr. Hidesaburo Hanafusa

Project Officer (NCI): Dr. Jack Gruber

Objectives: To conduct a systematic and comprehensive study to evaluate methods for the isolation of viruses from human neoplasia.

Major Findings: This is a new contract. Attempts will be made to isolate viruses from human sarcomas and other neoplastic tissues by use of information available from current studies on tumor viruses in other animals. Combinations of a variety of physical, chemical, and biological techniques will be evaluated.

Significance to Biomedical Research and the Program of the Institute: An essential element of the SVCP is the conduct of collaborative research related to the development and/or application of various methods for the detection of virus associations with human neoplasms. This project will provide useful, perhaps essential, information to this area of the SVCP.

human neoplasms are to be directly examined. The systematic examination t
be conducted may well succeed in detecting and/or isolating oncogenic viru
from human tumors, fulfilling a major goal of SVCP.

Proposed Course: The studies outlined above have been initiated and will
continued.

Date Contract Initiated: April 27, 1971.

ALBERT EINSTEIN COLLEGE OF MEDICINE (NIH-71-2251).

Title: Molecular Biology of Oncogenic Viruses and Malignant Transformatio

Contractor's Project Director: Dr. J. Thomas August

Project Officer (NCI): Dr. Timothy O'Connor

Objectives: To elucidate the molecular events involved in the adsorption
and penetration of oncogenic viruses into host cells, the integration of th
virus genetic information into the host cell genome, the replication of
virus, and the virus-induced malignant transformation of the cell.

Major Findings: This is a new contract. The project provides for the
following research activities: (1) In vitro studies on the activity of the
enzymes, nucleic acids, and protein components of the virus particle in
relation to the processes of adsorption, penetration, and intracellular
replication of the virus. (2) Determination of the functions of virus-
specific enzymes and the contribution of cellular enzyme systems in the
intracellular synthesis of viral nucleic acids and the viral replicative
process. (3) Elucidation of basic biologic mechanisms active in the
integration of the virus genetic information within the cell and in
malignant transformation of the cell. (4) Analysis of virion composition
and assembly and of host cell structures and components active in virus
replication. (5) Isolation and genetic characterization of mutant viruses
for use in the analysis of virus-specific reactions significant in virus
replication and cellular transformation.

Significance to Biomedical Research and the Program of the Institute:
It is important to program to determine the molecular events associated with
integration of the virus genetic message into cells and its expression in
virus replication and malignant cell transformation. Knowledge is also
required concerning repression and derepression of virus gene expression
within the cell. Such information may provide the basis for development
of rational measures for control of virus-induced malignancies. This project
proposes to concentrate the efforts of an outstanding group of investigators
in molecular biology on the fundamental molecular aspects of the tumor virus-
cell relationship.

Proposed Course: The studies outlined above have been initiated and will be continued.

Date Contract Initiated: April 26, 1971

Dr. Paul H. Levine, VLLB, Etiology Area, Chairman
Dr. Herbert J. Rapp, CG, Etiology Area, Vice-Chairman

CHILDREN'S HOSPITAL OF PHILADELPHIA (PH43-66-477)

Title: Interference and Immunofluorescence Studies with Cell Lines Derived from Leukemias and Lymphomas

Contractor's Project Director: Dr. Gertrude Henle

Project Officer (NCI): Dr. Virginia C. Dunkel

Objectives: (1) To assess by immunologic, virologic, and epidemiologic means, the relationship of EB virus to Burkitt's lymphoma and infectious mononucleosis; (2) To devise methods for improved propagation of the EB virus.

Major Findings: Immunofluorescence studies on cells from biopsies of Burkitt's lymphoma and nasopharyngeal carcinoma revealed that in only 5 of 79 Burkitt's lymphoma biopsies were cells found which contained EB viral antigens. When biopsy cells were placed in culture, EBV antigen-containing cells appeared within 3 to 7 days in 68% of the preparations representing from 0.1 to 5% of the total cell population.

Exposure of human lymphocytes to EBV derived from the HR1K cell line led to establishment of five continuous lymphoblastoid cell lines. Co-cultivation of Gibbon lymphocytes with x-irradiated EBV positive IM cells resulted in the establishment of lymphoblastoid cell lines. These cells were determined cytogenetically to be of Gibbon origin and contained EBV. Injection of such cells into autochthonous animals failed to produce tumors or any other signs of illness, but the animals developed antibodies to EBV within seven to ten days.

The separation of early antigen from viral capsid antigen necessitated retesting of sera from patients with various EBV-associated non-malignant and malignant diseases. Preliminary findings suggest that the early antigen (EA) is more disease-associated than the viral capsid antigen, and that patients with infectious mononucleosis, Hodgkin's disease, chronic lymphocytic leukemia, and Burkitt's lymphoma, were more likely to have antibodies to the early antigen even with low VCA titers. Normals generally did not have antibodies to the early antigen, even with high VCA titers. Two different patterns of immunofluorescence were observed: (1) Diffuse staining (D) of nucleus and cytoplasm of infected cells, and (2) restricted staining (R) of masses in the cytoplasm. The transitory

anti-EA response in infectious mononucleosis is almost exclusively of the anti-D type. In Burkitt lymphoma (BL) and nasopharyngeal carcinoma (NPC) both anti-D and anti-R are frequently present, but in BL anti-R is usually dominant or the only antibody whereas in NPC the reverse seems to be true.

Significance to Biomedical Research and the Program of the Institute: Herpes-type viruses have been shown to cause lymphoreticular diseases in chickens, rabbits, and monkeys. This contract has contributed a great deal of knowledge to our understanding of EBV infection and the definition of antigens and antibodies relating to EBV. The studies performed by the contractor support the hypothesis of an etiologic role for EBV in lymphoma.

Proposed Course: The contractor will concentrate on the characterization of the early antigen and the evaluation of its importance in human lymphoma by seroepidemiological studies.

Date Contract Initiated: January 1, 1966

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER (NIH 70-2076)

Title: Seroepidemiology Studies of Nasopharyngeal Carcinoma and Burkitt's Lymphoma

Contractor's Project Director: Dr. G. Blaudin de The

Project Officer (NCI): Dr. Robert H. Depue

Objectives: To investigate the relationship of EBV to nasopharyngeal carcinoma, Burkitt's lymphoma, and other malignancies.

Major Findings: Several lymphoblastoid cell lines were developed from patients with nasopharyngeal carcinoma. The culture conditions for optimum production of EB virus have been determined, and two selective lines have been sent to Pfizer for large scale production and standard antigens for the IF test. The radio-labeled antibody test (PRILAT) has been developed in conjunction with immunofluorescence tests for studying of EBV antibodies.

Significance to Biomedical Research and the Program of the Institute: High EBV antibody titers have been associated with nasopharyngeal carcinoma and Burkitt's lymphoma. Since fulfillment of Koch's postulates in humans is unethical, the most direct evidence for etiology can be obtained by combining

epidemiological and virological expertise. This contract, developed through collaboration of investigators of international repute, provides a program through which evidence of the etiology of EBV to PNC can be evaluated.

Proposed Course: Case control studies on PNC will be developed. The sera collected will be tested for EBV-related antibodies by PRILAT and immunofluorescence.

Date Contract Initiated: January 1, 1971

JOHNS HOPKINS UNIVERSITY (NIH 71-2109)

Title: Anti-tumor Reactivity in Patients with Leukemia and Lymphoma

Contractor's Project Director: Dr. George W. Santos

Project Officer (NCI): Dr. Ronald B. Herberman

Objectives: To develop information regarding principles of tumor immunology in man that may be rationally employed in the design of immunotherapy trials. To correlate the results of several tests of cellular and humoral anti-tumor reactivity to autochthonous leukemia and lymphoma in man with the clinica course of the disease.

Major Findings: Contract recently implemented.

Significance to Biomedical Research and the Program of the Institute: The demonstration and reliable measurement of tumo specific antigens in human tumors and immunity to same in patients at different stages of disease will form the basis fo rational immunotherapy. Measurements of the tumor immune stat also will permit close monitoring of the patient's response to the conventional and current methods of therapy. The use of simultaneous tests on the same patient material will permit evaluation and correlation of tests using different methods in various laboratories.

Proposed Course: To test selected patient specimens for cellular and humoral anti-tumor reactivity, thus leading to full implementation of proposed objectives.

Date Contract Initiated: May 1, 1971

UNIVERSITY OF MINNESOTA (NIH 69-2061)

Title: Tumor-specific Transplantation Antigens in Solid Tumors

Contractor's Project Director: Dr. Charles F. McKhann

Project Officer (NCI): Dr. Charles W. Boone

Objectives: To detect and characterize tumor-specific antigens in human tumors, and to characterize the serum-mediated and cell-mediated immune response to the tumor-specific antigens.

Major Findings: A reproducible *in vitro* assay for cell-mediated immunity was developed. This test appears to work well for both animal and human systems. Using this assay, the presence of a blocking antibody was demonstrated in a patient with Rhabdosarcoma. The *in vitro* immunization of normal mouse spleen cells against two syngeneic methylcholanthrene induced sarcomas was carried out successfully.

Significance to Biomedical Research and the Program of the Institute: By analogy to animal model systems of viral oncogenesis, if a virus is related to cancer causation in humans, a cross-reacting tumor-specific antigen may be involved. The search for, and characterization of, cross-reacting tumor-specific antigens in human tumors has already provided leads as to which morphological type of tumor is most likely to have a virus causation.

Proposed Course: The principal investigator will intensively study patients with tumors of a given histologic type for their cellular and humoral immune responses during the application of the customary modalities of therapy such as surgery, x-irradiation, etc. He will also apply his *in vitro* assay to a large number of human cancer patients, looking in particular for cross-reacting tumor-specific antigens.

Date Contract Initiated: April 14, 1969

RESEARCH FOUNDATION OF STATE UNIVERSITY OF NEW YORK (NIH 71-2137)

Title: Haptene-induced Immunotherapy of Epidermal Tumors

Contractor's Project Director: Dr. Edmurd Klein

Project Officer (NCI): Dr. Charles W. Boone

Objectives: To extend Dr. Klein's findings concerning the cure of some superficial basal cell carcinomas and squamous cell carcinomas of the skin to more deeply seated cancers of the skin, vulva, vagina, mucosa of the head and neck and esophagus. To study the interaction of autologous lymphocytes with tumor cells in vitro.

Major Findings: The principal investigator has developed a method of curing basal cell carcinomas and squamous cell carcinomas of the skin through the application to the tumor of dinitrochlorobenzene and other skin sensitizing compounds in patients previously made sensitive to the compound. The tumor-destructive effect appears to be a typical delayed hypersensitivity reaction, and occurs over a concentration range of the sensitizer which appears harmless to normal tissues.

Significance to Biomedical Research and the Program of the Institute: The determination of the applicability of techniques which have controlled or cured certain types of human cancer to other types of human cancer is of primary importance to general cancer control.

Proposed Course: Implementation of proposed studies.

Date Contract Initiated: May 25, 1971

ROBERT B. BRIGHAM HOSPITAL - MASSACHUSETTS (NIH 71-2172)

Title: Tumor-specific Transplantation Antigens in Solid Tumors

Contractor's Project Director: Dr. John David and Dr. W. H. Churchill

Project Officer (NCI): Dr. Charles W. Boone

Objectives: To search for and characterize tumor-specific antigens in human tumors using the macrophage migration inhibition assay.

Major Findings: In the last year, studies on a guinea pig hepatoma system showed that the interaction of sensitized lymphocytes with tumor cells does indeed result in the release of macrophage migration inhibitory factor (MIF). The separation of guinea pig MIF from lymphotoxin was achieved. At present, a large series of human cancer patients are being tested for evidence of cellular immunity to their own tumor as shown by inhibition of macrophage migration.

Significance to Biomedical Research and the Program of the Institute: In vitro assays for the degree of cellular immunity in human cancer patients are urgently needed so that (1) anti-tumor immune responses may be followed during the course of the disease and (2) so that large numbers of human tumors can be screened for tumor distinctive antigens. The finding of cross-reacting tumor antigens can provide leads as to which morphologi cal type of tumor is most likely to have a virus causation.

Proposed Course: To continue to screen human tumors for distinctive antigens using the MIF assay.

Date Contract Initiated: March 12, 1969

UNIVERSITY OF TEXAS - M.D. ANDERSON HOSPITAL (NIH 71-2178)

Title: Immunological Reactivity in Humans with Neoplastic Disease

Contractor's Project Director: Dr. Joseph G. Sinkovics

Project Officer (NCI): Dr. Berton Zbar

Objectives: To apply an in vitro cellular immunity test developed by the principal investigator to a number of patients with malignant diseases to demonstrate whether this test can be standardized and applied in a variety of neoplastic diseases. To demonstrate how often patients show evidence of cell-mediated immunity against their own tumor, and whether humoral factors can block the lymphocyte-mediated immune response. Subsequent to standardization the test may be applied in epidemiological studies to determine if an environmental agent is responsible for the disease. Also, a survey of tumor-specific antigens can be undertaken.

Major Findings: Evidence has been provided by the principal investigator that he can demonstrate specific and non-specific lymphocyte-mediated immunity in vitro on a small number of tumors. This contract began very recently, thus is in process of implementation.

Significance to Biomedical Research and the Program of the Institute: The identification of tumor-specific antigens is of primary importance to the etiological studies of human cancer. The etiology of a human tumor may be identified when a practical, immunological technique is combined with epidemiological studies. It is hoped that the test developed by this contractor may be one of such techniques.

Proposed Course: As the contract is implemented, more patients representing a variety of malignant diseases will be tested, leading to standardization of the test described.

Date Contract Initiated: April 30, 1971

TRW SYSTEMS GROUP (NIH 70-2200)

Title: Viral Antigens and Anti-Viral Antibodies

Contractor's Project Director: Dr. Norman Weliky

Project Officers (NCI): Drs. Vincent W. Hollis and Tibor Borsos

Objectives: To purify, separate, and characterize the antigens in Rauscher leukemia virus and the corresponding anti-viral antibodies.

Major Findings: High capacity immunoadsorbents, prepared by crosslinking mouse serum with ethyl chloroformate or glutaraldehyde, or cyanogen bromide activated Sephadex G-200, are effective in removing all mouse serum antibodies from antiserum except that to a Beta-lipoprotein. Mouse Beta-lipoprotein isolated with dextran sulfate inhibits the antiserum reaction, but it does not absorb antilipoprotein antibody as an immunoadsorbent after chloroformate crosslinking. The lipoprotein antigen was not found in the supernate from a chloroformate crosslinker mouse serum preparation. The large amounts of crosslinking reagents in the system are likely to block the antigenic determinants.

Significance to Biomedical Research and the Program of the Institute: Antigens crossreacting with murine and feline leukemia virus have been reported in human leukemic cells, and immunofluorescence and two human cell lines by immunodiffusion (GS III). This contract, by purifying and separating all the antigens associated with a known animal leukemia virus (Rauscher leukemia virus) will provide direct evidence to confirm or refute the existence of the cross-reacting viral antigens in animal and human tumors.

Proposed Course: Adsorbents will be prepared by coupling serum and Beta-lipoprotein to higher capacity particles by a variety of coupling methods, including aldehyde, bromo, activated carboxyl, and diazonium coupling. Physical adsorption, followed by chemical coupling, will be pursued.

Quaternary ammonium Sephadex and other polymers will also be used. Preparations will be made using purified Beta-lipoprotein so that its reactivity may be better determined. Retention of the antigenicity of Beta-lipoprotein with blocked side chain reactive groups will be observed.

Date Contract Initiated: June 15, 1970

SPECIAL ANIMAL LEUKEMIA ECOLOGY SEGMENT
Dr. Michael A. Chirigos, VBB, Etiology Area, Chairman
Dr. George J. Burton, VBB, Etiology Area,
Vice-Chairman (Acting) and Executive Secretary

CORNELL UNIVERSITY (NIH 71-2508, formerly PH43-65-620), ITHACA, NEW YORK

Title: Leukemia Studies in the Cat

Contractor's Project Director: Dr. Charles Rickard

Project Officer (NCI): Dr. M. A. Chirigos (Acting)

Objectives: Cats with spontaneous leukemias and sarcomas will be subjected to virus isolation procedures, in an attempt to obtain C-type virus strains from a broader spectrum of neoplastic diseases in the cat. Oncogenic virus strains were derived from lymphocytic leukemia/lymphoma or fibrosarcoma. Viruses from other leukemias and sarcomas are sought. The incidence of spontaneous leukemia and sarcoma virus infections in various populations of cats will be investigated by virus isolation in tissue culture, followed by demonstration of complement-fixing group-specific antigen (COCAL test), electron microscopy, demonstration of "helper" activity or interference in focus-forming tissue culture procedures, and kitten inoculation.

Cats which have been in contact with human cases of leukemia or sarcoma will be examined for presence of cat leukemia or sarcoma viruses. A virus isolated from such a cat can be used in serological tests of sera from the associated human patient and his family, to investigate the possibility that cat leukemia or sarcoma viruses have infected humans. The sites of replication of cat leukemia and sarcoma viruses, and their possible mechanisms of horizontal transmission will be investigated. Insect transmission of these diseases will be attempted. The infectivity of various cat leukemia and sarcoma viruses will be tested in cats, dogs, and tissue cultures derived from various animal species.

Major Findings: (1) A direct fluorescent antibody (FA) test has been developed for the demonstration of the group-specific antigens of feline leukemia and sarcoma viruses. This is a sensitive method for the detection of infected cells from animals or tissue cultures. It provides a means of isolation of feline leukemia and sarcoma viruses by inoculation of tissue cultures, enhancement of viral concentrations by propagation for 2-3 weeks, and application of the fluorescent antibody test. The same method permits titration of the viruses by inoculation of tissue cultures with serial 10-fold dilutions, growth for 10-20 days, and examination of the cultural cells by the FA technique. Cat embryo cells were the most susceptible to infection by minimal doses (10^{-7}) of feline leukemia virus, dog embryo cells somewhat less (10^{-6}), and human embryo cells still less susceptible (10^{-4}), in a comparative study. The FA test was significantly more sensitive and discriminating in the demonstration of feline leukemia and sarcoma infections than the agar gel immunodiffusion, complement fixation, and electron

microscopic techniques. It was used effectively in serum neutralization tests.

(2) Feline leukemia virus was demonstrated in 20 of 25 cats with lymphoma/lymphocytic leukemia, 3 of 4 cats with myeloproliferative disease, and 1 of 4 cats with mast cell neoplasia. Virus was not found in single cases of spontaneous feline osteosarcoma and liposarcoma. Budding C-type virus resembling feline leukemia virus was found in 1 of 2 mammary adenocarcinomas; its oncogenic activity was not determined. No virus was demonstrated in 15 animals which did not have a history of association with other cats with leukemia; however, leukemia virus was found in 3 of 6 clinically normal animals from a high leukemia incidence cat colony, and in 5 of 16 contact controls in experimental litters in which other kittens had been inoculated as newborns with feline leukemia virus.

(3) Serums from 17 cats with spontaneous lymphoma, myeloproliferative disease, or mastocytoma were tested for antibodies in agar gel immunodiffusion reactions against antigens from their strain of leukemia virus and the Gardner strain of sarcoma virus (with its "helper"). Six sera gave a precipitin line against the leukemia virus, 4 against the sarcoma virus, and 2 against both viruses. A study is under way to determine more specifically which antigens these spontaneous cat antibodies were directed against. The sera from 3 non-leukemic cats which gave agar gel precipitin lines against the sarcoma virus also had neutralizing activity against the same virus, suggesting that the agar gel tests detected antibody against viral coat antigens.

(4) Of 73 newborn or fetal dogs inoculated with feline leukemia or sarcoma viruses, 27 developed lesions of leukemia or sarcoma. The FSV-induced sarcomas tended to regress; dogs carrying tumors had undetectable or low antibody titers against the feline group-specific antigens. Feline leukemia virus induced lymphocytic leukemia/lymphoma in 10 dogs of 17 inoculated in 3 litters, representing 3 successive cell-free passages. The lesions were disseminated lymphosarcomas involving the thymus gland, various body lymph nodes, and other lymphatic tissues. C-type virus was readily demonstrated by E.M. in the neoplastic tissues. The incubation periods averaged about 60 days. This experimental lymphocytic leukemia of the dog is of sufficiently high incidence and short latent period that it should be useful for chemotherapy and immunotherapy studies. Some of the dogs inoculated with feline leukemia virus had serum antibodies demonstrable in the agar gel precipitin test, but the titers and identification of the antibodies have not yet been determined.

(5) A feline liposarcoma virus has apparently been demonstrated in a tissue culture inoculated with their strain of feline leukemia virus. It had been reported earlier (J.N.C.I., 42:987-1014, 1969) that liposarcomas occurred in experimental kittens in the first and second passages, but not in later passages. In the original and later experiments, 6 of 60 kittens in the first and second passages developed liposarcomas. In the present work, cell-free virus from the neoplastic thymus gland of the original spontaneous case (cat F-161) was inoculated into a cat embryo cell tissue culture. After 25 tissue culture passages, the cells transformed (whereas

uninoculated cultures did not). Cell-free tissue culture fluid from the transformed culture, when inoculated into newborn kittens, produced multiple liposarcomas, lymphosarcoma, or both. It would appear that both liposarcoma and lymphocytic leukemia viruses are present in the transformed tissue cultures.

(6) The group-specific internal antigens of feline leukemia and sarcoma viruses have been studied, in part with the collaboration of Professor Werner Schäfer of the Max-Planck Institute for Virus Research in Tübingen, Germany. One component with a molecular weight of about 15,000 was found only in feline leukemia and sarcoma viruses, was considered specific for the feline species, and was referred to as feline "gs.-spec. antigen". Another component with a molecular weight of about 33,000 was found to be shared by leukemia viruses of other mammalian (but not avian) species, and was called the "gs.-interspec. antigen". Evidence was obtained for the presence of the gs.-interspec. antigen in the 1.16 density gradient band of tissue culture fluid from a culture of bovine lymphosarcoma cells, and from two human neoplasms--the Levine III tissue culture from a case of mammary carcinoma and 6410 from a case of chronic myelogenous leukemia.

(7) Sera from many leukemic or clinically normal cats gave precipitin lines in Ouchterlony tests against various strains of feline leukemia and sarcoma virus. There is evidence of different serotypes in that a single serum can react with one or more strains, but not all strains of the viruses. Some, but not all, sera that produce a positive agar gel precipitin line, also neutralize 2 or 3 logs of the same viruses in tissue culture. The agar gel test was thus more sensitive than the serum neutralization test, or detected a different kind of antibody. Certain normal cat sera neutralized two serotypes of feline leukemia virus. Three cats maintained in an isolation ward apparently gave evidence of seroconversion: they initially gave negative Ouchterlony tests, which were positive one month later. The positive sera from normal or leukemic cats show precipitin lines of identity with purified virus surface ("v") antigen, but not with purified gs-antigens of feline origin or with disrupted murine leukemia virus. Sera from one human leukemic and three human members of his household gave negative Ouchterlony tests against a feline leukemia virus isolated from the human leukemic's pet cat which also had leukemia, and against other strains of feline leukemia and sarcoma viruses and purified gs-antigens.

(8) More specific antisera have been prepared against the gs-interspecies ("gs3") antigen, which has been found in the leukemia viruses of the mouse, cat, hamster, and rat. Such reagents are expected to be useful to search for evidence of leukemia virus antigens in human, cow, and dog neoplasms and tissues. A gs-interspecies antiserum was conjugated for use in a direct fluorescent antibody test. It gave positive tests for gs-interspecies antigen in human, cat, and dog cells infected with feline leukemia or sarcoma viruses, and also with mouse tissue culture cells inoculated with Moloney murine sarcoma virus. The same conjugate gave negative FA tests with the same mouse tissue culture cells (3T3) which had not been inoculated, normal dog embryo tissue culture stimulated by PHA, tissue cultures from a dog with erythroleukemia (with and without PHA), a dog mammary tumor, a dog eye tumor, a bovine lymphosarcoma tissue culture, NC-37 cells infected with

the Mason-Pfizer monkey virus, two normal human tissue culture lines (HWE 2836 and NC-37), a tissue culture from a human melanoma, a tissue culture from a human retinoblastoma, normal mouse primary kidney tissue cultures, and normal dog embryo (13th T.C. passage).

(9) Feline leukemia or sarcoma viruses, or infected cells, have been supplied to 18 other research workers. There have been 13 publications.

Significance to Biomedical Research and the Program of the Institute: The close association of cats with humans suggests that the possible common etiology of human and cat leukemia and sarcoma be carefully investigated. In addition, cat cancer studies will provide the most useful and practical model systems for research. It appears that cat leukemia virus can under certain circumstances, cross species barriers to induce tumors in the dog, which is also closely associated with man.

Proposed Course: The incidence of feline leukemia and sarcoma viruses will be determined in various cat populations, using isolation in tissue culture and fluorescent antibody testing of these cultures. Serological tests for antibody in cat sera will be investigated further, and the antibody characterized by serotype. Attempts will be made to demonstrate the "gs.-interspec." antigen in leukemia and sarcoma viruses, infected cells, and other suspect materials from various animal and human neoplasms. Further refinement of reagents for these tests will be carried out. Field isolates of C-type viruses from feline lymphocytic leukemias, myeloproliferative diseases, mast cell tumors, various sarcomas, and other sources, will be compared in neutralization tests in tissue culture for evidence of distinctive serotypes. Experimental kittens will be used to establish the viral etiology of additional feline leukemias, sarcomas, and other neoplasms. Newborn and fetal dogs will be inoculated with feline viruses to determine oncogenicity and antigenicity in the dog.

Date Contract Initiated: June 23, 1965

UNIVERSITY OF INDIANA (NIH-69-2048) INDIANAPOLIS, INDIANA

Title: Characterization of the Twiehaus Agent of Avian Reticuloendotheliosis

Contractor's Project Director: Dr. Alvin S. Levine

Project Officer (NCI): Dr. Michael A. Chirigos

Objectives: (1) To increase Twiehaus virus production in tissue culture. (2) To develop an *in vitro* assay for Twiehaus virus, including immunofluorescence techniques. (3) To continue efforts to concentrate virus from infected bird tissues and possibly from tissue culture. (4) To continue studies on the immunological, physical, and chemical characteristics of Twiehaus virus. (5) To continue oncogenicity studies of Twiehaus virus in hamsters and to further define the potential of this virus to induce tumors in mammals.

the technique of negative staining (PTA) demonstrated the following ultrastructural characteristics: (1) A sequential disassembly of the structures of the virion was observed. (2) The virion appears to be triple-layered: a central shell; a dense homogeneous, intermediate layer; and an outer envelope bearing short, compact, fiber-like projections. (3) The overall diameter of the viral particle measures 750-900 Å in thin-section, and is estimated as 800 Å in the PTA preparations of virus receiving no prior treatment.

Reticuloendotheliosis virus was purified from liver and spleen homogenates and chick embryo fibroblast Rollacell cultures by a series of differential, velocity and sucrose density gradient centrifugation methods. This enveloped virus was found to have the following properties: (1) A diameter of approximately 100nm. (2) A buoyant density in sucrose of 1.16-1.18 g/cc. (3) Single-stranded RNA at an estimated concentration of 8 percent. (4) DNA polymerase activity.

Immunological studies on purified reticuloendotheliosis virus from infected chick embryo liver cell culture fluids gave the following results: (1) Sucrose gradient banded virus from chick embryo liver cell culture fluids did not cause death on injection into day old chicks. (2) The purified virus on inoculation into chick embryo fibroblast cultures gave positive immunofluorescence by the indirect method using fluorescein isothiocyanate labeled rabbit anti-chicken gamma globulin. (3) Two non-chick antigen lines were obtained in immunodiffusion slides with chicken anti-reticuloendotheliosis virus sera. (4) Examination by electron microscopy of the denser of the two precipitin lines showed intact viral particles. (5) The denser of the two precipitin lines stained with Sudan Black B (lipid stain). (6) Enhanced immunocytolysis with rabbit anti-reticuloendotheliosis virus serum was observed in reticuloendotheliosis virus infected quail embryo fibroblasts. The antiserum was absorbed with normal chicken liver powder.

Tests to confirm induction of a tumor by reticuloendotheliosis virus in hamsters are in progress: (1) eighty newborn hamsters were inoculated with a preparation of 20 percent homogenate of hamster growth having a chick ID_{50}/ml REV titer of $<1 \times 10^{1}$. No tumors have been observed among 51 survivors. (2) Fifty newborn hamsters were inoculated with supernatant fluid from chick embryo liver tissue cultures made from nineteen day old embryos that were inoculated *in vitro* with reticuloendotheliosis virus. The infectivity titer of REV in the supernatant fluid was 2×10^{3} chick ID_{50}/ml. No tumors have been observed among 37 survivors. (3) Forty-nine newborn hamsters were inoculated with supernatant fluid from chick embryo liver tissue cultures having an infectivity titer of REV of 9×10^{3} chick ID_{50}/ml. No tumors have been observed among 34 survivors.

Collaborative studies are in progress with Life Sciences, Inc. under Contract No. PH69-63, to determine if any relationship exists between Marek's disease herpesvirus and RE virus. Preliminary data suggest that chicken REV immune sera from Indiana University (Levine) neutralized Marek's disease herpesvirus *in vitro*. The specificity of the test is not known. Gel diffusion tests using the above chicken REV immune sera did not give precipitin lines with MDHV antigen. Serum from an uninoculated contact control chicken caged

with [illegible] as measured by reduction of focus forming units and by production of a precipitin line against MDHV antigen in the gel diffusion test. The prevalence of MDHV in straight line Indiana Farm Bureau chickens is not known.

A survey of modes of horizontal transmission of reticuloendotheliosis virus, strain T, in chickens has been completed: (1) Titration of different organs (liver, spleen, lung, brain) from a moribund bird shows the level of infectivity of virus is essentially the same throughout the chicken. (2) Individually, moribund birds vary widely in the titer of infectious virus recovered. A range from 1.85 to 5.5 ID_{50}/ml (log base 10) by titration of spleen-liver specimens was observed. (3) Feather dandruff was always found negative for infective virus. (4) Relative susceptibility of day old cockerels were tested. The most susceptible routes were found to be intramuscular, subcutaneous, intraperitoneal, and intracardial. The least susceptible routes were found to be by the alimentary canal, intranasal, and intracranial. (5) Contact transmission studies show: (a) Simulated barnyard contact ratio of forty inoculated to forty uninoculated gave a 33 percent serologic conversion of the contact controls without evidence of reticuloendotheliosis in any bird but those inoculated. (b) Supported on wire above litter the contact ratio of forty inoculated to forty uninoculated gave a 13 percent serologic conversion of uninoculated. (c) Supported on wire above litter the contact ratio of one-hundred twenty inoculated to thirty uninoculated gave a 25 percent serologic conversion of the uninoculated. (d) Fifty uninoculated controls held in the same chicken room as the inoculated birds, but not in contact (cages kept at a distance), over a period of forty-two days, gave no evidence of disease or serologic conversion in the sera taken from thirty birds at random.

Reticuloendotheliosis virus (REV) has been reported to lack the gs-antigen of the avian sarcoma-leukosis complex. A repeat of these studies using purified virus which had been pelletized, resuspended in buffer, and centrifuged on a sucrose density gradient for 20 hours, gave a negative CoFAL test and a negative microimmunodiffusion precipitin reaction when hamster antisera to Rous sarcoma virus, strain Schmidt-Ruppin (RSV-SR), was tested with purified REV; however, when purified virus was treated with 1% sodium dodecel sulfate (SDS final concentration), positive tests were obtained when the treated virus was reacted with hamster anti-RSV-SR sera. These results have been confirmed using virus purified from infected chicken livers and spleens, as well as tissue culture-produced virus in chick embryo fibroblasts from SPF flocks. Control preparations from uninfected chicken livers and spleens, as well as from uninfected chick embryo fibroblasts, gave negative results. The immunodiffusion precipitin test, using chicken antisera to purified REV in 1% Noble agar with 8% NaCl, gave negative reactions when tested against purified Rous sarcoma virus and avian myeloblastosis virus; however, sodium dodecel sulfate-treated Rous sarcoma virus and avian myeloblastosis virus gave precipitin lines when reacted with chicken anti-REV sera. The positive control test gave precipitin lines with purified and SDS-treated REV. The negative control test, using preparations from uninfected chickens or sera from unimmunized chickens, did not give precipitin lines.

cross-immunofluorescence studies in tissue cultures of chick embryo fibroblast and chick embryo liver cells did not give cross-reactions with the following strains of Rous sarcoma virus: Bryan, Carr-Zilber, No. 599, Harris, Schmidt-Ruppin and RAV-2. The cross-immunofluorescence tests with Marek's Disease Herpes Virus were also negative. Polyacrylamide gel electrophoresis studies have shown common bands when purified reticuloendotheliosis virus strain T was compared with Rous sarcoma virus and avian myeloblastosis virus, after the sucrose gradient purified viruses had been treated with sodium dodecel sulfate plus 8M urea and stained with aniline black. There remains a need for a rapid and easy in vitro assay of REV. At this time the immunofluorescence test is most useful, but requires considerable attention and time for good results. Serial passage of virus in chick embryo fibroblasts has not been carried beyond the third transfer. Third, fourth, and sixth blind passages have proven negative by test for infectivity, immunofluorescence, and immunizing ability.

Significance to Biomedical Research and the Program of the Institute: The avian reticuloendotheliosis virus is a leukemogenic virus prevalent in many flocks of chickens, and is said to be transmitted both horizontally and vertically. The biologic characteristics and the understanding of the chemical, physical and immunological nature of the agent may be applicable to the possible role of viruses as etiologic agents in human neoplasia. Of equal importance is the determination of whether oncogenic viruses of animals have the capabilities to replicate and induce neoplastic diseases in man. The avian viruses must be evaluated for this potential.

Proposed Course: Develop a rapid and easy in vitro assay system for reticuloendotheliosis virus, strain T. Repeat and extend the chemical studies on the nature of the nucleic acid composition of the reticuloendotheliosis virus, strain T. Attenuation of reticuloendotheliosis virus, strain T, for vaccine strain(s). Study the interaction of reticuloendotheliosis virus, strain T, with Marek's disease herpesvirus in vitro and in vivo to determine if interference or potentiation results. These studies are being carried out in collaboration with Life Sciences, Inc., St. Petersburg, Florida.

Date Contract Initiated: May 8, 1969

IVERSITY OF CALIFORNIA (NIH-70-2048), DAVIS, CALIFORNIA

tle: Comparative Leukemia and Sarcoma Viral Studies

ntractor's Project Director: Dr. Leo K. Bustad

oject Officer (NCI) : Dr. Michael A. Chirigos

jectives: (1) To determine the oncogenicity, comparative pathogenicity
d the in vitro species infectivity of subhuman primate leukemia and sarcoma
ruses; (2) continue studies to compare, in vitro, the species infectivity
ectrum of feline sarcoma and feline sarcoma-leukemia virus complex in cell
nes of various species; (3) determine the pathogenesis of the diseases in-
ced by the sarcoma virus and the sarcoma-leukemia virus complex in differ-
t species, clinically and pathologically; (4) continue studies on the
iology and transmissibility of the feline myeloproliferative syndrome
d initiate these studies on spontaneous and radiation-induced osteogenic
rcoma; and (5) perform cooperative studies on the radiation-induced myelopro-
liferative syndrome in dogs.

jor Findings: Extracts of a spontaneous fibrosarcoma in a woolly monkey
re used to infect cultures of normal woolly monkey muscle cells. C-type
irus was isolated in an early passage and is being replicated. Electron
icroscopic examination of the cultures after the third and sixth passages
owed a number of budding and mature virions measuring approximately 95 mμ.
turation stages and buoyant density very closely resemble those of cat
irus, size being the major difference. A suspension culture of lymphocytes
om a gibbon ape with spontaneous lymphosarcoma is in the 14th passage.
lectron microscopic examination has shown large numbers of extracellular
rticles morphologically similar to mature C-type oncogenic virions. The
rticles are approximately 95 mμ in diameter with an electron dense nucleoid
65 mμ and come to rest in sucrose solution at a density of 1.14-1.16 g/cm^3.
dding forms of the particle have not been detected. Immunodiffusion tests
ith rabbit anti-feline-leukemia virus-gs1-antigen serum showed no reaction
ith the simian agents. Fluorescent antibody tests using dog anti-feline-
rcoma-serum, on cells replicating the simian virus, showed a positive
eaction only with the gibbon ape lymphoid cells. Tests for RNA-dependent
NA polymerase showed positive reaction in woolly monkey virus as with AMV
d FeLV. Gibbon ape lymphosarcoma virus was assayed for RNA-dependent DNA
olymerase activity over a range of detergent concentrations. Incorporation
nto a DNAse-sensitive, RNAse-insensitive, and acid-insoluble product was
etectable but low, when compared with avian myeloblastosis virus which was
sed as a control. Species infectivity studies with woolly monkey virus
owed positive infection in two human and two nonhuman primate cultures, but
irus was not observed in bovine, feline or canine cultures. Bovine anti-FSV
erum prepared by inoculating bovine cells transformed in vitro with FSV into
ne autochthonous host, manifest titers of 1/1012 by passive hemagglutination
or intact virions and 1/16 by immunodiffusion against disrupted FeLV. The
nti-FSV serum was found to neutralize greater than 95% of FSV focus-forming
nits at serum dilution of 1/1250. The serum globulin fraction conjugated
ith fluorescein isothiocyanate (FITC) and ferritin was found to react
pecifically against FSV and FeLV viral envelope and associated antigens.

detected by using an *in vivo* assay. The titer of neutralizing antibodies in the bovine anti-FSV serum exceeds any of the titers in cat sera examined thus far. In collaborative studies with Dr. Max Essex, Karolinska Institute, an indirect membrane fluorescent test for detecting cell membrane antigen associated with infection of cells by FeLV, showed that kittens with rapidly progressing malignant tumors or uninoculated controls usually failed to develop any detectable antibody; whereas antibody was detected in animals with regressive tumor. This correlates with *in vivo* assays.

C-type virus identical to feline leukemia virus was found in association with bone marrow cells in a variety of feline myeloproliferative diseases. Tumor tissues of myeloproliferative disease and osteosarcoma origin were maintained in tissue culture; none of these cell cultures have indicated the elaboration of virus-like particles detectable by tritiated uridine labeling and sucrose density gradient centrifugation. Cocultivation of these cells with MDCK cell cells have thus far failed to promote the appearance of labeled virus-like particles. In collaboration with the Radiobiology Laboratory, three ^{226}Ra-induced osteosarcomas in Beagles were successfully transplanted by intrauterine fetal inoculation of material from primary and metastatic lung tumors. Tumor growths in recipient pups occurred about 30-40 days after birth. The transplantable tumors were detectable radiographically. Of six additional spontaneous feline fibrosarcomas examined by electron microscopy, two contained C-type viruses. Cell-free tumor material prepared from three of the six cats induced tumors in 17 of 20 kittens inoculated. Electron microscopic examination showed the presence of C-type budding viruses. Kittens given multiple subcutaneous inoculations had tumors whose size was related to the total quantity of inoculum administered. Studies on cell mediated immunity in cats with regressing tumors are under way, as are *in vitro* studies on the isolation of defective FSV in cells of several species. Efforts are being continued to establish a possible viral etiology in canine leukemia.

In *in vitro* infectivity studies, FSV has infected cultures from cat, dog, cow, monkey, and man. All but the human and monkey cultures produced infectious virions which could readily transform cat cells even after 10-20 passages. The virions from infected human cultures, detectable by electron microscopy, did not cause morphologic alterations of feline cultures after several passages. However, cat cells co-cultivated with infected human cells did cause morphologic alteration of feline cultures after several passages. Clones of FSV-infected, transformed bovine and feline cultures have been isolated and will be assayed for possession of a defective FSV genome that could be used to determine or rescue a leukemogenic agent from spontaneous cases. Relative to the effects of dose and host age on the pathogenesis of feline fibrosarcomas, it was found that decreasing the dose had similar effects to increasing the age. Characteristics observed were a progressive increase in the latent period, an increase in the percentage of tumor regressions, a decrease in the maximum tumor size attained, a decrease in metastatic potential, histologic changes in tumor morphology, and a decrease in the ease of locating viral particlesby electron microscopy. An apparent increase was noted in resistance to tumors by kittens raised on queens previously used to raise FSV- or FeLV-inoculated kittens. Attempts to produce FSV in sheep were

successful only in sheep less than 60 days of fetal age, which had not yet acquired immune competence. The three tumors so produced all regressed within 30 days.

Canine lymphosarcoma and myelogenous leukemia were transplanted by in utero inoculation of fetal puppies. Whole cell suspensions from tumors were used to transmit lymphosarcoma. Gross and histopathologic evaluations on first and second passage lymphosarcoma recipients are complete. Chromosome determinations show cellular invasion of all recipients' organs by donor cells, and the emergence of a hypodiploid cell line. Preliminary electron microscropic examination of tissue from pups with myelogenous leukemia has not shown any virus-like particles. About 12 liters of bovine plasma was collected and concentrated by continuous flow-zonal ultracentrifugation for attempts to determine whether virus-like particles isolated from inoculated cattle can be considered as a leukemogenic agent.

Significance to Biomedical Research and the Program of the Institute: The proposed research objectives are relevant to human leukemia in that they wil enable determination of whether the oncogenic animal viruses under study can infect and transform cells of different species, including man, or induce neoplastic changes in selected animal species. The suspected viral etiology of two subhuman primate tumors are of special significance because of their obvious close relationship to man. The popularity of cats and dogs as pets has increased tremendously, and their importance in viral oncology as models and vectors is still of great interest and was reviewed in last year's proposal. The human population is in close and constant association with dogs and cats, which are both known to be carriers of communicable diseases. Of particular concern, too, is that children have the most intimate association with these pets. The use of milk from leukemic dairy cattle as a possible hazard to man has been reviewed frequently. Whether bovine, feline, and canine neoplastic diseases are communicable to man is a question that must be determined. It is possible that the cat is a reservoir for leukemic viruses and that leukemia in man may result from incidental infection. The fact tha the group-specific antigen of FeLV is also common to other animal leukemias suggests that the cat leukemia virus may be the prototype leukemia virus for all leukemias, including those of man. Although the evidence is incomplete, it enables speculation that cats may serve as excellent models for a full understanding of lymphoreticular neoplasia, sarcomas, and myeloproliferative disorders of man. The radiation-induced myelogenous leukemia in dogs now under study appears to be the best model for the study of this disease in ma

Proposed Course: (1) Characterization and species infectivity studies of the woolly monkey fibrosarcoma and gibbon ape lymphosarcoma agents will continue and include biochemical, biophysical, and serological as well as biological activity of the virus in vitro and in vivo. The development of assay methods for the detection of oncogenic simian virus will be continued. Sera against these viruses are now being evaluated. (2) Investigation wil continue on feline sarcoma virus specified antigen using immunofluorescent, immunodiffusion, gel electrophoresis, and isotopic labeling techniques. (3) Study of humoral antibody responses of cats to FSV will be continued. An effort will be made to correlate the presence of neutralizing antibodies wi antiviral titers detectable by indirect hemagglutination or fluorescent

queens and kittens in the experiment on the nature of the maternal transfer of resistance to FSV will be examined. (4) Experiments are being continued to detect the presence of cellular immunity in tumor regression in FSV-inoculated cats. (5) Studies will be performed to characterize the oncogenic RNA viruses in regard to the polymerase systems, and to determine if there is evidence for a canine oncogenic virus associated with the myeloproliferative syndrome and osteosarcoma using (a) hybridization of RNA material from dog tumor tissue to ^{3}H-DNA produced by known oncogenic viruses and (b) search for RNA-directed polymerase activity in tumor extracts. Possible methods separating viral DNA-dependent from viral RNA-dependent DNA polymerase activity will be examined. (6) Attempts will be made to rescue a "latent" viral agent by cocultivation with canine embryo cells and UV-inactivated Sendai virus fusion of these cells and those of MDCK with tissue culture cells of tumor origin. (7) Studies on spontaneous tumors of feline, canine, simian, and other animal species will be continued for the establishment of their viral etiology. (8) Collaborative studies will be continued with Dr. Max Essex. Department of Tumor Biology, Karolinska Institute, Sweden, to determine if true strain differences exist between the FSV isolates under study. These studies are continuing to determine how the level of viremia is correlated with the antibody response to the FeLV-FSV membrane antigen.

Date Contract Initiated: November 16, 1969.

TRW HAZLETON LABORATORIES, INC. (NIH-69-2079), VIENNA, VIRGINIA

Title: Etiology of Cancer in Dogs

Contract's Project Director: Dr. Erling M. Jensen

Project Officer (NCI): Dr. Wilna Woods

Objectives: The overall objective of this contract is to determine whether any of the cancers of dogs are caused by a virus(es) and whether there is any etiological relationship between canine cancer and human cancer. Among specific objectives are cellular and cell-free transmission of canine lymphomas and sarcomas; histopathologic, immunologic, serologic, hematologic and virologic, tissue culture and electron microscopic studies on spontaneous and induced canine neoplasias, as well as studies to determine whether a canine leukemia/sarcoma complex exists in the canine system.

Major Findings: Whereas emphasis previously had been placed on malignant lymphomas, the program has now been broadened to investigate the etiology of all cancers in dogs. Seventeen different tumor tissues have been acquired, including mammary tumors, perianal gland adenocarcinomas, osteosarcomas, fibrosarcomas and mast cell tumors. Cultures of many of these have been initiated for in vitro studies, and serum specimens have been collected for serological studies, including immunofluorescence for the presence of g.s. 3 antigens. Similarly, the serum from tumor-bearing dogs was also tested for g.s. 3 reactivity against FeLV-infected cells. In no instance was g.s. 3

positive reactions. Cells from dogs with undifferentiated sarcoma, osteo genic sarcoma, hemangiosarcoma and a fibrosarcoma reacted strongly with t autologous serum (with the exception of the fibrosarcoma) and with a var of other sera from tumor-bearing dogs. None of these specimens reacted w a normal dog serum. Similarly, a variety of normal dog cells did not rea with the sera from tumor-bearing dogs.

Tests employing the tritiated uridine labeling technique have been conducted on the 32043 cell culture of a transplantable canine lymphosarcoma mast cell tumor. A very strong peak of radioactivity at a density of 1.1 to 1.18 grams/ml indicated the presence of RNA-containing particulate materials in the cell homogenate. Cell-free passage of the peak fraction into dog embryo fibroblast cultures resulted in similar uridine uptake wh was not observed in the control. Electron microscopy by thin section of the pelletized peak fraction from 32043 cells revealed numerous spherical membrane-bound particles, many with dense nucleoids, with a diameter of 5 to 90 mμ. Although many of the particles bear a striking resemblance to rus particles, many are also suggestive of mycoplasma elementary bodies, and this possibility must be investigated.

Preliminary rescue studies with the known murine mixed culture system, ME MSV-HT-1, and Rauscher leukemia virus were successfully conducted to demo strate the validity of the techniques used. Stock feline leukemia and sa coma viruses have been prepared and titrated. Rescue studies on seven different dog tumor cell systems have been initiated using three rescue systems. It was observed that feline leukemia virus can be detected by t mixed cell culture cytopathic method (XC assay). This effect was observe with both infected feline embryo fibroblasts (FEF) and a canine fibrosarc cell line (M-132). The effect with M-132 was striking in that the develo ment of syncytial giant cells was observed within 24 to 48 hours, with mo giant cells showing numerous nuclei. Preliminary titrations with this ce line showed that this method is as sensitive for the detection of endpoin dilutions as is the CF test.

Since previous experience with <u>in vivo</u> passage of tumors has been limited primarily to lymphomas, additional studies are now being conducted with a wider variety of tumors by <u>in utero</u> inoculation. Mast cell tumor and tra missible venereal tumor (TVT) specimens received from Dr. C. Rickard of Cornell University have been inoculated both <u>in utero</u> and into young pups In three attempts the mast cell inoculum killed the feti within a few day It is felt that this effect was due to the histamine normally found in th cells. No tumors have developed after 57 days in two pups inoculated at days of age. Similar abortion problems have been encountered in three attempts to establish the TVT <u>in utero</u>. The reason for this problem is unknown. Inoculation of one adult dog did result in a tumor which was pa into three 27-day-old pups. Tumors are developing and will be passed to additional newborn pups.

All of the dogs in long-term holding are currently being reviewed careful with an aim to terminating approximately 60 to 70 of the current 169 dogs

uridine uptake, immunofluorescence, and freezing and storage of tissue culture cell lines, have been performed for the NCI as a service function during this period.

The combined effectiveness of chemotherapy and immunotherapy on canine lymphomas is being investigated. Previous reports from this laboratory have described a transplantable lymphosarcoma-mast cell tumor which has now been through 11 passages in newborn dogs. A tissue culture line derived from this tumor and transplantable in dogs will be very useful in in vivo therapy studies, since it affords a well characterized and reproducible tumor system. Experiments have been conducted to determine the optimal inoculum dose and age of inoculation for such studies. Results to date show that tumor induction requires at least $1x10^6$ cells administered intraperitoneally or $1x10^5$ cells intramuscularly, and that tumors do not develop when inoculated later than 10 days post partum.

Since combined chemotherapy and immunotherapy studies are also anticipated in dogs bearing spontaneous tumors, eight lymphosarcomatous dogs have been obtained, and five of these have been used in dose-response studies with cyclophosphamide. The three remaining dogs are being evaluated to determine the course of their disease prior to administering therapy. Once the optimal regimen of cyclophosphamide therapy is established to gain remission of the tumors, immunotherapy will be initiated. Results thus far show that a single dose of 20 mg/kg of cyclophosphamide, administered either before or after antigenic stimulation, causes a marked decrease in the total peripheral leukocyte count but does not affect either the primary or secondary humoral immune response. Studies are now in progress to evaluate the drug effect on the cellular immune response. Specifically, it will be determined whether the compound affects the ability of dogs to become sensitized to dinitrochlorobenzene.

Significance to Biomedical Research and the Program of the Institute: Because of the close relationship of dogs to man, as an intimate member of his household, it is important to determine whether canine tumors are induced by viruses, and whether such viruses can infect other species, including man. By isolating, identifying, and studying viruses found in both canine and human leukemia, lymphomas, and sarcomas, similarities may emerge which may link the disease in dogs with that of man. As a corrolary, it is also necessary to determine whether oncogenic viruses of cats can pass to man through dogs.

Proposed Course: (1) Continuation of the screening of spontaneous tumor tissues and serum for the presence of either g.s. 3 reactivity or the presence of common tumor antigens and attempt to determine the significance of the reactivity already observed in these systems. (2) Studies to determine the identity and significance of the virus-like particles seen in the tritiated uridine labeled fractions of the 32043 cells. (3) Virus-rescue studies with canine tumor tissues will be continued. (4) The demonstration of the applicability of the XC cell assay system to feline viruses and the unique properties of the M-132 cell system is a very significant observation and the various parameters of this system will be explored further.

(5) Since Herpesvirus saimiri is indigenous in one species of monkey, i.e., squirrel monkeys, but oncogenic in other species, i.e., owl monkeys and marmosets, a similar situation may exist in the family Canidae. This possibility will be explored using canine herpesvirus in other canine or related species. Arrangements are currently being made to obtain gray foxes, jackals, and dingos. Other members of the Canidae family being considered are the red fox, wolf and coyote. In vivo and in vitro studies of the effects of the virus on these various species will be studied.

Date Contract Initiated: May 26, 1969.

UNIVERSITY OF MIAMI SCHOOL OF MEDICINE, (PH43-67-1187) CORAL GABLES, FLORIDA

Title: Immunization Studies on Avian Leukosis and Related Problems

Contractor's Project Director: Dr. M. Michael Sigel

Project Officer (NCI): Dr. Gary Pearson

Objectives: (1) Development of in vivo and in vitro assays for transplantation antigens. (2) Studies on protection against Rous-RAV-1 with small amounts of virus. (3) Attempts to purify tumor antigen derived from chicken tumors. (4) Studies on Rous tumor antigens obtained from hamster tumors.

Major Findings: (1) Successful immunization against tumor induction by RSV(RAV-1) can be achieved by immunization with either low doses of RSV (RAV-1) or with doses of RAV-1 in the range of 10^1 to 10^6 infectious units. Thus, the protection achieved by low dose RSV (RAV-1) immunization could probably be attributed to the RAV-1 component present in excess in stocks of RSV(RAV-1). (2) In every experiment in which SPAFAS chickens were immunized with low dose RSV(RAV-1) or RAV-1 there was a good correlation (about 90 percent) between resistance to tumor induction and neutralizing antibody. This correlation does not establish a causal relationship between antibody and protection, as there have been instances where protection was evident in the absence of antibody. (3) Protection can be achieved with a broad range of RAV-1 doses, but the critical factor in successful immunization appears to be elapsed time from start of immunization to challenge. Thus, only partial protection can be achieved when challenge is 20-30 days after the initiation of immunization, full protection requiring a lapse of at least 35 days. This requirement is probably compounded of time periods necessary for virus replication, production of protective transplantation antigens, and evocation of cellular immunity. Protection lasts at least 112 days, the latest time tested to date. (4) In two experiments, one employing low dose RSV(RAV-1) as the immunizing agent, and the other RAV-1, significant protection, 36% and 69% respectively, was obtained against tumor formation by SR-RSV. This extension of protection induced by subgroup A virus to challenge with subgroup B virus has now been demonstrated on 5 separate occasions. Overall, protection against SR-RSV ranges from 42-71%, with RAV-1 immunization apparently being more efficient in this regard than RSV(RAV-1). Homologous

protection within subgroups has now been found for subgroup B viruses. Birds immunized with live RAV-6, a subgroup B leukosis virus, show protection against challenge with a subgroup B tumor virus, SR-RSV, at the rate of 80%; moreover, RAV-6 immunization can also protect against challenge with the subgroup A virus RSV(RAV-1) as evidenced by increased lag in tumor induction, and by reduction in incidence of tumor (43% versus 100% in controls). The finding of both homologous and heterologous protection in these two virus subgroups offers evidence for the concept that tumor-specific transplantation antigens are involved in the natural Rous host system.

In all of the experiments described there is a high incidence of antibody in prechallenge sera to the immunizing virus, while there is no antibody to the heterologous challenge virus. This finding argues against the presence of the heterologous virus in stocks as an explanation for cross-protection, although other possibilities cannot be excluded at this time. An experiment with a formalin-killed preparation of RAV-1 was performed to attempt to elucidate the role of antibody in protection in their system. All birds receiving this vaccine, even those injected with 6 doses in Freund's complete adjuvant, failed to resist challenge or produce antibody to RAV-1. Using a model hamster tumor system, attempts are being made to detect virus-induced surface antigens through antibody-induced cytotoxic reactions, as measured by release of ^{51}Cr. In an experiment with serum from SR-RSV tumor-bearing inbred hamsters, the cytotoxic assay was carried out using syngeneic and allogenic SR-RSV-transformed cells and syngeneic SV_{40}-transformed cells as targets. Two of the 3 sera showed cytotoxic effects against syngeneic (36% and 18% ^{51}Cr release) and allogeneic SR-RSV-transformed cells (64% and 69% Cr release) while there was no cytotoxicity against syngeneic SV_{40}-transformed cells (-1.0, 1.5% ^{51}Cr release). A control serum was devoid of activity against any target cells.

Significance to Biomedical Research and the Program of the Institute: The overall goal of the SVCP is the control of cancer. From the immunological viewpoint, it would be extremely important to clarify the issue as to the possible existence of a transplantation antigen and its role in the disease process. Basic studies are also needed on immunity in chickens in order to understand fully the immunologic implications in avian tumors, and possible application to mammals.

Proposed Course: The following are the major areas of investigation for next year: (1) Duration of immunity induced by immunization with live virus. (2) Expansion of the studies on specificity of the protective immunity using other subgroups of the leukosis system (heterologous combinations of immunizing and challenge viruses). (3) Humoral factors other than neutralizing antibody, such as the migration inhibition factor. (4) Cell-mediated immunity in hamsters and in chickens. In the former, a syngeneic SV_{40} model already developed will serve as a point of reference for the studies with the hamster Rous sarcoma system. In the latter, there is a need for obtaining basic information about the operation of cell-mediated immunity. (5) Further investigations on killed virus using concentrated virus.

Date Contract Initiated: June 23, 1967

Title: Studies of the Rat Mammary Tumor Derived Virus (RMTDV or BV)

Contractor's Project Director: Dr. Victor V. Bergs

Project Officer (NCI): Dr. Michael A. Chirigos

Objectives: To determine whether purified C-type particle preparations of RMTDV will induce leukemia or other malignancies when administered to conventional and germ-free rats under natural and immunologically altered conditions of the host; and (2) to determine whether the 9H virus (9HV), previously isolated from rat leukemia, will exert a helper or inhibitory effect upon co-infection with RMTDV in vivo and in vitro.

Major Findings: The growth kinetics of RMTDV in cells of the established REL line of Sprague-Dawley rat embryo cells were determined. Following infection at a multiplicity of 1.0-0.01 $TCID_{50}$ per cell, the peak of the virus titer was reached by day 4 post-infection. High-titered (10^7 - 10^9 $TCID_{50}$/0.1 ml) RMTDV is now being routinely prepared from infected REL roller bottle cultures by sedimentation of the partially purified virus harvest at 78,000 x g. The virus is stored in 0.05M potassium citrate at -70 degrees C. for future use. Storage in 0.05 citrate caused an apparent aggregation of the virions of RMTDV, which segregated upon dilution with culture medium at 37 degrees C. Appreciable aggregation did not seem to occur in culture, and at the time the virus was harvested and sedimented, but only upon storage in 0.05 citrate at -70 degrees C; however, storage of RMTDV under these conditions is necessary to avoid marked loss of infectivity. Infectious RMTDV banded at average buoyant densities of 1.12 and 1.25 gm/ml in a potassium tartrate gradient. Virus banding at a density of 1.25 gm/ml represented aggregated virions or virions attached to cellular components. RMTDV centrifuged in a preformed gradient of 50% and 10% sucrose by means of a Spinco SW 39 rotor at 38,000 rpm for 180 minutes, banded within a buoyant density range of 1.04 - 1.12 (average 1.08) gm/ml. If RMTDV was deliberately mixed with the 9H virus (9HV) and the mixture centrifuged under these conditions, RMTDV again banded within the above range; whereas 9HV banded at a density of 1.15 - 1.21 (average 1.18) gm/ml. The two viruses may therefore be separated by this method.

High-titered (10^7 - 10^9 $TCID_{50}$) RMTDV was inoculated intraperitoneally into several groups of newborn Wistar/Furth and Sprague-Dawley rats 3-5 months ago. To date no grossly detectable biological effects have been observed in any of these animals; however, an early disease developed within 15-32 days in W/Fu rats inoculated at birth with a mixture of RMTDV and 9HV. Histologically, it was characterized by focal coagulative necrosis and fatty change in the liver, accompanied by focal cell infiltrates which resembled leukemic cells of the lymphocytic type. Such a response did not occur in W/Fu rats inoculated with either RMTDV or 9HV alone. In one experiment, animals inoculated with a potent preparation of 9HV combined with RMTDV, developed typical peliosis hepatis within 12-15 days, but none of the other

changes. Control animals inoculated with 9HV alone also developed peliosis hepatis, and the ones inoculated with RMTDV alone showed no response. It appeared that in this case 9HV, because of its high potency, produced lethal peliosis hepatis before an expression of the changes caused by RMTDV occurred. Subsequent experiments showed that (a) 9HV antiserum blocked all the changes which may occur following a simultaneous inoculation of a mixture of RMTDV and 9HV as described above, and (b) RMTDV could be isolated from liver-spleen tissue extracts of animals that showed the focal necrosis, fatty change and cell infiltrate in the liver and had been previously inoculated with an RMTDV-9HV mixture. These extracts, however, did not cause any disease when inoculated into newborn rats, presumably due to the fact that infectious 9HV was absent or at low level as a result of high serum 9HV-antibody levels of the donor.

The WF line of Wistar/Furth rat embryo cells was established in their laboratory about 3 years ago. Recently cytopathological manifestations in this cell line were observed. Upon examination with the electron microscope, many typical C-type particles were seen budding from the cell membrane. Further investigations disclosed that lesions, consisting of transformed fusiform and rounded cells, appeared 4-7 days after seeding in culture vessels, provided the cultures were not refed. If the cells were refed or subcultured every 3-4 days no appreciable cytopathology could be detected. Similarly, if a culture showing some lesions was subcultured, the cells that grew out looked normal again, but such a culture again began to show altered cells after the 4th day if not refed earlier. The WF cells were highly malignant, since 90-100% of the inoculated suckling W/Fu rats developed rapidly growing tumors within 4-5 weeks and displayed infiltration of cells into vital organs. A group-specific murine leukemia anteserum, received from Huntingdon Research Center, Inc., failed to react significantly with RMTDV grown in REL cells in complement fixation tests. Since no positive antigen control was available, the results are not conclusive. These studies will be henceforth carried out in collaboration with Dr. C. Rickard.

Comparative titrations of RMTDV in WF cultures and in REL cultures previously inoculated with rat C-type virus from the natural carrier cultures WF (rat embryo cell line) or RMTL-9 (rat mammary tumor cell line), suggested slight interference to RMTDV. This was indicated by 0.5 - 1.0 $\log_{10}$ lower virus titers as compared to those in control REL cultures. REL cultures inoculated with C-type virus of the RMTL-9 cell line, produced 16 to 32-fold lower HA titers of 9HV, upon challenge with the latter, than did control REL cultures. In another study, fetal calf serum was found to have some stimulatory effect upon the synthesis of RMTDV in REL cultures, resulting in a 1-2 $\log_{10}$ increase in titer. At the same time, it markedly protected the cultures from RMTDV-induced cell alterations, thus obscuring the microscopical detection of RMTDV. This agrees with earlier findings in a collateral project that bovine amniotic fluid or estrogen stimulated the synthesis of RMTDV in WF cell cultures; whereas progesterone had no effect. A rat inoculated with high-titered RMTDV about 6-1/2 months ago developed an intraperitoneal adenocarcinoma, which is being studied now. Attempts to propagate RMTDV in suspension cultures of two human leukemic cell lines and one normal cell line have been unsuccessful to date; however, another line of normal human

blood cells appeared to have yielded progeny RMTDV, but this will have to be confirmed in repeat experiments.

Large quantities of concentrated high-titered RMTDV stocks have been sent to Drs. L. Bustad and C. Rickard for RNA dependent DNA polymerase and gs-antigen studies, respectively. Some of this virus was also used to initiate preliminary investigations in germfree and SPF rats in collaboration with Dr. J. Warren.

Significance to Biomedical Research and the Program of the Institute:

Although rats are highly susceptible to spontaneous cancers as well as to the induction of cancers by chemicals and irradiation, and C-type viruses similar to those which cause cancers in other species have been found in rats by electron microscopic studies, no such virus of rat origin has yet been proven to cause a cancer in this species. In addition to the important possibility of having a new laboratory model in another species for guiding approaches to the human virus-cancer problem, the proposed studies would also provide an important test of the Huebner hypothesis that C-type viruses are the determinants of most cancer in all species, including rats and humans for which oncogenic viruses have not yet been demonstrated.

Proposed Course: (1) Complete identification studies on RMTDV. (2) Further *in vivo* and *in vitro* studies on co-infection with RMTDV and 9HV in regard to time and dose relationships. (3) Attempts to potentiate RMTDV by frequent serial transplants of leukemic cells and extraction of the resulting tumors for virus. (4) Attempts to potentiate the WF cell agent by serial *in vivo* passage of the cells, accompanied by inoculation of rats with cell-free extracts of the tumors. (5) Characterization of the agent present in the WF cell line and development of a suitable host system for its assay. (6) Further purification studies on RMTDV and subsequent characterization of its nucleic acid. (7) Studies on the possible infectivity of the nucleic acid of RMTDV. (8) Completion of studies on RMTDV in human cells.

HOWARD UNIVERSITY SCHOOL OF MEDICINE (NIH 70-2178) WASHINGTON, D. C.

Title: Immunological Studies on Human Mammary Tumors and Other Neoplasms

Contractor's Project Director: Dr. Michael V. Viola

Project Officer (NCI): Dr. Paul Levine

Objectives: (1) Survey of neoplastic breast cancer tissue culture lines for tumor antigens using fluorescent antibody and complement fixation techniques. Sera will be surveyed for tumor specific antibodies, also. This work will be done in collaboration with Dr. Robert Ting, Bionetics Research Laboratories. (2) Procurement of selected tumor specimens and establishment of neoplastic tissue culture lines from selected cancer patients, particularly

those with leukemia, lymphoma and breast cancer. (3) Procurement of sera specimens from patients with neoplastic and non-neoplastic diseases and their families. Patients at Freedmen's Hospital as well as all inhabitants of Quitman County, Mississippi (population 23,000) will have sera collected and stored.

Major Findings: Specimens were procured and delivered to the following investigators at the NIH: Dr. Harish Chopra: four breast carcinoma cell lines, lymphoma cell line, two rhabdomyosarcoma cell lines, one effusion from Burkitt tumor, one pancreatic carcinoma cell line, one lung carcinoma biopsy, one breast carcinoma biopsy, one Burkitt tumor biopsy, one effusion from breast carcinoma; Dr. B. Eddy: four lung carcinoma biopsies; Dr. P. Levine: lymph node imprints from patient with Hodgkins Disease, and 50 sera specimens from patients with Hodgkins Disease and matched controls; Dr. P. Gerber: 2 lymphocyte preparations on 2 patients with Hodgkins Disease; Dr. Rapp: 1 lymphocyte preparation from a patient with chronic lymphocytic leukemia, and 1 lymphocyte preparation from a patient with breast carcinoma; Dr. Plata: 2 tumor biopsies from patients with breast carcinoma, 1 cell line derived from breast cancer patient with a pleural effusion, and 1 serum specimen from a patient with breast cancer; Dr. Ting: 35 specimens from patients with breast cancer and controls for Levine line studies, 49 other sera from breast carcinoma patients, and 2 large plasma specimens on breast cancer patients with positive anti-belev titers; and 250 sera specimens and clinical histories from patients with cancer.

Tumor specific immunity in humans with breast cancer and other malignancies was investigated using a mixed lymphocyte-tumor cell culture technique. Incorporation of tritiated thymidine into lymphocyte DNA was measured as an indication of cellular immunity to tumor antigens. Cells from neoplastic effusions were used as the main source of tumor cells. Eighteen patients have been studied; at present completed data have been obtained on the first six patients studied. Lymphocyte stimulation by tumor cells in excess of control cultures were obtained in the following patients:

Diagnosis	#pos. / # tested
lymphosarcoma	1 / 1
breast carcinoma	1 / 1
lung carcinoma	1 / 1
non-neoplastic effusions	0 / 3

An American patient with the clinical and histo-pathological characteristics of Burkitt's lymphoma was studied. No virus particles were found by electron microscopic examination of tumor specimen and neoplastic effusion. EB virus antibody titers ranged from 1:8 to 1:320. Indirect immunofluorescence using the patient's acetone-fixed tumor cells and high-titered anti-EB sera (from a patient with nasopharyngeal carcinoma) was negative; however, mixed lymphocyte-lymphosarcoma cell culture revealed stimulation of normal lymphocytes by neoplastic cells; therefore, it appears that this American patient with Burkitt's tumor has tumor specific antigens, probably unassociated with the EB virus.

A number of tissue culture lines were established from tumor specimens: (1) One rhabdomyosarcoma monolayer cell line. (2) One lymphoblastoid cell line derived from lymph node of American patient with Burkitt's lymphoma. (3) A long term cell line has been established from a human stomach carcinoma specimen. The cell line is epithelioid, heteroploid, does not stain for mucin, and causes tumors when inoculated into the cheek pouch of cortisonized hamsters. The line is being assayed for the carcinoembryonic antigen by Dr. Gold. (4) Two cell lines have been established from a pleural effusion from a patient with breast cancer, are being screened for virus particles by Dr. Chopra, and have been supplied to Dr. Plata for immunological studies. Since considerable tumor cell death occurs in the mixed cultures, a method to detect lymphocyte cytotoxicity using Cr^{51} release from target solid tumor cells, is being studied. A soluble membrane preparation of the stomach carcinoma cell line is being prepared for in vitro lymphocyte stimulation and in vivo skin testing of patients.

Studies have been continued on lymphocyte function of patients with breast carcinoma and other neoplasms. The decreased lymphocyte response to PHA in patients with b.c. (advanced) is due to a lymphocyte abnormality and not to a serum factor that depresses lymphocyte function, as had been previously reported. Sera from 5 patients with decreased response to PHA did not inhibit blastic transformation of lymphocytes from normal individuals. Three of 15 patients studied have shown marked stimulation of their lymphocytes by autologous tumor cells. One patient had lymphosarcoma and the other two had breast carcinoma. The pleural fluid from one patient with a malignant breast effusion was shown to stimulate lymphocytes from three other patients with breast carcinoma. One control lymphocyte suspension tested did not stimulate.

Significance to Biomedical Research and the Program of the Institute: In the light of recent observations of virus-like particles in specimens from breast tumor patients (e.g., pleural effusions), including C-type and smaller particles, and in view of the recent isolation of C-type particles in a spontaneous mammary tumor in a rhesus monkey, follow-up studies on mammary tumors in humans (and other species) currently represents a high priority in the Special Virus Cancer Program.

Proposed Course: (1) Lymphocyte sensitivity to tumor antigens will be studied further using: (a) Additional patients with neoplastic and non-neoplastic effusions, (b) Trypsinized solid tumors and neoplastic cell lines, (c) Cell-free effusion fluid as a source of antigen. (2) In patients demonstrating positive in vitro lymphocyte response, membrane antigens will be isolated from tumor cells and stored in liquid nitrogen. This antigen source will be used for serial testing of patients throughout the course of their disease. (3) Transfer factor will be isolated from lymphocytes from patients with positive response to isogeneic tumor cells. Unresponsive lymphocytes from a patient with a tumor of the identical cell type will then be sensitized with dialyzable transfer factor from the responsive patient. Positive in vitro transfer to tumor specific immunity by transfer factor would indicate common tumor antigens in tumors of the same cell type, and would be the first step in the clinical evaluation of the usefulness of transfer factor. (4) Tumor specimens and sera from cancer patients will be

collected for NIH and SVCP laboratories desiring specimens. (5) Additional tumor specimens and cell lines derived from tumors will be supplied to Dr. H. Chopra for electron microscopic screening.

Date Contract Initiated: April 27, 1970

ALBERT EINSTEIN MEDICAL COLLEGE (PH43-65-612) BRONX, NEW YORK

Title: Research on Immunological Factors in Susceptibility to Murine Leukemia Viruses

Contractor's Project Director: Dr. Frank Lilly

Project Officer (NCI): Dr. M. A. Chirigos (Acting)

Objectives: To determine the role of genetic and immunological factors in susceptibility of certain animals to candidate murine and human leukemia viruses specifically, the following studies were planned: (1) Examination of a cross involving tail-kinks gene and Fv-2 gene to determine on which side of dilute in linkage group 11 Fv-2 is situated. (2) Further studies to determine whether Fv-1 and Fv-2 control separate viral components (SFFV and LLV) in the Friend virus complex. (3) An experiment designed to clarify the basis for the observed loss of FMR and certain H-2 complex antigens from spleen cells during the course of the Friend disease. (4) Further studies with the apparently new virus line isolated by passaging F-B virus through B10.D2 mice. (5) Studies on the immunological unresponsiveness of certain strains of mice to the H-2.2 and to the FMR antigens. (6) Investigation of the genetic basis of the differential hematopoietic response in BALB/c and C_3H mice to F-B virus.

Major Findings: (1) A conference-workshop on "Genetic Factors in the Friend Virus Disease" was held in Bethesda on 22 January 1971, bringing together a number of workers with results which bear upon the interaction of virus genome in determining the outcome of infection with murine leukemia viruses. A broad area of agreement exists concerning the property of N-, B-, or NB-tropism of the viruses and response to the virus in vivo and in vitro as a function of the host's genotype at the Fv-1 locus. It was recognized that Fv-2, on the other hand, appears to influence the host response mainly to the SFFV component of Friend virus, and probably not to the LLV component. (2) Dr. Lilly's contribution to the correlation of the NB system with the Fv-1 system consisted in showing that (a) the FV strains used in vivo to define the Fv-1 system differ in the NB system in vitro in the manner predicted; (b) the congenic mouse strains used to define the Fv-1 system in vivo respond to N- and B-tropic viruses in vitro in the manner predicted; and (c) all available data on susceptibility of various inbred mouse strains to FV in vivo correlated perfectly with the results. (3) Comparison of the congenic BALB/c ($H\text{-}2^D$) and BALB.B ($H\text{-}2^b$) mouse strains with respect to the quantitative expression of FMR antigen on their spleen cells shows that the antigen appears at the same time after virus infection and to almost the same extent; however, thereafter the amount of FMR on BALB/c cells declines

enormously; whereas the amount on BALB.B cells declines only slightly. (4) B10.BR ($H\text{-}2^k$) and C3H.0H ($H\text{-}2^0$) mice are both highly susceptible to GV leukemogenesis; whereas their F_1 hybrids are quite resistant. Analysis of the F_2 generation (N=194) of this cross implies that this phenomenon of genetic non-complementation is due to two independent genes, neither of which is associated with H-2; however, further analysis shows that H-2 type significantly modifies this response, but not in an all-or-none manner. This is relevant to the recent findings that HL-A type is significantly but not completely correlated with human leukemia occurrence. (5) Studies on skin tumorigenesis by vaccinia virus and methylcholanthrene (MC) in a number of inbred mouse strains, and also in various crosses of the BALB/c and AKR strains, indicate that MC painting may induce (a) skin tumors or (b) leukemia, but rarely both, and that, if it induces skin tumors, one might infer that no covert infection with leukemia virus is present. (6) Absorption of FV by bone marrow cells of different genotypes. Suspensions of femoral bone marrow cells from three strains of mice were incubated with aliquots of a preparation of F-S virus and the supernatants assayed for residual virus activity in DBA/2 mice. By comparison with the unabsorbed control virus preparation, bone marrow cells from BALB/c and C57BL mice did not absorb significant amounts of virus, but cells from C3H mice removed all detectable virus activity (i.e., a reduction of at least 100-fold, by comparison with the controls). (7) Computer analysis of cytotoxic assays: Considerable effort has gone into the attempt to establish a computer program for the analysis of data obtained in the titration of isoantisera by the Cr^{51}-labeling method. Initial observations using this system appear to indicate that the cytotoxic assay is perhaps a more reproducibly quantitative technique than previously considered to be. (8) Since F-B virus always induces polycythemia in BALB mice but frequently induces anemia in C3H mice, they have examined the hematocrits of mice of the (C3H X BALB) X C3H mice at various times after virus inoculation. There is a significant difference in the mean hematocrit values of homozygous $H\text{-}2^k/H\text{-}2^k$ mice and heterozygous $H\text{-}2^k/H\text{-}2^d$ mice of this population which develops only during the third week after infection, and at about 23 days. The kd mice show a mean value at least 10 points higher than the kk mice in each of two experiments. Thus H-2 type is a factor in the polycythemic response to FV, and this should prove useful in analyzing the mechanism of the H-2 effect on leukemogenesis. (9) Although they had previously shown that intact FV particles do not possess FMR antigen on their surfaces, they have now found that when the virus particle is disrupted, there is a great deal of FMR activity detected. Most of this activity is still attached to fragments of the virus, because it is sedimentable by centrifugation at 100,000 G, but a portion of the activity is present in soluble form and can be recovered from Sephadex G 150 columns, mostly in large molecular form. Studies on the smaller molecular form of the antigen (25-35 thousand MW) obtained from infected spleen homogenates indicate that its isoelectric point is about 7.5-8.0; this is markedly different from that of the gs-1 antigen, which was about 5.5-6.0 in published studies.

BALB/c mice were inoculated with a mixture of (a) the N-tropic F-S virus (a low dilution, so that by itself it produced no disease in any BALB mice), and (b) the B-tropic B/T-L virus (which induces the Gross type of leukemia, rather than the Friend type). This mixture induced Friend disease in all

recipients less than 2 months of age, and two passage lines of virus from these animals have been established. Titration of these new strains of virus, called F-T virus, shows high infectivity in BALB and D2.R^S mice and low infectivity in DBA/2 mice. Thus the new virus strain has exactly the opposite host range as the original Friend virus (F-S). Comparative titration of F-B virus in the H-2-congenic pair of mouse strains, BALB/c (H-2^d) and BALB.B (H-2b), gave a very similar titer in both kinds of mice by the spleen focus assay method, but a significantly different titer (higher in BALB/c by more than a 10-fold difference) by the spleen palpation method. This confirms that the H-2 effect on leukemia virus susceptibility is exerted at a late stage in the disease process, not at the early stage of cellular infection by the virus. Preliminary findings indicate that cells of a passaged solid tumor line, derived from the spleen of a late survivor among BALB mice infected with F-B virus, (a) have lost all detectable FMR tumor-specific antigen, but (b) now show a new antigen not present on FMR-positive BALB spleen cells. Antibodies prepared by immunizing BALB mice with this BALB solid tumor are cytotoxic for the solid tumor cells, but not cytotoxic for BALB F-B-infected spleen cells. Paradoxically, the antiserum is cytotoxic for BALB normal spleen cells, but not for C57BL normal spleen cells. These studies will be repeated and extended when a new batch of the same antiserum is available. There will be available very soon data on the cell surface mapping of the relative position on BALB F-B-infected spleen cells of the FMR antigen with respect to various H-2 antigens.

Significance to Biomedical Research and the Program of the Institute: One of the basic facts about tumor biology is that the genetic mechanisms of the host exert strong control over the expression of oncogenicity. This contract represents the only in-depth study being done on the genetics of susceptibility to viral oncogenesis. The knowledge derived from this study and the mouse strains developed will have broad applications both to the SALES program and the general effort in virus-cancer problems.

Proposed Course: The following experiments are planned for next year: (1) Comparison of further immunogenetic and viral parameters of infection by Friend virus in BALB/c and BALB.B mice. (2) Further studies of the polycythemia-anemia problem in BALB and C3H mice. (3) Absorption of F-S and F-B viruses by spleen cells of various mouse strains. (4) Attempts at final purification of the FMR antigen. (5) Mapping of the Fv-2 gene with respect to theta, mdh and Trf. (6) Having shown that the leukemogenic, B-tropic B/T-L virus can rescue SFFV in BALB mice and give rise to a B-tropic Friend virus, they wish now to try the same experiment with some naturally occurring B-tropic viruses (isolated by Dr. W. P. Rowe) which are not leukemogenic in vivo, although they give a fairly high titer in the XC cell assay.

Date Contract Initiated: May 13, 1965

GERMFREE LIFE RESEARCH CENTER (PH43-65-95) FT LAUDERDALE, FLORIDA

Title: Germfree Research and Operation of a Collaborative Germfree Tumor Virus Laboratory

Contractor's Project Director: Dr. Joel Warren

Project Officer (NCI): Dr. M. A. Chirigos

Objectives: The activities under this contract are of three different types, each involving the special germfree and environmentally controlled SPF facilities and capabilities of the contractor: (1) Service type activities in general support of SVCP and intramural research requiring clean, defined, animals and viral reagents; (2) Participation in collaborative research primarily of other groups, but in which germfree and/or rigid SPF environmental control are required for definitive results; and (3) Research primarily by contractor scientists, but of mutual interest to the SVCP or collaborative with other SVCP groups where back-up in other special disciplines are necessary. Specific objectives under (1) are: (a) Production of tumors in germfree avian and rodent animals by chemical carcinogens and propagation of such tumors by transplantation as a resource for studies of such tumors by other investigators. (b) Production of oncogenic viruses in germfree animals as a source of "reagent grade" virus for use in SVCP research, including that in this contractor's facility. (c) Supply of limited number of germfree animals to other investigators where feasible, as a spin-off from maintenance of the foundation colonies.

Major Findings: Employing the helper-free, non-dependent RSV Beta-O strain of virus, they have completed a series of four replicate experiments designed to correlate the size of infecting dose with level of extractable virus in the ensuing tumors. There is a marked correlation between extractable virus and initiating dose. Regardless of the nature of the factors that might be essential for infectious virus replication within cells (including the cell-associated chf, and the virion-associated RNA-dependent DNA polymerase, the possibility exists that provirus can be integrated at multiple genetic loci, and that the amount of virus produced is quantitatively related to the number of such parasitic viral genomes incorporated per cell, as well as the number of cells embodying such productive intergrations, both of which might be expected to be related to input multiplicity of virus.

The amount of RSV in cell-free tumor extracts is highly dependent upon whether the host is germfree or not. Tobacco tar concentrate administered in a single dose will accelerate tumor formation with an associated increase in virus yield. These same experiments have uncovered the interesting observation that dimethylsulfoxide, administered into the breast muscle prior to inoculation of Rous virus, markedly increases the multiplicity of infectious virus in such tumors. Whether this effect is similar to that observed by others with DEAE remains to be established. A similar effect was found in vitro, where the presence of DMSO in monolayer cultures markedly enhances focus formation by this virus. The infected foci in DMSO-treated cultures are markedly different from those observed in

untreated controls.

To determine whether a chemical carcinogen, methylcholanthrene, was active in a germfree, "C" particle-free host, a colony of Fischer rats was reared germfree and certified as "C" particle-free by the NIH. When 18 of such animals were inoculated with methylocholanthrene, all but one developed large tumors in approximately 100 days. One of these tumors, transferred through four generations in tissue culture, induced a sarcoma in conventional Fischer rats 73 days later. The cell-free extract of the culture is now on test in this host. The biological properties of spontaneous leukemia in germfree Fischer rats are being compared with those of a chemically-induced (Shay) and radiation-induced (Jones) leukemia.

Work has continued on a comparison of properties of host-helper and virus-helper dependent properties of RSV strains. Using the low-dose technique of Bryan et al., they are investigating the A-variants of the Prague and Schmidt-Ruppin strains in germfree quail to determine whether the latter behave similarly to helper dependent RSV strain. They are also attempting to obtain the recently discovered RAV-0 agent by rapid passage of RSV tumors in quail. Further experiments substantiate the finding that RSV-0 multiplies to higher levels in muscle tissue of conventional animals than in germfree. Removal of birds from an isolator leads to rapid change in the host, resulting in increased virus proliferation in muscle. Similar experiments with non-helper-dependent RSV viruses are underway, as well as studies to determine whether the use of sterilized diets modifies the susceptibility of conventional animals.

A colony of germfree rodents has now reached an age at which spontaneous tumors are beginning to appear at an increasing rate. These, as well as MCA-induced neoplasms, are being studied in tissue culture in an attempt to isolate a transmissible agent.

Significance to Biomedical Research and the Program of the Institute:
The study of many of the most important problems in the virus causation of cancer in animals requires the use of certified animal hosts free of extraneous viruses and other pathogenic microorganisms, as well as skilled technical operations within environmentally controlled facilities, including completely germfree isolators for some purposes.

This contract is one of two established under the SVCP for supporting critical studies in such animals of interactions among viruses in dual infections (e.g., interference, enhancement, "helper" action), and co-carcinogenic activity between viruses and chemical compounds, both of which underlie the development of knowledge and technology for the detection and isolation of oncongenic viruses of man.

Future Course: To continue to develop, maintain, and distribute colonies of germfree and SPF mice, rats, and hamsters as required by SVCP projects. To continue to serve as the nucleus colony for other contracts and intramural laboratories. To continue the production of "reagent grade" lots of the Vogt strain of RSV in germfree quail for use in research in this and other SVCP contracts; appropriate bacteriological and histopathological monitoring

will be continued. To continue the propagation of chemically induced tumors in germfree animals, investigate that, and supply either such tumors or the tumor-bearing animal to other participants in the SVCP. To continue to develop instrumentation for gnotobiology on a time-available basis. Particular attention is being given to aerosol and smoke chambers for germfree rodents. To test GF and SPF derived tissues in vitro. To maintain SPF animals on GF diets. To continue studies on determination of low dose response in DMSO-treated quail, and on the general usefulness of DMSO for viral isolation. To develop a pneumotropic strain of RSV-SR.

Date Contract Initiated: April 16, 1965

LIFE SCIENCES, INC. (PH43-69-63) ST. PETERSBURG, FLORIDA

Title: Studies on Marek's Disease as a Model for Herpesvirus Associated Oncongenesis

Contractor's Project Director: Dr. Jack Frankel

Project Officer (NCI): Dr. M. A. Chirigos

Objectives: (1) To determine the exact nature of the role of the herpesvirus associated with Marek's disease in the etiology of this disease, i.e. whether it is direct, or indirect (e.g. by interacting with some other agent, or by activating a covert viral oncogene--the Huebner hypothesis); (2) To establish Marek's disease as a model system for herpesvirus associated oncogenesis, using reagent grade SPF avian hosts (free of overt leukosis virus and other pathogens); and (3) To operate an avian virus testing laboratory for monitoring both hosts and viral materials used in experiments for freedom from extraneous viruses, including infectious leukosis virus.

Major Findings: (1) Techniques for extraction of DMHV in high titer from feather shaft base of MDHV infected chickens have been developed. (2) Detection of MDHV antigen in feather tips by agar gel double diffusion tests after two weeks coincided with the initial appearance of infectious MDHV. (3) MDHV neutralizing antibodies in response to infection were detected within six to seven weeks, and infectious MDHV could no longer be isolated at this time. (4) Electron microscopy during the period of MDHV isolation from feather shaft base consistently showed herpesvirus particles, inclusion bodies and small membranous particles to be present in the feather follicles. (5) Optimal conditions for assay of MDHV infectivity are: dilution in tryptose phosphate broth, absorption onto 48 hour old LSI-SPF chick embryo fibroblast (CEF) cultures (primary, secondary, tertiary), pretreatment with 50 mcg of diethylaminoethyl-dextran, and maintenance with tissue culture medium at an initial pH of 7.0. (6) CEF ($10^{3.7}$ FFu/ml) and chick kidney ($10^{3.5}$ FFu/ml) cultures and the CAM of LSI-SPF embryonated chicken eggs ($10^{3.2}$ PFu/ml) were found equally susceptible. (7) Titration of MDHV in LSI-SPF chicks showed that the pool contained $10^{3.2}$ LD_{50} per ml. (8) MDHV infectivity was retained following filtration through a 0.45u Millipore

filter. (9) MDHV has been stored at -70 degrees for eleven weeks without infectivity loss. (10) Foci formation and pocks on the CAM were significantly reduced by serum derived from MD infected chickens. (11) Prior infection of CEF cultures with MD cells (tumor and CEF passage 5) significantly reduced plaque formation by influenza virus (strain WSN). (12) A monocontaminated research farm facility is in operation and provides an area for the housing of infected chickens and for studies involving natural exposure to DMHV. (13) The biocontainment facility (LSI, Building No. 3) containing eight Reyniers steel isolators, is available for studies with MDHV and other viruses. (14) The isolators prevented spread of a virulent virus (Newcastle disease, strain Roakin), from infected LSI-SPF chickens and quail to other birds in the outside environment and to birds housed in other isolators. (15) In the isolators, LSI-SPF and LSI germfree chicks responded to MDHV infection the same as LSI-SPF chicks housed at the monocontaminated research farm facility.

MDHV superinfection of primary cultures of LSI-SPF chicken embryo fibroblast cells (CEF), infected with 10^2 - 10^4 $TCID_{50}$ per ml of RAV-2, resulted in an 87 - 94 percent reduction in MDHV foci as compared to control cultures inoculated with MDHV alone. Interference with focus formation was RAV-2 dose-dependent, since no inhibition of MDHV foci was observed in cultures infected with relatively low concentrations of RAV-2 ($10^{0.5}$ - 10^1 $TCID_{50}$ per ml). Total inhibition of focus formation occurred in secondary cultures infected with large doses of RAV-2 (10^5 - $10^{5.5}$ $TCID_{50}$ per ml). Interference with focus formation was evidenced when RAV-2 was added to cultures together with or 48 hours after MDHV. Interference has also been observed with RAV-1, RAV-50, and ALV. On the other hand, super-infection of primary and secondary RAV-2 infected cultures failed to inhibit plaque formation by herpes simplex and vesicular stomatitis viruses. It is of particular interest that MDHV superinfection of RAV-2 infected cultures resulted in significant enhancement in COFAL titers compared to cultures infected with RAV-2 alone. These increases did not always parallel interference with MDHV focus formation. RAV-50 and ALV interference with MDHV focus formation also resulted in similar increases in COFAL titers; however, the latter response was not observed with RAV-1.

Significance to Biomedical Research and the Program of the Institute: No satisfactory animal model is currently available for studying the role (i.e. whether direct or indirect) of herpes-type viruses in the induction of neoplasia. Since herpes-type viruses are associated in very high frequency with two types of human cancer, namely Burkitt's lymphoma and post-nasal carcinoma, an animal model system for developing approaches to and guiding studies on the human problem is urgently needed.

Since Marek's disease of chickens is also associated with a herpes virus (MDHV), and this virus can now be isolated and worked with systematically in the laboratory, it seems likely that this chicken disease can be developed into the desired laboratory model.

Proposed Course: (a) A variety of methods will be explored to increase the efficiency of extraction of MDHV from feather shaft bases and to stabilize MDHV infectivity during handling and storage. (b) The optimal conditions

for detection of MDHV infectivity in tissue cultures and eggs will be determined and complement fixation and fluorescent antibody techniques will be developed. (c) Potent MDHV will be employed in interference and enhancement studies with a variety of viruses including viruses studied previously with MD cells. Studies with MDHV, herpes simplex and other herpesviruses will be emphasized. (d) The pathogenesis of Marek's disease in LSI-SPF chicks will be studied by sequential changes in histopathology, serology and virology. (e) Concentrated MDHV will be treated with formalin, ethylene oxide and beta propiolactone to determine the efficacy of inactivated vaccines to protect chickens against this herpesvirus.

Date Contract Initiated: November 1, 1968

UNIVERSITY OF PENNSYLVANIA (PH43-65-1013) NEW BOLTON, PENNSYLVANIA

Title: Research in Experimental and Natural Transmission of Bovine Leukemia

Contractor's Project Director: Dr. Robert Marshak

Project Officer (NCI): Dr. M. A. Chirigos (Acting)

Objectives: To determine the etiology of bovine leukemia. To attempt experimental transmission of bovine leukemia through use of cell-free materials prepared from selected leukemic milk and solid tissue. To investigate natural transmission of the disease through reciprocal foster nursing experiments.

Major Findings: Three experiments (differential centrifugation + sucrose density gradient centrifugation (SDGC); polyethleneglycol precipitation + SDGC; and rate zonal centrifugation + SDGC) were conducted in an attempt to isolate, concentrate and purify the VLP in cell line NBC-6. In all three experiments, band three (3-5 bands were present in each preparation) contained occasional C-type VLP. Thus far, none of the procedures is deemed suitable for producing highly concentrated virus preparations, possibly because VLP appears to occur in large aggregates in the cell cultures. The presence of budding particles in PHA treated and untreated buffy coat (BC) cells from leukemic cow BF044 is now routinely demonstrated. C-type particles were present in BC cells from 4/7 cows in their multiple-case herd and in 0/5 cows in a leukemic-free herd. Identical budding C-type particles have been shown to be present in 3 different leukemic bovine cell lines.

Helper Assay and Tissue Culture Studies: Using the *in vivo* system described by Steeves (JNCI 44: 587, 1970), they showed helper activity in five of six extracts from PHA-treated BC cultures from BF044. Two untreated cultures have given questionable results and helper activity was also present in four extracts of BC cultures from a normal cow (2 PHA-treated and two untreated). The greatest helper activity was observed in a cell-free extract prepared from NBC-6 cells cultured 48 hours in E20FCI. Such cultures contain replicating C-type virus. The presence of helper activity in the presumably

virus-free preparations remains to be explained. In cooperative studies with Dr. Bassin (NIH), attempts to demonstrate helper activity by candidate bovine materials in the MSV system were unsuccessful.

In an attempt to find culture conditions under which budding particles might be detected in buffy coat (BC) cultures from cow BF044, the cells were cultured with and without PHA in Eagle's MEM without fetal calf serum. C-type VLP were present in both PHA and control cultures at 6 hours. Distinct budding particles were seen in both cultures at 12 and 18 hours and in the control culture at 24 and 48 hours. Enveloped A particles were observed in some cultures and, surprisingly, as much or more virus was present in the control culture as in the PHA-treated culture. In another series of experiments in which BC cultures were grown in Eagle's MEM with and without PHA, the amount of virus in the untreated cultures was at least equal to that in the PHA-treated cultures. Budding particles were observed as early as 3 hours in the PHA cultures, and at varying intervals thereafter in both the treated and untreated cultures. In another experiment in which different BV044 BC cell concentrations were used, the results suggest that a significantly higher total virus yield may be obtained from the culture initiated at the higher cell concentration if the viability of the 2 cultures is comparable. It was also found that the appearance of IF antigen(s) in cell line NBC-6 parallels the appearance of the C-type virus.

Immunological Studies: Cell line NBC-6 was examined by indirect immunofluorescence using acetone-fixed cell preparations. In the first series of experiments, cells showing a granular and/or diffuse brilliant fluorescence in the cytoplasm were consistently found when tested with the serum from a regression case of bovine leukemia (cow #27-125). The percentage of fluorescent cells was significantly higher in the cultures maintained in E20FSI than in the cultures maintained in M20HSI. Subsequent EM and IF tests, performed in the same cultures of NBC-6 cells grown in E20FSI or in M20HSI, confirmed the close correlation between cells showing VLP and fluorescent cells. Sera of normal cow (BI 205) and of a normal calf from a leukemia-free herd were negative. Preincubation of fixed NBC cells with the serum or 6-globulin fraction of the regression case (27-125) completely blocked the fluorescence activity of FITC conjugated y-globulin from 27-125. No blocking activity was found in the serum or y-globulin from normal cow BI-205. A preliminary serological survey showed the presence of fluorescent antibodies to NBC-6 cells in the sera of 11 out of 13 leukemic animals (12 cows and one calf). Ten additional sera from normal cows in a leukemia-free herd were tested and none showed IF antibodies.

Studies on Bovine Syncytial Virus (J-BSV) - On the basis of 1,452 sera (from 24 herds) which have been studied to date, the following limited conclusions are drawn: (a) The distribution of J-BSV infection varies greatly from herd to herd regardless of the herd's leukemia status; (b) There appears to be a significantly greater rate of infection in multiple case herds when compared to contact herds, and both of these show significantly higher rates than the control herds tested so far; as yet, no relationship between J-BSV infection and persistent lymphocytosis is apparent.

Reciprocal Foster Nursing Experiment - Oldest animals are 37 months old;

youngest are 14 months. No leukemias have been detected so far. Though too early for definitive analysis of the lymphocytosis status of the various categories of animals, as yet there have been no alterations in absolute lymphocyte counts which can be related to the types of foster dams upon which the calves nursed.

Studies on the Experimental Production of Leukemia - To date, no clinically detectable leukemias have developed, but collectively the cattle in the gross filtrate, leukemic suspension culture cell line (CL) and the leukemic thoracic duct lymph (TDL) transmission experiments have all shown significantly elevated absolute lymphocyte counts (ALC). A significant drop in the ALC of four out of five cattle in the leukemic cell line experiment may be significant because, in spontaneous cases, such a drop in ALC commonly precedes the appearance of tumor masses. Chromosome studies in several animals which received i.v. infusions of CL and TDL also suggests transmission of the disease. A new transmission experiment in which colostrum-deprived neonatal calves are receiving NBC-6 suspension culture cell grown in Eagle's medium with 20% FCS (such cells are rich in budding C-type virus) is underway.

Significance to Biomedical Research and the Program of the Institute: Since there is a close association between cattle and their products, and man, the etiology of bovine leukemia is of utmost importance. This program has been making progress, and there has been active collaboration with many other investigators.

Proposed Course: (1) Virus isolation and concentration experiments will be extended. (2) An investigation of the presence of RNA-dependent DNA polymerase will be conducted using virus preparations obtained from NBC cell lines and from short term BF044 BC cultures. The presence of the enzyme in cells from previous cultures as well as in lymphocytes from leukemic and non-leukemic cattle will be determined. (3) Search for the gs-antigens of leukemia viruses in preparations from NBC cell lines and BC PHA treated cultures. (4) Attempt to transmit bovine C-type viruses to bovine embryo fibroblasts, NBC-10 cell line, and other established cell lines. (5) Attempt to develop an *in vitro* infectivity assay for the bovine C-type viruses.

Date Contract Initiated: June 18, 1965

NATIONAL CENTER FOR DISEASE CONTROL (NCI-VCL-(68)-42) ATLANTA, GEORGIA

Title: Etiologic Studies of Leukemia and Related Diseases Occurring in Unusual Epidemiologic or Genetic Situations

Contractor's Project Director: Dr. Clark W. Heath

Project Officer (NCI): Dr. A. Gazdar

Objectives: This is a collaborative program of the National Center for Disease Control (CDC) and the National Cancer Institute (NCI) in which

epidemiologic and virologic studies are conducted of leukemia and related illnesses (cancer, congenital defects) occurring in unusual epidemiologic or genetic circumstances: community case clusters, human-animal case associations, and situations in which cases appear associated in families. In selected population areas, case surveillance programs are operated to furnish accurate data on current leukemia incidence patterns, and to assist in the prompt detection of unusual epidemiologic or genetic events. Each unusual event is investigated in detail, and when appropriate, specimens are collected from patients, persons with associated illnesses, family members and possible case contacts. While such investigations are pursued with a wide range of etiologic possibilities in mind, they focus primarily on viral oncongenesis. Specimens are examined both in the contractor's laboratory and in that of collaborating contractors, including bone marrow, serum, buffy coat cells, and tissue biopsies.

a. Field Investigations: A total of 37 field studies were pursued, 9 being investigations begun originally in earlier years. Sixteen concerned case cluster situations, 8 cases associated with unusual animal contacts (4 with cats, 2 with dogs, and 1 each with horses and cows), 4 multiple case families, 3 leukemia in identical or like-sexed twins, and 2 marital leukemia.

In connection with these field studies, serum specimens were collected from 600 individuals, and blood and/or marrow cells for culture from 23. Of the serum specimens, 202 were obtained from a random sample of a high school population in California as part of an investigation of an apparent cluster there of osteogenic sarcoma, and 146 from a random sample of two grade school populations in Middletown, Connecticut, as part of a study of a case cluster of childhood leukemia in that town.

A cluster of three cases of lymphoma among children was found in the small town of DuBois, Pennsylvania. All three were from one Catholic parish and the two most recent cases were schoolmates during one school year at that parish's parochial school. Their onset coincided with a sharp outbreak of varicella that affected that school both sooner and more severely than other schools in the area. The association of cases in this town with a particular church/school population segment is similar to earlier observations made in Niles, Illinois, Middletown, Connecticut, and Kendall Park, New Jersey. Case reporting continued from seven areas in the United States (Connecticut, Rhode Island, Utah, metropolitan Atlanta, Houston, Kansas City, and Nassau County, New York). An additional surveillance project was begun involving childhood leukemia and lymphoma in the state of Colorado.

b. Laboratory Studies: Facilities at CDC for culturing cell specimens and performing virologic and immunologic studies on these and related sera were developed, starting in August 1970. Since that time 50 specimens of blood or marrow cells have been processed (preparation and storage of acetone fixed slides, in vitro tissue culture of cell materials), 6 in connection with ongoing field investigations, 12 on cultures received from Pfizer and developed there in past years in connection with earlier investigations, and 32 from patients with leukemia seen at local medical centers in Atlanta and north Georgia. Of these, 28 cell lines have been established and continued,

each being built up for freezing back, for preparation of further acetone-fixed slides and, in the near future, for virologic and immunologic testing (fluorescent antibody and cytotoxicity testing with patient/contact sera, electronmicroscopic screening, and immunologic screening against known oncogenic agents (EBV, etc.). Cytogenetic analyses are being made of each cell line being processed. Of the 28 cell lines derived from blood or marrow of patients with leukemia or their relatives, 22 have been screened with EM, five being found to contain EBV, and two a smaller as yet unidentified virus-like particle. Ten lines have been tested for immunoglobulin production, and two gave positive results. Thirteen have been cross-tested with corresponding homologous sera, and absorption experiments are now in progress. Eight specimens have been cocultivated with human embryo and Down's syndrome fibroblasts, yielding no evidence of viral activity. Laboratory work will largely focus on cross-absorption studies to delineate patterns of FA reactivity in collected cell lines and their related sera.

Earlier in the year 11 skin fibroblast cultures were developed from a large multiple-case cancer family in California. These have been examined cytogenetically and sent to the Melpar laboratories for transformation studies. An analysis was made in collaboration with Dr. Don Morton, NCI, of the distribution of antibody titers against human sarcoma antigen in the 202 sera obtained from a high school population in Santa Clara County, California. No patterns of titer distribution were found to account for the pair of cases of osteogenic sarcoma diagnosed during that same school year in two classmates at the school.

Significance to Biomedical Research and the Program of the Institute: Since this contract deals with epidemiologic and genetic studies on cases of human leukemia, and attempts to ascertain and clarify the etiology of human leukemia through intensive investigations of selected cases, it is of direct and immediate relevance to the aims of the Special Virus Cancer Program.

Proposed Course: Investigations of selected leukemia cases or cases of potentially related diseases will continue to be pursued, and appropriate serologic specimens will be collected. In the CDC Laboratory, serologic and immunologic tests will be continued on collected specimens with attention focused on: (a) search for oncogenic virus activity and (b) sero-epidemiologic studies to help interpret individual family, community, or animal-related case clusters.

Date Contract Initiated: July 1, 1967

UNIVERSITY OF NORTH DAKOTA (PH43-66-8) GRAND FORKS, NORTH DAKOTA

Title: Quantitative Studies on the Transmission of Feline Oncogenic RNA Viruses and Selected Herpesviruses by Certain Bloodsucking Arthropods

Contractor's Project Director: Dr. Robert G. Fischer

Project Officer (NCI): Dr. George J. Burton

Objectives: To determine whether the following diseases induced by certain oncogenic viruses can be transmitted from infected animals to healthy susceptible animals by bloodsucking arthropods with special reference to virus quantitation: feline leukemia virus (FeLV), feline sarcoma virus (FSV), murine cytomegalovirus (Henson), and Marek's disease virus(MDV). Specific objectives are: (1) To determine oncogenic virus levels in arthropods which have fed on experimentally infected donor animals of known titer (extrinsic incubation studies). (2) To determine whether the disease caused by each oncogenic virus can be transmitted biologically, i.e. by transfer of the virus via the salivary glands of the infected arthropod when taking a subsequent blood meal. (3) To determine the quantititative distribution of the virus in those arthropods demonstrating suitably high virus levels among various organs and tissues, including alimentary tract, hemolymph or blood, salivary glands, and in any other organ where virus may localize and multiply. (4) To determine whether the disease caused by each oncogenic virus can be transmitted mechanically through interrupted feeding, or by transfer of the virus on the legs and body of the arthropod (especialy in Marek's disease).

Major Findings: It has been established that FLV could be transmitted by several insect species which had partially fed on an FLV-infected mouse and subsequently completed their blood meal on a "normal" suckling BALB/c mouse. The minimum number of bites necessary to initiate infection by Aedes triseriatus, Culex pipiens quinquefasciatus or Stomoxys calcitrans is one, while to date at least two bites by Anopheles quadrimaculatus have been necessary to initiate a leukemic response. The initial attempt at establishing the laboratory transmission rate was made and reported in Aedes triseriatus (5/114 or 4.34 percent), Anopheles quadrimaculatus (18/152 or 2.56 percent), Culex pipiens quinquefasciatus (6/114 or 5.26 percent), and Stomoxys calcitrans (18/152 or 11.84 percent).

In experiments involving Friend leukemia virus (FLV), several extrinsic incubation studies (i.e., determination of FLV fate) have been conducted with the black salt marsh mosquito Aedes taeniorhynchus, the malaria mosquito Anopheles quadrimaculatus, and the cat flea Ctenocephalides felis. In current studies, there was no indication of FLV activity beyond two days in any of the insects held at normal rearing temperatures (23 degrees C - 27 degrees C). It was seen earlier that cooler insectary temperatures (particularly 13 degrees C) slowed the rate of FLV decay but the overall response was not affected. Collaborative effort with Drs. W. Turner and G. Burton, N.I.H., has been initiated to determine whether the FLV complex ingested by Stomoxys calcitrans is altered with time in this fly, so that subsequent introduction of this insect-virus mixture into an animal system

results in an occasional splenomegalic response without the concommitant formation of splenic foci. One group of 10,000 flies has been allowed to feed directly on intact viremic BALB/c mice; whereas the second group (6,000 flies) was fed from sterile sponges soaked with FLV virus-spleen-blood mixtures.

Extrinsic incubation studies of feline leukemia (lymphoma/lymphosarcoma) virus (FeLV) and feline fibrosarcoma virus (FSV) in the cat flea, Ctenocephalides felis have been performed in collaboration with Dr. C. Rickard, New York State Veterinary College at Cornell University and Dr. G. Burton, N.I.H. Groups of fleas have been fed through membranes in vitro on either FeLV-human blood or FSV-human blood mixtures on 9, 6, 3, 1, and 0 days before dissection. Both FeLV and FSV were viable for at least 24 hours in the cat flea.

In additional experiments, fleas fed directly on FeLV-viremic kittens in vivo were noted to ingest larger blood volumes (3X) than those which fed artificially through membranes. Groups fed on viremic kittens 9, 7, 3, 1, and 0 days earlier were used in additional studies.

Arthropod transmission of Herpesvirus saimiri (HVS), which induces reticulum cell sarcoma in marmosets, is being investigated with the collaboration of Dr. F. Deinhardt, Presbyterian-St. Luke's, and Dr. G. Burton, N.I.H. They included initial extrinsic incubation studies of HVS in relation to the stable fly, Stomoxys calcitrans, and the malaria mosquito, Anopheles quadrimaculatus; as well as investigation of the possibility of transmission from an infected marmoset to a susceptible recipient during interrupted feeding activity of the stable fly. HVS has been found to be viable in the midgut of the stable fly and malaria mosquito for 6-6.5 hours after feeding on an infected marmoset. An in-depth collaborative effort with Drs. W. Turner and G. Burton, N.I.H, was performed to determine whether the Friend leukemia virus (FLV) complex ingested by Stomoxys calcitrans is altered with time in this fly, so that subsequent introduction of this insect-virus mixture into an animal system results in an occasional splenomegalic response without the concommitant formation of splenic foci. Eight samples (10% spleen homogenates) were prepared from mice presenting with varying degrees of splenomegaly (210 mg-617 mg). Results of this study are forthcoming pending the completion of the X-C testing by Dr. Turner.

Marek's disease transmission studies are being conducted with the collaboration of Drs. J. Frankel and V. Groupé of Life Sciences, Inc., and Dr. G. Burton, N.I.H. Two experiments have been conducted at St. Petersburg. They include an initial extrinsic incubation study in Stomoxys calcitrans and Ctenocephalides felis. In addition, two natural association experiments have been conducted in "germ-free" isolator units. Extrinsic incubation studies of mouse cytomegalovirus (Henson) in relation to several mosquito species are now in progress. They include studies at 0, 3, 4, 5, 7, 8, 10, 11, 12, 14, 15, 19, 22 and 26 days for Aedes aegypti; 0, 4, 7, and 11 days for Aedes taeniorhynchus; 0, 4, 7, and 11 days for Anopheles quadrimaculatus and 0, 4, 7, and 11 days for Culex pipiens quinquefasciatus. An investigation to determine possible SFFV and/or LLV activity in certain insects is being conducted in collaboration with Dr. R. Steeves, Roswell Park Memorial Inst. as regards FLV. In the EM laboratory of the subcontractor in Yonkers, N.Y.,

i.e., Boyce Thompson Institute, FLV particles were detected in the midgut of the mosquito Anopheles stephensi infected with Friend LV by feeding on leukemic BALB/c mice with a titer of FLV $10^{-5.4}SED_{50}$, following post-feeding periods of 30 hours and 3 days. Various developmental stages of FLV virions were found in engorged donor blood cells. Extracellular virions of tailed mature, as well as immature, forms were also observed in the midgut lumen. The findings indicate that the insects may act as potential carriers of this virus. Even if they remain unable to transmit it by biting, they still may play a role in transmission if crushed, or defecating.

Although extracellular FeLV-like particles were observed in the midgut of Ctenocephalides felis 4 hours after the virus acquisition, no virus particles were detected in various organs of the fleas, following post-feeding periods of 1, 3, 6, and 9 days. Electron microscopic examination of the cat fleas infected by a single feeding, as well as multiple feedings, on diseased donors is now under way. Various organs of the mosquito Aedes taeniorhynchus were excised 4 days after virus acquisition on FSV donor kittens. EM examination of the bonemarrow, blood, and sarcoma tissues of the donor kittens is in progress.

Electron microscopy work with the Henson strain of mouse cytomegalovirus (CMV) has been initiated. A group of adult fleas, C. felis, was fed for one hour on CMV-infected mice following four additional multiple feedings at a 24-hour interval. Various organs of the insects were removed 8 days after the initial virus acquisition. The bonemarrow, blood, and salivary glands of the donor mice were also removed. Preparations of the samples for the electron microscopic examination are now under way. Studies of oncogenic viruses in invertebrate cell cultures have been initiated. To date, a standard method for the flea cell culture has been established, providing active cell growth in vitro from ovarian tissue fragments of the young adult fleas.

Significance to Biomedical Research and the Program of the Institute: Arthropods have been investigated in relation to the transmission of human diseases for many years. Viruses, which are obligate parasites, may be transferred by arthropod vectors, and such transmission may occur biologically or mechanically. Arthropods such as mosquitoes, biting flies, fleas, ticks and mites may suck blood from both man and from domestic animals (including birds) closely associated with man. It is, therefore, important to investigate whether leukemogenic viruses present in infected animals can be transferred to humans. If such vector or vectors are found, then measures can be taken to control the vector and to prevent contact between vector and human or animal hosts.

Proposed Course: If positive transmission results are obtained with herpes saimiri virus, then it is expected that priority will be given to investigations utilizing arthropods feeding on marmosets infected with this virus. As many species of common bloodsucking arthropods will be used as can be procured and reared in large numbers. Further investigations on Marek's disease virus, murine cytomegalovirus (Henson) and feline oncogenic viruses will depend on initial results. It is expected that this contract will be

terminated at the end of 1971.

Date Contract Initiated: October 27, 1965

ST JUDE CHILDREN'S RESEARCH HOSPITAL (NIH 71-2134) MEMPHIS, TENNESSEE

Title: Studies on the Etiology of Selected Amphibian Tumors

Contractor's Project Director: Dr. Allan Granoff

Project Officer (NCI): Dr. Gary Pearson

Objectives: This project is aimed at determining the etiologic role of viruses, particularly herpes-type virus, isolated from the renal adenocarcinoma of Rana pipiens (Lucké tumor). Both in vitro and in vivo host-virus relationships will be investigated using pertinent biological, biochemical, immunological, and physical techniques. Characterization of the virions will be made using physical and chemical methods to establish the possible relationship of these agents to oncogenic mammalian and avian viruses. Studies concerning natural transmission, epidemiology, and the role of environment, particularly temperature, in tumor formation will be carried out concurrently or subsequently, as applicable. Studies will also be carried out on the possible viral etiology of the lymphosarcoma of the South African toad, Xenopus laevis, if time permits.

Major Findings: This contract was initiated on May 13, 1971, only 1-1/2 months prior to the end of FY '71. During this time equipment and supplies were ordered and the project was put into initial operation.

Significance to Biomedical Research and the Program of the Institute: The role of herpes-type viruses in benign and malignant diseases of man and animals has been under investigation, i.e., in Burkitt's lymphoma, cervical carcinoma, infectious monomucleosis, and Marek's disease. The ubiquitous distribution of HTV requires that the relationship of this group of virus to cancer be unequivocally established. Since it appears that temperature acts as an inducing factor in the case of the herpes-type frog virus, perhaps this may have more general application to other DNA virus-cancer systems. Th Lucké tumor offers an experimental system for proving directly that HTV is an oncogen, rather than by sero-epidemiology alone. The presence of other viruses in the Lucké tumor suggests also that defective-helper virus may be involved in the etiology of the tumor. Recent studies have shown that at least one antigen of the Burkitt herpesvirus is identical to an antigen of the herpesvirus present in the Lucké tumor of frogs. The significance of this find has been under investigation. It is possible that the information to be obtained by this contract will have some application in the etiology of human cancer.

Proposed Course: Rana pipiens whole embryo cell lines (RPE) and adult frog kidney cell lines (AKRP) will be used. Rate of herpes-type virus attachment, effect of temperature on attachment and the amount of cell-associated and

released virus will be ascertained. The temperature of virus replication (4°C to 30°C) will be studied, since HTV infection in tumors in vivo appears to be temperature-dependent. Development of HTV will be studied concomitantly by electron microscopy. A variety of mammalian, avian, piscine, and amphibian cells will be tested for susceptibility to transformation by HTV at low and high multiplicities, at various temperatures for 4°C to 37°C. Infected cells will be transplanted into the anterior eye chamber of the frog to test for growth of transformed cells. The number and function of structural and virus-induced antigens will be determined with in vitro cell systems and with naturally occurring Lucké tumors free of detectable virus. If tumor induction is accomplished by absence of virions, tumors will be examined for virus-specific antigens. Hybridization experiments to detect viral specific RNA will be performed using the methods of Gillespie and Spiegelman and Green. Studies will be made on a papova-type virus isolated from Lucké tumors. Attempts will be made to induce tumors in R. pipiens embryos and adults utilizing HTV and PTV. If time permits studies will be initiated in attempts to determine the possible viral etiology of lymphosarcoma in the South African toad Xenopus laevis.

Date Contract Initiated: May 13, 1971

RUTGERS UNIVERSITY (NIH 71-2077) NEW BRUNSWICK, NEW JERSEY

Title: Test for Genetic Acquisition of Oncogenic Potential and Cell-Transforming Capacity by RNA Animal Viruses

Contractor's Project Director: Dr. Robert Simpson

Project Officer (NCI): Dr. Willie Turner

Objectives: The objective of the contract is to determine whether a non-oncogenic RNA animal virus can gain tumor-producing or cell-transforming ability as a consequence of one or more of the following genetic events:

1. Host-induced genotypic modification of viral RNA.
2. Intracellular persistence of incomplete but functionally active RNA viral genomes.
3. Chemically-induced mutation(s).

Non-oncogenic RNA viruses showing oncogenic potential after conversion by such genetic mechanisms would be investigated to determine the nature and origin of the genetic information involved.

Major Findings: Initiation of the contract program on February 15, 1971, was followed by efforts to procure all major items of equipment, hire project personnel, and arrange for necessary physical alteration of the research facilities. All major items of equipment have now been obtained. The alteration of laboratory rooms, including installation of a self-contained

cell culture module, should be completed by July 15, 1971. Project personnel, with the exception of an animal caretaker, have been hired, and their training has progressed very satisfactorily.

Most of the research carried out thus far has been devoted to preparation of reagents, such as purified stocks of candidate viruses, development of specialized cell lines to be used for bioassay, and preliminary experiments concerning the intracellular persistence of incomplete viral genomes. Milligram quantities of gradient-purified stocks of influenza WSN virus and vesicular stomatitis virus (VSV) have been prepared and stored at -70°. Substantial amounts of hyper-immune sera against inactivated (beta-propiolactone) VSV, have been prepared in rabbits and titrated for neutralizing antibody. Immunization trials have also been started, using influenza virus as antigen. Wild-type stocks of influenza virus, VSV, Sindbis virus, and specific conditional-lethal mutants of these viruses, have been cloned under conditions designed to eliminate adventitious agents such as avian leukosis virus or mycoplasma. A line of guinea pig embryo cells, now in its 22nd consecutive passage, is being maintained as a potential system for bioassay of oncogenic mutants. VSV, specifically inactivated with the compound Bayer A 139, is being used in attempts to integrate a non-infectious replicating genome into animal cells. Cells thus infected with inactivated virus are being monitored for production of virus-specific proteins and changes in growth characteristics.

Significance to Biomedical Research and the Program of the Institute: The genetic acquisition of oncogenic potential by RNA animal viruses is an intriguing possibility and one of great importance, since it is possible that the "human cancer virus" could be a member of an RNA virus group which normally causes non-oncogenic diseases in man. Although supporting evidence for this phenomenon in animal RNA virus is quite scarce, there are persistent observations which suggest that normal non-oncogenic viruses may have oncogenic potential. Thus, a thorough investigation of the capacity of non-oncogenic viruses to genetically acquired oncogenic potential is warranted. These studies would fit well in the scope of the SVCP, since one of the specific aims of this program is to determine the etiology of human cancer or uncover viruses with oncogenic potential which could be possible candidates for an etiological role in human neoplasms.

Proposed Course: Test viruses: Total emphasis will be put on the use of enveloped RNA viruses, based on the class properties of most of RNA viruses. The Contractor has gathered a sizeable collection of mutants of various enveloped RNA viruses which carry useful genetic markers. Temperature-sensitive (ts), host-restricted (hr), thermostable, and drug resistant mutants as well as plaque-forming recombinants of representative members of the myxovirus, arbovirus, rhabdovirus and reovirus groups will be employed in these studies. Stock of the above viruses will be grown in cells recognized to yield maximum titer of infectious viruses. Large volume of gradient purified virus will be prepared from cells propagated and maintained in roller bottle production units. Newborn and young adult non-inbred guinea pig (GP), secondary GP embryo fibroblast cell culture, as well as a developed continuous cell line of GP kidney cells will be

employed in these studies. In addition, attempts will be made to establish cell lines from tumors induced in GP by the new genetically converted oncogenic viruses. The GP was selected as the animal of choice in these studies because of the extremely low incidence of spontaneous tumors in these animals in age groups under 3 years. The Contractor plans to employ "Rif-free" embryonated eggs as a source of embryos for cell culture of whole embryos or specified tissue thereof, for use in these studies. In addition, cell culture derived from tissue from newly hatched chicks ("Rif-free" eggs), as well as newly hatched chicks, will be employed in this investigation.

Human primary cell lines such as human embryonic kidney and fetal skin/muscle cells and human continuous cell lines such as WI-38, etc., will be employed to detect the newly acquired oncogenic potential of RNA animal virus treated as described above. Other cell lines, both of human and animal origin, will be included in these studies; i.e. 3T3, FL, BHK 21, etc.

Date Contract Initiated: February 15, 1971

DUKE UNIVERSITY (NIH 71-2132) DURHAM, NORTH CAROLINA

Title: Study and Production of Avian Leukosis Virus

Contractor's Project Director: Dr. Joseph W. Beard

Project Officer (NCI): Dr. Michael A. Chirigos

Objectives: The objectives of this project are: (1) to continue quantity and quality production of BAI strain A avian tumor virus; (2) to continue investigations on RNA avian leukosis viruses. The Contractor projects a monthly production of 15 gms wet weight of plasma and 30 gms wet weight of tissue culture grown BAI strain A avian tumor virus. They will investigate in in vivo (chickens) and cell culture systems other avian viruses which include: sarcoma (without hematopoietic disease); erythroblastosis (and associated growths); myeloblastosis (and associated growths); and myelocytomatosis (and associated growths). Avian leukosis virus strains BAIA, R, ES4, and MC-29 will be employed in these studies. The Contractor will employ a multidisciplinary approach to the studies of avian tumor viruses including: biochemistry, tissue culture, virology, electron microscopy, pathology, and immunology.

Major Findings: The major work has been the production and distribution of BAI Strain A (myeloblastosis) virus and leukemia myeloblast cells. A total of 45,473 mg wet weight of plasma virus, and 149,255 mg wet weight of tissue culture virus has been supplied to other investigators: D. Baltimore, 1,040 pv and 2,131 tcv; B. Burmester, 3,071 tcv; F. Deinhardt, 256 pv; J. L. Eidinoff, 4,457 tcv; Robert Gallo, 5,827 pv, 1,368 tcv; D. Gardner, 1,156 tcv; M. Green, 7,172 tcv; S. E. Grossberg, 34 pv; J. Hurwitz, 1,605 pv, 5,423 tcv; K. Jenson, 1,056 tcv; Walter Keller, 512 pv; F. Portugal, 34 pv; Charles Rickard, 879 tcv; R. Rutman, 554 pv; P. Sarma, 2,030 tcv; A. Schrecker, 34 pv;

M. M. Sigel, 812 tcv; S. Spiegelman, 24,702 pv, 112,092 tcv; K. Stomberg, 1,034 pv; A. Tannenberg, 1,067 tcv; G. Todaro, 5,736 pv; Robert Wells, 4,991 tcv; R. Winzler, 1,556 tcv; Paul Zamecnik, 4,105 pv. Dr. S. Spieglman has also been sent 1,227 ml of washed, packed myeloblasts from leukemic chickens, and Dr. R. Rutman, 40 ml of the same. Dr. Spiegelman has received 4,800 ml of labeled myeloblast tissue culture fluid with virus, and 1 x 10^{11} myeloblast cells. Dr. Gallo has received 1,895 ml and 4.6 x 10^{10} cells.

Localization of RNA-dependent DNA polymerase activity in fractions of BAI A leukemia myeloblasts was demonstrated in a large particle ("mitochondrial") fraction, and was slight or absent in small particle ("microsomal") and soluble fractions. Studies with use of non-ionic detergent suggested that the enzyme was associated with a lipoprotein, probably membrane, structure. The nuclear fraction has not yet been assayed accurately because of the overwhelming DNA-dependent DNA polymerase activity. The quantitative aspects of various kinds of detergent disruption of RNA tumor viruses are being further investigated by changes in light scattering followed spectrophotometrically. The data will aid in the selection of procedures of virus disruption for study of virus structure, and will aid in the estimate of virus mass.

Heating of 60S RNA at different temperatures affected the physical properties, differing from findings by others. Much work will be required to establish the nature of the changes induced by heating, and to determine the importance of environmental influences. Contamination of BAI-A virus samples by cell-derived components may interfere with purification of the agent and evaluation of biochemical data. Efforts are being made to find the means for eliminating such components from both blood plasma and tissue culture virus preparations. Comparison of the respective sensitivities of the CF and agar gel precipitation tests, for detection of gs-antigen with RSV antiserum from the pigeon, showed that the CF reaction was 16 times as sensitive as the agar gel reaction. The gs-antigen analysis with pigeon serum against disrupted AMV antigen by immunoelectrophoresis also gave satisfactory results.

Significance to Biomedical Research and the Program of the Institute:
Avian tumor viruses induce lymphoproliferative and neoplastic diseases in domestic chickens. One of the major functions of the SALES Segment of the SVCP is to fully explore all important animal model systems for the determination of the possible viral etiology of cancer in man. Avian tumor viruses induce a variety of diseases similar to those which occur in man (erythroblastosis, myeloblastosis, myelocytomatosis, reticuloendotheliosis, and sarcomas); the causative viruses have been isolated and the disease can be induced in vivo under controlled conditions which permit the study of the immunology, virology, biochemistry and therapy of the tumor virus complex; the avian tumor viruses may be used as a prototype for a potential viral vaccine in the event that a virus causing cancer in man is isolated. A limited field trial of a Marek's disease vaccine is presently in progress. The recent reports showing that certain animal tumor viruses contain a unique enzyme, RNA-dependent DNA polymerase, has been largely based on the use of the BAI strain A avian tumor virus as a model system. Subsequent biochemical and enzymatic studies will depend largely upon purified,

concentrated virus, which the Contractor will supply.

Proposed Course: Since this report is based on only 2-1/2 months' work, the next year's work will be essentially a combination of activities described above.

Date Contract Initiated: April 19, 1971

FLOW LABORATORIES, INC. (NIH 71-2341) ROCKVILLE, MARYLAND

Title: Animal Holding Facility to Support Intramural Research on RNA Viruses and Autoimmune Diseases

Contractor's Project Director: Dr. William A. Knapp

Project Officer (NCI): Dr. John W. Pearson

Objectives: The objective of this contract is to support ongoing activities in the Virus and Disease Modification Section, VBB, NCI and the Viral Pathology Section, VLLB, NCI. The nature of the above activities require supportive services which this contract will provide. In general, the contractor will receive and maintain mice, rats, hamsters and other small animal species as required for the purpose of observation and experimentation during the aging process for the following research studies: (a) Development of autoimmune disease, (b) Relationship of autoimmune disease to oncogenic viruses, (c) Development of spontaneous cancers and their modification to chemo-immunotherapeutic agents, and (d) Immunologic responsiveness to immuno-enhancers and/or suppressors.

Major Findings: Since this contract was initiated only 15 days prior to the end of FY '71, a report of findings is not applicable at the present time.

Significance to Biomedical Research and the Program of the Institute: The major goals of SVCP program are: (1) to detect and isolate tumor viruses, (2) the prevention and control of virus-induced malignancies in man as well as in animals associated with him. Current in-house efforts are underway to investigate the relationship of the aging process and autoimmune disease states to spontaneous and virus-induced malignancies. In addition, ongoing investigation utilizing chemo-immunotherapeutic approaches are being studied in both rat and mouse leukemia and tumor model systems. It is expected that the information resulting from this research will have considerable application in studies on similar diseases in man.

Proposed Course: It is intended to engage in inoculation, palpation, blood smears, sera collection, eye bleedings, autopsies, and harvesting and processing of tumors in mice, rats, and hamsters, and to continue maintenance of these animal colonies for long-term studies on (1) autoimmune disease in New Zealand mice, (2) development of spontaneous cancer in AKR mice and their modification by chemo-immunotherapeutic agents, and (3) immunological responsiveness to immunoenhancers, i.e. BCG, Corynebacterium granulosum,

interferon stimulators, and/or immune cell transfer alone or in combination with drug therapy in various mouse and rat leukemia tumor systems.

Date Contract Initiated: June 15, 1971

Program Management Segment
Dr. John B. Moloney, ASDVO, ET, NCI, Chairman
Dr. Louis R. Sibal, OASDVO, ET, NCI, Executive Secretary

ATOMIC ENERGY COMMISSION (NCI-FS-13)

Title: Co-carcinogenesis Program

Contractors Project Directors: Dr. F. T. Kenney
Dr. G. D. Novelli
Dr. M. G. Hanna
Dr. E. S. Rogers
Dr. R. L. Tyndall
Dr. R. W. Tennant

Project Officers (NCI): Dr. James T. Duff
Dr. Timothy O'Connor

Objectives: In January 1963 an interagency agreement was established between the National Cancer Institute and the Oak Ridge National Laboratory for joint carcinogenesis studies. During the past 2 years the agreement has been funded by both the Carcinogenesis and Viral Oncology Program Areas. Only the Viral Carcinogenesis Program of the FS-13 agreement is reported here. The broad objectives are (1) to study the interaction of RNA tumor viruses with the host immune mechanism (Hanna), (2) to conduct investigations on the biochemical mechanisms involved in the initiation of cancer (Novelli), (3) provide fundamental understanding of the mechanisms by which gene expression is regulated in mammalian cells (Kenney), (4) to study cellular changes during carcinogenesis by the Shope papilloma virus, and the development of the use of passenger viruses to transmit specific genetic information (Rogers), (5) to investigate the commonality between embryogenesis and oncogenesis and its possible significance in the control of cancer (Tyndall), and (6) to study the functional and antigenic properties of cells infected or transformed by murine RNA tumor viruses, and the nature of the interactions between cells, viruses and carcinogens (Tennant).

Major Findings: Immunology of Viral Carcinogenesis. Early histologic and ultrastructural changes in lymphatic tissue were studied in adult BALB/c mice given Rauscher virus (RLV). It was found that (1) endogenous C-type virus is localized as antigen in antigen-retaining reticular cells of germinal centers, (2) it is possible that some forms of virus destruction occurs in these extracellular regions, (3) virus replicates in the parenchymal immunoblast compartment of the germinal centers, (4) amplification of this compartment by other antigens

enhances RLV splenomegaly, (5) maturation arrest in the immunocompetent cell compartment correlates with RLV-induced immune suppression, (6) viropexis of C-type virus between immunoblast and hematopoietic cells of the spleen was found. In these experiments it was also found that there may be a synergistic effect of passenger lactic dehydrogenase virus (LDV) with the RLV virus in causing the pathology associated with "Rauscher disease." LDV was found to replicate in the thymic dependent area of the lymphatic tissue. LDV completely destroyed the thymus parenchyma 48 after injection directly into the thymus.

In a collaborative study with J. H. Coggin (FS-7), it was found that humoral antibodies, generated as a result of immunization with isologous fetal tissue, depress the growth of RLV-infected spleen cells and plasma cell tumors in BALB/c mice. The data suggests the presence of an embryonic antigen which crossreacts with neoantigen of both the RNA virus-infected spleen cells and the plasma cell ascites tumor. These embryonic antigens can immunize adult animals, making them capable of suppressing RNA virus-induced tumor growth and pathogenesis. Adoptive transfer of fetal antigen-primed spleen cells can suppress RLV-induced splenomegaly.

RFM mice have a low spontaneous leukemia incidence but are quite susceptible to radiation induced leukemia. In these mice antigens of RNA tumor viruses can be detected in the thymus after 2 weeks of age and reach a peak at 7 weeks of age. Detection of the virus in the spleen occurs between 4 and 5 weeks of age followed by a marked drop at 10 weeks. The appearance of virus in the spleen correlates with the development of immune competence and capacity to trap antigen. These results are interpreted as an immune development to the virus and can be correlated with the development of glomerulosclerosis which occurs in these mice after 20 weeks of age; virus antigen and antibody can be detected in the kidney during this interval. When C_3H/Bif mice become immunologically mature they lose susceptibility to Gross virus-induced thymoma. This susceptibility is restored after low levels of irradiation. The incidence of thymomas in irradiated adult C_3H/Bif mice is markedly suppressed if the animals are injected with newborn thymus cells or with the Gross virus. These results clearly demonstrate that immune suppression is a factor in susceptibility of C_3H/Bif mice to Gross virus-induced thymoma.

Germfree mice, unlike their conventional counterparts, do not develop plasma cell tumors after mineral oil injection. An experiment is underway to determine if this inability to develop plasma cell tumors is a result of the absence of target cells in Peyer's patches.

Enzymology of Carcinogenesis. A study of changes in isoaccepting tRNAs and embryonic tissues was completed. A survey of tyrolsyl- and asparty1-tRNAs was carried out in 6 transplantable tumors, 5 spontaneously occurring primary tumors and 3 cultured tumor cell lines. All of these tumors showed 2 types of asparty1 and tyrosyl tRNAs: the normal differentiated cell type, and the abnormal "stem" cell type, in various proportions according to the individual tumor. The abundance of the abnormal "stem" cell type appeared to be proportional to the malignancy of the tumor. These abnormal tRNAs are not, however, unique to tumor tissue; they are also detected in embryonic tissues.

A study was initiated of the host factors acting to regulate expression of oncogenic viruses. In initial work the RNA dependent DNA polymerase was used as an indicator of virus expression. Template requirements, metal ion requirements, and other parameters of polymerase activity in extracts of purified RLV were carefully investigated to determine the optimal assay conditions. The results of these studies suggest that chain initiation is rate limiting for this polymerase. Normal and RLV infected mouse spleens were fractionated and the fractions tested for capacity to inhibit or activate added RLV polymerase. Several such components were found. A major inhibitory component of the normal spleen is not present in the RLV-infected spleen. These preliminary results suggest that there may, indeed, be cellular host factors acting to regulate virus expression via controlling the RNA-dependent synthesis of DNA.

Biochemistry of Carcinogenesis. Two cancers arising from the virus-induced papillomas of rabbits have been transplanted over a period of many years. The Vx-7 cancer still has virus and the virus induced arginase after 115 transplant generations. The Vx-2 cancer has lost both virus and arginase. Each has specific and unusual amino acid requirements which may lead to metabolic control of these cancers using specific antagonists and dialysis against specific enzymes. Upon reintroduction of the virus into Vx-2 amino acid requirements and compositions change to resemble closely the Vx-7.

Specifically hybridized Shope virus messenger RNA has been isolated from papillomas and separated into 5 fractions by equilibrium density centrifugation. The capacity of these RNAs to code for arginase or other virus proteins is being examined using an *in vitro* ribosomal system capable of appreciable and precise protein synthesis.

Cultured fibroblasts from children suffering from arginase deficiency disease have been inoculated with the Shope virus. Preliminary results indicate some induction of arginase in such cells.

Regulation of Gene Expression. Carcinogenic agents apparently act by freeing from restraint many genes that are normally kept repressed within differentiated tissue. Evidence for such derepression is found in the appearance of fetal and viral associated antigens in differentiated cells transformed by carcinogens. This activity concentrates on the molecular mechanisms operating to regulate expression of specific genes in mammalian cells. As the products of gene expression, the cellular enzymes, can be regulated in amount by degradative as well as synthetic processes; both of these are being studied. Hepatoma cells (H35) growing in culture provide the focus for much of this work. A new effort was initiated wherein techniques and concepts developed in previous research on hormonal regulation of gene expression are being applied to the mechanisms regulating expression of oncogenic virus components.

Work on the model regulatory system, tryosine transaminase induction in H35 cells, continued with emphasis on the mechanism by which steroid hormones effect the release of transcriptional repression to permit accelerated synthesis of specific mRNA. Specific steroid binding proteins were partially purified and results obtained which support the conclusion that binding to nuclear receptors is a necessary prerequisite to enzyme induction. Preliminary fractionations indicate that the receptor is located in chromatin. The chromatin from steroid-treated cells has increased capacity to serve as template for synthesis of RNA. Synchronized cells were found to be incapable of responding to the steroid inducer during both G2 and M phases of the cell cycle. It was found that transaminase synthesis and degradation are both changed by high concentrations of L-leucine. This effect is independent of hormone action; preliminary evidence for a leucyl-tRNA species which requires high concentrations of leucine for changing will be examined further.

The enzyme alanine transaminase, known to be inducible by steroid treatment *in vivo*, was found to respond to hydrocortisone addition to cultured H35 cells in a comparable fashion. Differences between the response of this enzyme and that of tryosine transaminase suggest that there may be sequential rather than coordinate effects of the steroid on transcription processes. Factors regulating the activity of ornithine decarboxylase, an enzyme which undergoes large increases in cells and tissues stimulated to rapid growth, were examined in rat liver. A number of hormones were tested for effects on activity levels of this enzyme; results indicates that none of the hormones tested is acting directly to induce ornithine decarboxylase.

Studies of regulation of virus expression were initiated with concentration on two immediate objectives: 1) development of an experimental system in which expression of oncogenic viruses can be observed and can be manipulated experimentally and 2) development of a sensitive, precise and specific assay for virus expression. Most of the initial effort was toward the latter goal where it was asked whether the viral type RNA-dependent polymerase might serve as an appropriate marker. In further work polymerase assays will be correlated to serologic assays for gs antigen activity. It is hoped that the sensitivity of the enzyme assay will be complemented by the specificity of immunological assay.

Viral Carcinogenesis. Analogies between fetal and malignant tissues prompted an investigation of the degree and specificity of Gallium localization during embryogenesis. Isotope uptake in embryos was determined directly and by autoradiography. About midway during gestation, ^{67}Ga uptake in embryonic tissues was equivelent to or surpassed that previously detected in leukemia tissues of adult mice. Uptake of ^{3}H-thymidine was not comparable to that of ^{67}Ga, demonstrating that gallium uptake is not a reflection of cell turnover. In vitro cytotoxicity of fetal antigen-primed spleen cells was demonstrated against cells infected with Moloney leukemia virus. The irradiated fetal tissues used for immunization in these studies were negative for leukemia virus antigens by several tests. The spleen cells from immunized mice were found to incorporate thymidine upon exposure to thymidine labeled target cells suggesting a true immune reaction. These results suggest that cells infected with MLV possess antigens similar to those of certain isologous embryonal cells.

Effects of fusion of permissive and nonpermissive cells on leukemia virus synthesis have been examined. This approach is based on the premise that susceptibility may be regulated intracellularly. Techniques have been developed for simultaneous autoradiography and fluorescence microscopy, and cell fusion. Preliminary results are suggestive that incorporation of a nucleus from a human cell reduces the capacity of the recipient cell for virus synthesis.

Proposed Course: Develop a concerted, interdisciplinary research program in the central aspects of both chemical and viral carcinogenesis.

Significance to Biomedical Research and the Program of the Institute: This effort focused on the phenomenon of carcinogenesis per se, considering this as a fundamental biological problem with common features whether the carcinogenic stimulus be a chemical, or radiation, or a virus. Special attention, however, is given to particular areas such as carcinogenesis and cocarcinogenesis in the respiratory tract and the role of both

C-type viruses and cellular control mechanisms in murine leukemias.

Date Contract Initiated: July 1, 1963 (Viral Oncology Funding: July 1, 1970)

OAK RIDGE NATIONAL LABORATORY MAN PROGRAM (AEC-NCI FS-7)

Title: The Joint AEC-NCI Molecular Anatomy Cancer Program

Contractor's Project Director: Dr. Norman G. Anderson

Project Co-Investigator: Dr. Josphs H. Coggin, Jr.

Project Officer: Dr. Charles W. Boone

Objectives: The NCI portion of this contract is divided into five problem areas--cancer biology and immunology including 1) cancer prevention, 2) cancer detection, 3) cancer cell typing, 4) cancer patient immune status monitoring, and 5) cancer immunotherapy.

Part I. Studies are oriented toward chemically defining cancer neoantigens and describing the mechanism for their activation in a variety of cancer model studies in rodents. Methods for interpreting viral and chemical carcinogenesis employing tumor and fetal antigens are being exploited. Tumor-specific antigens from model and autochthonous human tumors are being prepared and tecnniques developed for the rapid detection and quantitation of tumor related immune reactions.

Part II. The feasibility of typing human and rodent tumors from pathological material using antisera developed against antigenic extracts from tumors and fetal cells is being investigated.

Part III. A program to monitor the immune reactivity of cancer patients to their tumors employing techniques and information derived from model tumor studies continues in progress.

Part IV. The pattern and character of immune responsiveness to virally-induced and transplanted tumors is being investigated intensively. The relationship of antibody responses and the activation of cellular immunity have and will continue to be examined in an effort to understand the reason for tumor progression and to develop mechanisms for promoting tumor rejection by immunological stimulation or modification.

Major Findings: In hamster, mouse and human embryo tissues during early and mid gestation, there exist antigenic components capable of inducing antibody responses and transplantation rejection potential against two-virus stimulated autochthonous tumors and against several other types of tumors. Male and female animals receiving irradiated fetal vaccines show strong antibody responses to fetal tissues (syngeneic and xenogeneic) but only male animals develop cell-mediated immunity and tumor resistance. Antigen isolation studies (preliminary) indicate that tumors and fetus posses identical antigenic fractions. Irradiation of the fetus is essential to permit antigenic expression adult tissues.

Significance to Biomedical Research and the Program of the Institute: The programs listed constitute a direct approach to the human cancer problem including prevention, detection, typing, therapy monitoring, and immunotherapy.

Proposed Course of Action: The contractor will: 1) check spectrum of tumor antigen cross-reactive with fetal antigens in other model systems and in human tumors, 2) develop methods for immunizing against autochthonous cancer (chemically induced and spontaneous) employing fetal antigens, 3) isolate fetal and tumor antigens and prepare useful antibody preparations for detecting and quantification of these antigens, 4) establish a tumor and patient serum bank for use in evaluating new tumor and fetal antigen assays, 5) develop a battery of tests for routine early cancer detection, and 6) organize and publish the Proceedings of the Second Conference and Workshop on Embryonic and Fetal Antigens in Cancer on February 13-19, 1972.

Date Contract Initiated: July 1, 1962

BAYLOR COLLEGE OF MEDICINE (NIH 71-733)

Title: International Conference on Virology, Budapest, Hungary

Contractor's Project Director: Dr. Joseph Melnick

Project Officer: (NIAID) Dr. Earl Chamberlayne

Objectives: To make possible the attendance of U. S. scientists at an international conference on viral oncology.

Date Contract Initiated: March 1, 1971

BIONETICS RESEARCH LABS., INC. (NIH 69-2160)

Title: Support Services for SVCP

Contractor's Project Director: Dr. Robert Ting

Project Officers (NCI): Dr. George Todaro
Dr. Paul Levine
Dr. Robert Bassin

Objectives: To provide a laboratory that will collect, process and test cancer specimens from human and animal sources suspected of containing virus associated antigens.

Major Findings: EBV studies were carried out under the supervision of Dr. Paul Levine. One study initiated and completed during this year was a seroepidemiological study comparing EBV titers in American patients with Burkitt's lymphoma and age-and sex-matched patients with acute lymphocytic leukemia, African Burkitt lymphoma, and non-malignant diseases. The African Burkitt sera were significantly higher than the American Burkitt sera ($P<0.005$). The role of EBV in human lymphoma was evaluated by immunological techniques detecting humoral and cellular immunity to the virus. The importance of careful clinical evaluation was emphasized by a study of twenty American patients with Burkitt's lymphoma and age and sex matched controls. Treatment and prognosis correlated with EBV titers in both lymphoma and leukemia patients, indicating that seroepidemiological studies which include single samples on a patient may be misleading. The studies clearly demonstrated that American patients with Burkitt's lymphoma, although their histopathology is indistinguishable from African patients, have different immune patterns to EBV.

Five individuals with low titers to EBV who were identified on an earlier study of Hodgkins disease were followed over a three year period. Half the patients developed high titers while the other half maintained low titers.

A study of leukemia in identical twins was initiated to determine whether an antigen could be detected in the cells of a leukemia twin which would not be identified in his normal HLA identical twin. Leukemia-associated antigens were detected in four of the seven families studied to date using the lymphocyte cytotoxicity test. In the animal system, this test is positive only when the lymphocytes are presensitized by an antigen, so that the reactivity of the family members against

the leukemic patient's cells but not against the normal twin's cells suggest that an environmental agent, perhaps a virus, is present.

Sera from 43/102 (42%) of breast cancer patients had antibodies to BeLev antigens. Sera from 29% of patient's with sarcomas had detectable antibodies, whereas, 13% of patients with benign breast diseases and 3.6% of normal blood bank donors reacted.

Significance to Biomedical Research and the Program of the Institute: Provides opportunity for systematic, large-scale effort to detect viruses or viral antigens in human or animal materials using tissue culture, immunological, biochemical and EM techniques. This is a major objective of the SVCP.

Proposed Course: Although this contract will continue to supply necessary supportive services to SVCP, the workscope has recently been divided into three major areas, each being co-directed by a senior investigator at Bionetics and an NCI project officer. Drs. Rein and Todaro will attempt to isolate, characterize, and purify the factor(s) in serum which overcome contract inhibition and regulate the growth of normal and transformed 3T3 cells in culture. Drs. Pienta and Bassin will attempt to rescue and isolate a viral genome in undifferentiated sarcomas from untreated patients by co-cultivation, hybridization, and other techniques. Drs. Levine and Ting will continue studies to detect tumor specific antigens in patients with leukemia, lymphoma and breast cancer. In the leukemia studies, special emphasis will be placed on testing patients who have an identical twin; in the lymphoma studies, the serums of patients in selected disease groups will be tested for antibodies to EBV.

Date Contract Initiated: June 27, 1969

MASSACHUSETTS INSTITUTE OF TECHNOLOGY (NIH 71-2149)

Title: Studies on RNA-Dependent DNA Polymerase

Contractor's Project Director: Dr. David Baltimore

Project Officer (NCI): Dr. George Todaro

Objectives: To characterize DNA polymerase and its product, to study its mechanism of reaction and formation of viral RNA during infection.

Major Findings: None reported yet, this is a new contract.

Significance to Biomedical Research and its Program of the Institute: The objectives above have highest priority in the SVCP. The results may provide very sensitive techniques for finding cancer virus genetic information in human tumors.

Proposed Course: Continuation with addition of EM capability.

Date Contract Initiated: May 1, 1971

MELOY LABORATORIES, INC. (NIH 70-2047)

Title: Cell Biology Facility

Contractor's Project Director: Dr. John E. Verna

Project Officers (NCI): Dr. George J. Todaro
Dr. Charles W. Boone

Objectives: To provide research facilities for the Cell Biology Section, Viral Biology Branch, NCI (Part A), and the Viral Leukemia and Lymphoma Branch, NCI (Part B), in support of the following programs:

Part A: (1) Influenza virus mediated enhancement of tumor immunity, (2) testing the carcinoembryonic antigen hypothesis, (3) use of the paired label technique to characterize tumor surface antigens of AKR virus-induced rat lymphoma cells, and (4) isolation of plasma membranes of EB virus-infected cells.

Part B: (1) An examination of viruses as etiologic agents of human cancer, (2) genetic studies of SV40 virus and SV40 DNA, (3) elucidation of the difference between normal and "transformation prone" human cells, (4) development and characterization of BALB/3T3 and BALB/3T12 lines, (5) transformation of mouse and human cells by RNA tumor viruses, (6) extend studies of RNA dependent DNA polymerase, and (7) examine serological relationship between RNA dependent DNA polymerase associated with leukemia viruses of a variety of species as well as host cell enzymes, (8) characterization of host cell and viral RNA dependent DNA polymerases.

Major Findings: Part A: (1) Cell-free tumor homogenates ordinarily not capable of inducing immunity in BALB/c mice induced immunity if the cultured tumor cells were first infected with influenza virus. This phenomenon of influenza virus-mediated enhancement of tumor immunity was also produced when formalin inactivated tumor homogenates infected with influenza virus were employed.

(2) In the mouse system, inoculation of large groups of BALB mice with early embryonic tissue did not induce tumor immunity, a finding at variance with the generality of the carcinoembryonic antigen hypothesis.

(3) The density and antibody binding affinity of cell surface G antigen sites on rat AKR lymphoma cells was determined using the paired label antibody assay plus certain titration techniques.

(4) The main immunofluorescence antigen (Klein in Burkitt lymphoma cells containing EB virus may be a part of the virus and not an integral part of the plasma membrane (i.e., a host-coded neoantigen). Work continues to confirm this point.

Part B: (1) The ability to infect and transform human cells with SV40 DNA is now well established. Different forms of DNA are being tested to define the smallest amount of viral genetic information required for biologic activity. The potential applications of integrating genetic information by means of introducing DNA into cells are presently being investigated.

(2) Following the initial discovery of an RNA dependent DNA polymerase in Rauscher sarcoma and Rauscher leukemia viruses by Temin and Baltimore, similar activity was detected in a large number of other sarcoma and leukemia viruses as well as in MTV. Methods have been developed which greatly increase the sensitivity of detection of the viral RNA dependent DNA polymerase using synthetic templates with manganese.

(3) Sera from animals with murine-virus induced tumors were found to contain an antibody to the murine viral polymerase. Antibody made in rabbits against the purified viral enzyme were shown to also inhibit the viral polymerase of several mammalian C-type RNA viruses. However, they do not cross-react with the enzymes of the avian tumor viruses nor of MTV.

(4) MSV and MuLV releasing cell lines are being studied as model systems to learn more about human tumors. How the viral information is carried in the MSV-transformed mouse cell is being studied in detail biochemically. Temperature-sensitive mutants are being characterized biologically and biochemically.

Significance to Biomedical Research and the Program of the Institute: Part A: The phenomenon of influenza virus-mediated enhancement of tumor immunity has the potential of use in the immunotherapy of human tumors. Primary tumor tissue which is removed from the patient, infected with influenza virus, homogenized, inactivated with regard to infectivity and then inoculated back into the patient could conceivably enhance the patient's immune resistance to residual tumor and to the development of metastases.

Part B: These studies offer the possibility of determining the sequence of events that occur when a normal cell is converted into a neoplastic cell, suggest a possible simple test for "cancer proneness" in man which would permit recognition of a "high risk" group for cancer in the human population and provide further information at the cellular and molecular level on the "Oncogene Theory" of cancer.

RNA dependent DNA polymerase activity was demonstrated in normal and transformed cells. Biochemical characterization of the enzymes revealed differences between normal cellular enzymes and virus-specific enzymes. Using these techniques, it should be possible to search for enzyme activity in human cells; normal and malignant as a possible screening for human cancer.

Proposed Course of Action:

Contract activities will continue essentially along the lines described above.

Date Contract Initiated: September 30, 1965

NATIONAL CENTER FOR HEALTH STATISTICS (D.C.) (FS-35)

Title: Childhood Death Certificates

Project Officer (NCI): Dr. Robert Miller

Objectives: To make available death certificates for epidemiologic studies on cancers of interest to the Special Virus Cancer Program.

Date Contract Initiated: July 1, 1970

UNIVERSITY OF NEBRASKA MEDICAL SCHOOL (71-2076)

Title: Studies on Temperature Sensitive Mutants of Oncogenic Viruses

Contractor's Project Director: Dr. Giampiero di Mayorca

Objectives: The broad aim of this proposal is to identify viral gene functions which are necessary for malignant transformation.

Major Findings: This is a new contract.

Significance to Biomedical Research and the Program of the Institute: The goals of this contract are to devise models for the investigation of possible viral etiology of human tumors and to gain insight into the mechanisms of carcinogenesis at the molecular level. The achievement for control of human tumor viruses is a major goal of the SVCP.

Date Contract Initiated: March 12, 1971

HET NEDERLANDS KANKERINSTITUT (71-678)

Title: RNA Viruses and Host Genome in Oncogenesis

Contractor's Project Director: Dr. L. M. Boot

Project Officer (NCI): Dr. Louis R. Sibal

Objectives: To make possible the attendance of U. S. scientists at an international conference on viral oncology.

RUTGERS, THE STATE UNIVERSITY (PH 43-68-1025)

Title: Cross Protection Studies Among Murine Leukemia Viruses

Contractor's Project Director: Dr. Nicholas C. Palczuk

Project Officer (NCI): Dr. Louis R. Sibal

Objectives: (1) To immunize BALB/c mice with killed murine leukemia virus vaccine prepared from available representative strains and to determine whether mice are protected from leukemia following challenge by virus from the strain used for immunization as well as each of the other strains. (2) To determine whether a susceptible strain of mice (C BL/K) can be protected from radiation induced leukemia by the administration of a killed virus vaccine before x-irradiation. (3) To determine whether a strain of high leukemic mice (AKR) can be protected from spontaneous leukemia by the administration of a killed vaccine prepared from virus isolated from this or other strains.

Major Findings: The data have indicated that: (a) mice immunized with Rauscher leukemia virus are protected only against Rauscher and Friend leukemia virus challenge; (b) mice immunized with Friend virus are protected only against Friend and Rauscher virus challenge; (c) mice immunized with Moloney virus are partially protected against Friend and Rauscher virus challenge; (d) mice immunized with Gross leukemia virus were not protected against Rauscher, Friend, Moloney, Buffet, Breyere-Moloney, or Gross virus challenge; (e) mice immunized with Breyere-Moloney virus are currently partially protected against Breyere-Moloney virus challenge; (f) mice immunized with Buffet leukemia virus currently are partially protected against Friend and Buffet virus challenge.

Immunization of female AKR mice with a killed Gross virus vaccine succeeded in delaying the leukemic time-to-death.

Significance to Biomedical Research and the Program of the Institute: The major goal of the SVCP is to control leukemia in man either by the use of vaccines or other methods. Several techniques used to approach this goal have resulted from laboratory experimentation with murine leukemia viruses. The development of an effective vaccine against murine leukemia would thus serve as a model for a human vaccine at a time when this becomes feasible.

Proposed Course: The work on this contract has been completed.

Date Contract Initiated: June 24, 1968

Current Contract Level: $22,676

WORLD COMMITTEE FOR COMPARATIVE LEUKEMIA RESEARCH (71-1033)

Title: Vth International Leukemia Symposium at Padua, Italy

Contractor's Project Officer: Dr. R. Dutcher

Project Officer (NCI): Mr. Thomas Lewin

Objectives: To make possible the attendance of U. S. scientists at an international conference on viral oncology.

Date Contract Initiated: June 10, 1971

Objectives: To obtain tissues and serum specimens from pediatric oncology patients and suitable controls for collaborative studies with the SVCP.

Major Findings: Tissue, sera, and appropriate clinical information were provided to investigators at NCI; Pfizer & Company, Inc.; Bionetics Research Laboratories, Maryland; and Meloy Laboratories, Virginia. Leukemic cells and pediatric solid tumors were useful for tissue culture, immunological work, and polymerase studies. Follow-up samples from patients and family members provided suitable material for a variety of investigators. Since this contract has been operating primarily as a resource, the major findings are being reported under other annual reports.

Specimens from 33 patients were provided in the last trimester to investigators within the Special Virus Cancer Program, including Dr. Robert Gallo, Dr. William Feller, Dr. George Todaro and contract investigators at Meloy Laboratories and Bionetics Research Laboratories. Full clinical forms were sent with each speciman. The quality of materials has been excellent and the variety of diagnoses included in the patient material have been neuroblastoma, osteogenic sarcoma, acute lymphocytic leukemia, Hodgkin's disease, Wilm's tumor, astrocytoma, rhabdomyosarcoma (three cases), glioblastoma and Ewing's sarcoma. The contractor has added a second clinical fellow during this contract period, which is partly responsible for the increase in the number and quality of the specimens provided to the SVCP investigators.

Significance to Biomedical Research and the Program of the Institute: This is a major pediatric research contract in the United States, which provides specimens to NCI and East Coast laboratories. Specimens obtained from this contract are being investigated by a number of NCI and SVCP laboratory workers.

Proposed Course: Continue to collect serum and tumor specimens as in the past. Immunological investigations, using fresh lymphocytes and tissues, will be initiated by the contractor. Additional personnel are being made available to collect specimens from adults with cancer, as well as children. In addition, individuals with high risk to neoplasms will be investigated for host factors which increase the possibility of developing cancer. Materials will be sent to NIH for virological and immunological studies.

Date Contract Initiated: June 18, 1969

UNIVERSITY OF CONNECTICUT (NIH 69-52)

Title: Development and Maintenance of a Specific Pathogen Free Flock of White Leghorn Chickens

Contractor's Project Director: Dr. Roy E. Luginbuhl

Project Officers (NCI): Drs. Robert Holdenried and Roy Kinard

Objectives: Establish and maintain a flock of chickens free of specified pathogens, including avian leukosis viruses, and to provide eggs for research use.

Major Findings: Approximately 14,000 eggs or cell cultures were provided to cancer research. A few of the recipients were Drs. J. Beard, M. Green, H. Morgan, F. Deinhardt, P. Sarma, and G. S. Beaudreau.

There is no serologic evidence of the following organisms or diseases in the SPF flocks: Mycoplasma gallisepticum and synoviae, Salmonella pullorum, Newcastle disease, avian infectious laryngotracheitis, avian encephalomyelitis, CELO virus, and the three serotypes of RSV. There have been no clinical cases of Marek's disease (MD); however, MD-associated herpesvirus (MDHV) antibodies have been detected using the agar gel precipitin test. Clinical MD has not been detected. Thirty-six birds of one flock have been identified as gs antigen negative and nine as gs antigen positive, and separate flocks have been reproduced from these identified birds. In third generation of selected birds, 90% appear to be gs negative.

Significance to Biomedical Research and the Program of the Institute: The methods being developed indicate that eggs free of specified infectious organisms can be produced. A significant portion of the avian leukosis research in the United States is dependent on the continued availability of this highly controlled and monitored flock.

Proposed Course: Continued maintenance of the flock, with development of genetic lines of chickens characterized for the susceptibility of their embryos to leukosis virus. Flocks free of MD virus and gs liver antigen are being established.

Date Contract Initiated: June 18, 1962

ELECTRO-NUCLEONICS LABORATORIES (NIH 71-2253)

Title: Development of Propagation Procedures, Purification and Characterization of Viruses

Contractor's Project Director: Mr. Irving Toplin

Project Officers (NCI): Drs. George Todaro, Stuart Aaronson and Robert Bassin

Objectives: To develop propagation procedures to produce high virus yields of cell cultures, purify, determine particle count per ml and otherwise characterize the produced virus.

Major Findings: Several cell lines are being propagated and viruses harvested.

Significance to Biomedical Research and the Program of the Institute: The search for evidence of viral etiology of human cancer includes studies of viruses present in animals including human cell cultures. Large amounts of these well characterized and purified viruses are used for preparation of specific antisera and for biochemical, immunological, and epidemiological investigations.

Proposed Course: To continue the propagation of cell lines and harvest virus as directed by the Project Officer.

Date Contract Initiated: May 26, 1971

EMORY UNIVERSITY (NIH 71-2256)

Title: Maintenance of Irradiated Monkey Colony

Contractor's Project Director: Dr. Harold McClure

Project Officer (NCI): Dr. Roy Kinard

Objectives: To determine the incidence of tumors in a unique group of irradiated aging rhesus monkeys and to supply tissue from tumors for transplantation, tissue culture and virus isolation to SVCP collaborators.

Major Findings: A group of 68 rhesus monkeys with adults ranging in age from 12 to 18 years remain from an earlier study on the effects of irradiation. Forty-six animals received irradiation in 1956-1958, and 12 are non-irradiated controls. The remainder are non-irradiated offspring born in the last four years.

Significance to Biomedical Research and the Program of the Institute: The SVCP conducts collaborative projects for the study of relationship between etiology of tumors of primates, including of course, humans. This project will provide useful information and tumor tissues to interested SVCP projects. If viruses can be isolated it may mean that these or similar viruses are responsible for human cancer.

Proposed Course: To continue maintaining and monitoring the colony for tumors.

Date Contract Initiated: May 1, 1971

FLOW LABORATORIES, INC. (PH43-65-1012)

Title: Maintenance of a Repository for Storage and Distribution of Reagents and Tissue Specimens

Contractor's Project Director: Mr. Jack W. Walker

Project Officer (NCI): Miss Marie E. Purdy

Objectives: To provide for the SVCP a centrally located low temperature storage and distribution center for viral reagents and tissues.

Major Findings: In 1970 there was a decrease from 1969 in outgoing shipments from 349 to 312; receipts dropped from 49 to 31. The number of items dropped from 16,458 to 10,220 outgoing and from 38,370 to 26,200 incoming. The heat problem generated by the large number of mechanical freezers has been corrected by installation of air conditioning.

Significance to Biomedical Research and the Program of the Institute: An efficient research program must have readily accessible adequately characterized resource materials. The storage and shipping facilities operated under this contract enable the scientist to have access to a large inventory of special research materials without the burden of procurement, storage, inventory, and distribution.

Proposed Course: We anticipate that the repository activities will remain at about this year's level.

Date Contract Initiated: June 22, 1965

HOSPITAL FOR SICK CHILDREN (PH43-65-97)

Title: Human Leukemic and Normal Tissue Collection and Preservation

Contractor's Project Director: Dr. Peter McClure

Project Officer (NCI): Dr. Paul H. Levine

Objectives: To obtain serum and plasma specimens for a wide variety of research purposes from pediatric leukemics, relatives of such patients, and non-leukemic controls.

Major Findings: In the past contract year, serum collection was broadened to pediatric patients with other solid tumors, in response to demands from SVCP investigators. The major contribution of the contract, however, was the identification of three leukemia patients who had clinically normal identical twins. The families were investigated at the Hospital for Sick Children, where establishment of identity was confirmed, using fingerprinting, teeth moldings, and mixed leukocyte cultures. The families were also admitted to NCI for thorough virological and immunological studies. Leukemia-associated antigens, possibly virus-related, were identified in two of the three leukemic twins. The one set of twins studied during a two-year remission showed no evidence of leukemia-associated antigens. The contractor has also identified three patients with concurrent infectious mononucleosis and acute leukemia, which has provided additional evidence immunologically for a distinct etiology for the two diseases.

Significance to Biomedical Research and the Program of the Institute: As the largest pediatric hospital in North America, this contractor can respond rapidly to a variety of SVCP needs. Although the shipment of certain specimens is made difficult by problems with customs, by referring patients to NCI for study and by collecting serum samples in which 24 to 48 hour delays are not critical, the contract provides a valuable service to SVCP.

Proposed Course: To continue the virological and immunological studies on the twin families already identified. The contractor will continue to look for other patients of interest, and will continue to collect serial specimens from pediatric cancer patients and family members.

Date Contract Initiated: February 3, 1965

HUNTINGDON RESEARCH CENTER (NIH 69-54)

Title: Development of Oncogenic Virus Diagnostic Reagents and Services

Contractor's Project Director: Dr. Roger E. Wilsnack

Project Officers: Drs. Robert Holdenried and Daniel J. Rubin (NCI), and Wallace P. Rowe (NIAID)

Objectives: To develop, produce, and characterize special diagnostic reagents for use in the SVCP, primarily antisera and antisera conjugates to viruses, gs antigens, globulins of various animal species, and to T-antigens of polyoma and SV40 in tumored hamsters.,

Major Findings: The anti-MSV(M) sera from tumored rats produced by this contractor remains, as in the former year, the most widely issued reagent. The anti-AKRV sera from tumored rats has shown inhibitory activity against polymerase. The anti-MSV(M) sera are reactive against interspec antigen and murine leukemias. These reagents are experiencing expanded application in cancer research.

Three host species--goat, rabbit and guinea pig--were immunized with Rauscher virus (human cell origin) intact, Tween-ether disrupted (ether and aqueous phases), cat leukemia virus intact and ether and aqueous phases of Tween-ether disrupted virus, Moloney leukemia virus--intact virus, enzyme treated, sodium dodecyle sulfate (SDS) disrupted, and Tween-ether (aqueous phase) disrupted virus. The antisera was characterized and evaluated in tests against groups of antigens to selected sera with antibody to group and type specific leukemic antigens and contaminating host cell and media antigens. Tests included immunodiffusion, counterelectrophoresis, complement fixation, direct and indirect immunofluorescence and neutralization. Test results have been tabulated for inclusion with shipments of antisera to research laboratories.

In general the following patterns were observed:

1. None of the sera are as satisfactory for CF tests as tumor bearing rat serum.

2. Goat antisera are the most satisfactory for counter-electrophoresis, immunodiffusion and immunofluorescence tests.

3. Guinea pigs are most sensitive immunologically to the calf serum contaminating antigen.

4. Rabbit and guinea pig sera react poorly to tumor extract antigens in counterelectrophoresis and immunodiffusion tests.

5. Selected sera from any of the test species neutralize virus but goat sera are frequently toxic to mouse and feline embryo cell cultures.

Antisera produced and shipped:

Tumored Fischer rat anti MSV(M) serum: 547 ml to 38 investigators.

Tumored Fischer rat anti AKRV serum: 283 ml to 9 investigators.

Antisera to cat leukemia virus: 159 ml to 13 investigators.

Antisera to human Rauscher virus: 128 ml to 15 investigators.

A variety of anti species globulin and special reagents to a large number of investigators.

Rhodamine contrast stain: 115 ml to 18 investigators.

Significance to Biomedical Research and the Program of the Institute: The reagents and test systems developed provide tools used in cancer research. The project functions in close collaboration with SVCP research projects and the Laboratory of Viral Biology, NIAID.

Proposed Course: Continue development, production, and characterization of serological test systems.

Date Contract Initiated: June 2, 1963

JOHNS HOPKINS UNIVERSITY (NIH 69-2008)

Title: Maintenance of a Flock of RIF-Free Chickens

Contractor's Project Director: Dr. Frederik B. Bang

Project Officer (NCI): Dr. W. Ray Bryan

Objectives: To maintain the small "closed" flock of leukosis-free White Leghorn chickens to supply fertile eggs for use in avian tumor virus studies.

Major Findings: The eleventh generation of the original flock is now in egg production. Chicks for the twelfth generation are hatched. About five dozen eggs per week are shipped to Life Sciences, Inc., St. Petersburg, Florida, for use in Marek's disease studies on Contract PH43-69-63. Additional eggs are used in cell culture and avian tumor virus studies at Johns Hopkins University.

Recent expansion of the contract covers the study and maintenance of a continuous chicken embryo cell culture. Continuous chicken cell lines are very rare and would be useful in avian tumor studies. The chicken embryo culture in its second to third year of continuous culture seems to carry Mycoplasma as a contaminate. The addition of tylosin to the culture medium prevents cell degeneration presumably by inhibition of Mycoplasma multiplication.

Significance to Biomedical Research and the Program of the Institute: This flock supplied the birds used by Dr. Bang in his important studies on avian tumor viruses. The genetic heritage must be maintained to permit the continued definitive research with this highly characterized flock.

Proposed Course: Indefinite

Date Contract Initiated: March 24, 1969

LIFE SCIENCES, INC. (PH43-68-711)

Title: Production of Germfree and Reagent Grade Specific-Pathogen-Free Animals

Contractor's Project Director: Dr. Wendall M. Farrow

Project Officers (NCI): Mr. John P. Kvedar and Dr. W. Ray Bryan

Objectives: To produce animals for research, both germfree and specific-pathogen-free (SPF) derived therefrom. The latter are maintained under environmentally controlled conditions which preclude intercurrent infection by pathogenic microorganisms or infestation by parasites and are referred to as "reagent grade" hosts (RG-SPF).

Major Findings:

(1) An inbred germfree colony of BALB/c mice is maintained as a foundation colony to furnish breeders for replacement of retired breeders of the SPF production colonies.

(2) RG-SPF colonies of BALB/c mice, derived from germfree foundation colonies, have been established in separate environmental control cubicles as follows:

a. Inbred RG-SPF colony for producing pedigreed mice for research.

b. Random bred RG-SPF (non-pedigreed but derived from BALB/c foundation stock) produce mice for general purpose use such as assays, virus passages, production of reagent grade virus for research, etc. in SVCP.

Both colonies have been tested and certified free of all common murine viruses tested for, except leukemia virus which is transmitted vertically and is inherent in all known mouse stocks.

(3) A SPF colony of NIH mice (Swiss) is maintained as insurance for the preservation of this important stock. Limited numbers of animals are supplied to intramural scientists of NCI.

(4) A RG-SPF laying flock of Japanese quail (Coturnix coturnix) has been established from initially germfree stock and is now producing eggs for research and hatching at a rate of about 700 per week. This flock provides eggs for germfree hatches used in limited numbers by SVCP programs.

(5) A foundation colony of leukosis-virus free White Leghorn chickens (LSI-C) derived from the University of Connecticut (Luginbuhl) stock now in the third generation since establishment from germfree chicks. It is maintained by pedigreed (random bred) pairs of males and females behind a tertiary barrier for environmental control. All birds in the foundation colony are monitored periodically both for leukosis virus and extraneous viruses, and embryos from this pedigreed source are continuously monitored for freedom from leukosis virus expression. Only fertile eggs from certified virus free pairs are used for maintenance of the expansion and production colonies which supply eggs and chicks for research.

(6) Secondary-barriers protect the expansion and production of chicken flocks. All available fertile eggs and chicks are used for the Marek's disease research under another contract (PH43-69-63). The production rate is at about 350 eggs (or chicks) per week.

(7) A viral testing laboratory monitors the foundation and production colonies of chickens.

Significance to Biomedical Research and the Program of the Institute: The use of the well genetically and microbiologically defined laboratory animals permits experiments with an increased

number of parameters under the control of the investigator. Virus can be produced in animals and derived cell lines free of contaminating adventitious viruses. The Marek's disease research can be performed in chickens with a known and controlled viral flora.

Proposed Course: This service type contract for the production of germfree and reagent grade SPF animals will be continued, with the flexibility of being reoriented as rapidly as possible to meet changing needs of SVCP activities as they occur.

Date Contract Initiated: February 8, 1968

UNIVERSITY OF LOUISVILLE (PH43-66-902)

Title: Preparation of Simian Foamy Virus Reagents and Antisera

Contractor's Project Director: Dr. Paul B. Johnston

Project Officer (NCI): Dr. Robert Holdenried

Objectives: To prepare and test reference reagents (virus and corresponding antisera) for the simian foamy viruses, types 1-7, and foamy virus from other laboratory species.

Major Findings: The seven types of simian foamy viruses have been prepared and packaged. These are being tested for homogeneity, potency, and purity by Dr. Johnston. He also identifies, and if needed will prepare reagents for foamy virus from species of cancer research animals other than primates. None of the seven simian foamy virus antisera neutralized the cat syncytium-forming virus.

Significance to Biomedical Research and the Program of the Institute: The simian foamy virus reagents will be used in the identification of viruses and viral antibodies in primates used for cancer research. The indigenous viruses of laboratory primates pose husbandry problems, in addition to contaminating test systems and complicating the attempts to recover oncogenic virus from tissues and tissue extracts. The specific antisera may also be useful in suppressing the growth of these adventitious viruses in primate tissue cultures. The identity of cat syncytial virus isolated is being determined.

Proposed Course: The packaged foamy virus reagents now in low temperature storage will be checked for titer stability at selected time intervals. This laboratory will also assist in the detection of foamy virus contaminates on a referral basis.

Date Contract Initiated: June 13, 1966

MAKERERE UNIVERSITY (PH43-67-47)

Title: Epidemiologic Study of Burkitt's Lymphoma

Contractor's Project Director: Dr. George Kafuko

Project Officer (NCI): Dr. Robert H. Depue, Jr.

Objectives: To conduct studies on the natural history, occurrence, and transmission of Burkitt's lymphoma (BL), with special reference to the etiologic role of Epstein-Barr virus (EBV).

Major Findings: A pilot study for a large scale prospective sero-epidemiologic study of EBV and BL in the West Nile region of Uganda has been completed. This pilot study demonstrated that the immunofluorescent titers to EBV are stable in children, and the cooperation of the population is sufficient to embark on the large scale studies proposed by the International Agency for Research on Cancer.

Significance to Biomedical Research and the Program of the Institute: The SVCP conducts many laboratory studies of EBV. The large scale sero-epidemiologic project is designed to help determine whether this virus is etiologic for BL and whether continued laboratory studies are indicated.

Proposed Course: This contract will cooperate in the proposed large study to determine the possible effect of co-factors with EBV necessary to development of BL.

Date Contract Initiated: September 26, 1966

Current Annual Level: $12,000

MARQUETTE UNIVERSITY (PH43-68-1010)

Title: Effect of Human Pregnancy Hormones on Breast Cancer Cells In Vitro

Contractor's Project Director: Dr. Ronald Pattillo

Project Officer (NCI): Dr. Robert H. Depue, Jr.

Objectives: To test the effect of co-cultivation of hormone-secreting human choriocarcinoma cell lines with breast cancer lines in an attempt to increase the yield of a virus detected in human breast cancer.

Major Findings: The contractor has established in vitro cell lines from human choriocarcinoma cases. These lines secrete in vitro human chorionic gonadotropin, lactogenic hormone, estrogen, and progesterone. One of these lines (BeWo) is being co-cultivated with the Levine 3 human breast cancer line (isolated by another contractor). No increase in the virus yield of Levine 3 has been detected by electron microscopy as yet; however, the morphology and culture characteristics of the line do change. Not all combinations of hormone yield have been tested yet. A test more sensitive to virus production than electron microscopy will be instituted as soon as feasible since EM is a slow and inefficient way to monitor these experiments. BeWo will be used to help isolate new breast cancer cell lines in an attempt to find one that yields more virus.

Significance to Biomedical Research and the Program of the Institute: The virus present in Levine 3 is the prime candidate for a human breast cancer virus, but it has not been isolated in sufficient quantity to use as an antigen to determine its significance and distribution. Therefore, methods to increase the yields are vital to further progress in this area.

Proposed Course: To continue co-cultivation studies, and to attempt establishment of new breast cancer lines.

Date Contract Initiated: September 19, 1963

UNIVERSITY OF MICHIGAN (PH43-65-639)

Title: Collection of Leukemia-Lymphoma Specimens

Contractor's Project Director: Dr. Chris J. D. Zarafonetis

Project Officer (NCI): Dr. Paul H. Levine

Objectives: To collect and distribute specimens and information from patients with leukemia or lymphoma.

Major Findings: Collection of specimens from this contractor has provided valuable support for SVCP virology studies. Six units of leukocytes from two patients with acute leukemia provided valuable information and source material for polymerase studies carried out by Dr. Stuart Aaronson and Dr. Robert Gallo (NCI), and Dr. S. Spiegelman (Columbia). Buffy coat and

white cell rich plasma from six other patients with high white counts have also been provided for polymerase studies. A total of 3,704 serum samples have been collected. Sera, skin biopsies, lymph nodes, and other tissues were distributed to a number of SVCP investigators.

A major effort was placed on the computerization of the serum bank under the auspices of Dr. Deward Waggoner. A total of 1,335 sera have been added in the past five months and when this bank is computerized, it will become a far more valuable resource. Serum is still being used for antibody studies and 439 sera have been utilized for RNA virus studies. The contractor is also screening patients for EBV in order to build up a bank of EBV negative and high titered EBV positive serum for the SVCP serum bank.

Significance to Biomedical Research and the Program of the Institute: Availability of clinical specimens and pertinent information on the cases is paramount in the achievement of a major goal of the SVCP, i.e., to identify, rescue, characterize, and propagate a candidate human cancer virus. Large volumes of leukemic cells are necessary for the biochemical characterization of the polymerases present in these cells, and a number of clinical contracts will be necessary to keep up with the biochemical demand. In addition to helping meet this need, the contractor is also collecting a large number of sera for the SVCP serum bank and has been able to meet new requests for a variety of specimens for SVCP investigators.

Proposed Course: Continuation as described.

Date Contract Initiated: June 21, 1965

MICROBIOLOGICAL ASSOCIATES, INC. (PH43-67-700)

Title: Development of Murine Virus Diagnostic Reagents and Services

Contractor's Project Director: Dr. John C. Parker

Project Officers: Drs. Robert Holdenried (NCI) and Wallace P. Rowe (NIAID)

Objectives: To develop reagents and tests for the detection of murine and other laboratory rodent and cat viruses; to apply these and other tools in the determination of the importance of the indigenous viruses in experimental systems; to study means for elimination of viruses from laboratory animal populations.

Major Findings: This contract project provides to the NCI a heavily used, highly skilled murine virus diagnostic laboratory. In the year ending January 31, 1971, 5,355 serological specimens were tested for an average of 8 viral antibodies per speciman. From February through May, 12,000 tests were completed. In addition, indirect tests (mouse antibody production) were performed on 94 specimens. In the last trimester, 49 specimens were processed by MAP. The laboratory has the capability of performing serological tests detecting infection with 34 known viruses or closely related groups of viruses. All serological procedures remain under evaluation with a spectrophotometric technique being developed to assist in reading complement titrations. This technique will increase the reproducibility of complement fixation tests. Complement fixation antigens have been prepared for 14 apparently distinct cat picornaviruses. Monotypic antisera is being prepared in germfree rats and hysterectomy derived and sterilely reared kittens. Tests and reagent development proceed for additional cat viruses. Four cat colonies are being systematically tested by serology for viral antibodies.

One technician from an animal diagnostic laboratory received a week of training in serological techniques.

The large spectrum of viruses encompassed in the virus detection work requires the production of a large and complex battery of viral reagents, control cells, and viral seed stocks. These materials are available to the scientific community on request through SVCP Resources. Research continues on the evaluation of the importance of various viral infections in laboratory animals to cancer research, both directly to cancer research work and in influencing the supply of healthy animals.

Significance to Biomedical Research and the Program of the Institute: The virus diagnostic capabilities provide the NCI with the ability to monitor laboratory rodent and cat colonies and laboratory animal produced viral reagents and tumors which have resulted in the production of highly characterized systems for cancer research. This contract provides assistance and guidance of particular importance for the detection of LCM in rodent systems. LCM virus, in addition to being infectious for humans, is difficult to detect. Significant contributions are being made to the knowledge of the natural history of several indigenous viruses of laboratory animals.

Proposed Course: Continue the rodent virus serodiagnostic service and develop a similar service for cat viruses. Improve the sensitivity and reliability of the tests. Apply the information developed to reduce and control viral infections in laboratory animal colonies and materials derived from animals.

Date Contract Initiated: April 10, 1961

Current Annual Level: $400,000

MICROBIOLOGICAL ASSOCIATES, INC. (PH43-66-914)

Title: Establish and Operate a BALB/c Mouse Colony

Contractor's Project Director: Mr. Wilbur Athey

Project Officer (NCI): Mr. Samuel M. Poiley

Objectives: To provide BALB/cAn mice for laboratory investigations supported by the SVCP, primarily for virus bioassays on Contract 43-67-697.

Major Findings: The contractor has provided the maximum numbers of mice that can be produced in 2,500 cages. All regulations of The Institute for Laboratory Animal Resources are followed carefully. Requests for animals based on age, sex, weight, suckling litters, or breeders, etc. have been consistently met.

Significance to Biomedical Research and the Program of the Institute: The availability of high quality BALB/cAn mice for biological assays of murine tumor viruses is important to the goal of understanding the role of viruses in oncogenesis.

Proposed Course: Mouse production will be continued at the current level.

Date Contract Initiated: June 16, 1966

UNIVERSITY OF MINNESOTA (NIH 71-2261)

Title: Study of Immunodeficiency Diseases and Cancer

Contractor's Project Director: Dr. John Kersey

Project Officers(NCI): Dr. George Todaro and Dr. Wade Parks

Objectives: The contractor sees a large number of patients with immunological problems who are at higher than normal risk to the development of malignancies. He will utilize the patients to determine susceptibility of cultures of their skin fibroblast to viral and chemical transformation. He will study the patient to evaluate cell mediated and humoral

factors in protection against tumors, search for oncogenic viruses in patients with premalignant disease and those on immunosuppressive therapy. The patients will be studied to determine the role of genetic factors revealed in in vitro transformation assays.

Major Findings: In the brief period since initiation of the contract, we have begun collecting clinical data on six new patients with immunodeficiency syndromes and explanted skin biopsies from all of these patients. SV40 transformation experiments with fibroblasts from immunodeficiency patients are progressing with Dr. Todaro. Explants of tumors from three cancer patients have been started and will soon be ready for virologic analysis.

Equipment purchasing and personnel hiring are proceeding according to plan.

Significance to Biomedical Research and the Program of the Institute: A knowledge of the immunological capability of humans and their response to viral oncogenes and to viral induced tumor antigens is of great importance to the development of effective means of human cancer control. This contract provides information and materials from carefully selected patients suffering from immunodeficiency diseases.

Proposed Course: Continue present work.

Date Contract Initiated: May 13, 1971

MONTREAL CHILDREN'S HOSPITAL (PH43-65-1020)

Title: Procurement of Serum from Human Childhood Leukemia

Contractor's Project Director: Dr. Ronald L. Denton

Project Officer (NCI): Dr. Paul H. Levine

Objectives: To obtain serum from a variety of pediatric oncology patients, family members, and controls for virologic study; to identify special cases for more extended workup.

Major Findings: The contractor has provided sera for a variety of SVCP investigators. The results of one serological study, using samples from Montreal Children's Hospital, were reported in a publication in J.N.C.I. One of these studies by Syracuse University investigators indicated that antibodies to avian leukosis virus were not present in sera from patients with acute

leukemia. Two other studies, one involving other animal leukemia viruses and another involving Australia antigen, are still under way. Samples from a patient with probable progressive multifocal leukoencepholopathy were provided to Dr. George Todaro to determine whether the virus-like particles identified in similar cases could be isolated and characterized. The contractor participated in a publication indicating that infectious mononucleosis was associated with a favorable clinical course when occurring in the course of acute leukemia.

Significance to Biomedical Research and the Program of the Institute: This is a resource contract for the supply of serum and tumor specimens from pediatric oncology patients and suitable controls. An increasing need for tissues and blood samples from pediatric oncology patients requires a number of resource contracts to meet SVCP needs.

Proposed Course: Continue to collect serum specimens, especially from selected leukemia patients, for identification of host factors associated with long-term survival.

Date Contract Initiated: September 24, 1965

NEW YORK STATE VETERINARY COLLEGE AT CORNELL UNIVERSITY
(NIH 70-2224)

Title: Feline Tumor Viral Diagnostic Laboratory

Contractor's Project Director: Dr. James H. Gillespie

Project Officer (NCI): Dr. James T. Duff

Objectives: To complete production and evaluation of cat and dog viral reagents; to monitor cat cell cultures and other materials associated with cat tumors for indigenous cat viruses and other microorganisms.

Major Findings: The contractor has established a feline tumor viral diagnostic laboratory and has available all known indigenous viruses of the cat. The feline reagents (virus seed stock and antisera) include 16 viruses. The contractor has tested the final packaged reagents for purity, potency, homogenicity, and complete reciprocal neutralization. Although the packaged viruses have undergone a drop in titer of about two logs, these reagents are satisfactory for distribution to other investigators.

Cross neutralization tests for 10 of the 14 cat picornaviruses have been completed and results reported. Neutralization titers for the previously prepared reagents of panleukopenia and herpesviruses are reported. Additional virus characterization information completed in the report period include the chloroform sensitivity of eight of the picornavirus strains and the resistance to chloroform treatment of the cat herpesvirus. The herpesvirus hemagglutinating activity is being utilized in development of an HAI test.

In the microneutralization technique, a procedure of adding cell culture suspensions to serum and virus mixtures in the wells was compared and found equally sensitive to the previously used method of inoculating the virus-serum mixtures on monolayers. The addition of the cells to the mixture will be used routinely since there is a saving in both time and material.

The service of monitoring cat tumors and cell derivatives for adventitious viruses has been established.

Significance to Biomedical Research and the Program of the Institute: An evaluation will be completed of reagents prepared for the feline viruses since these materials will be made available to NCI intramural and collaborating scientists as well as to the scientific community. The contract provides a central laboratory where materials isolated from neoplastic or normal cats can be sent to determine whether or not they contain indigenous feline agents, and/or for viral identification.

Proposed Course: (1) Initiation of a pathogenesis study of feline syncytial agent(s) in the domestic cat; (2) Monitoring of NCI-Cornell Specific Pathogen-Free Cat Colony for feline syncytial agent(s); (3) Study a feline syncytial agent and a feline adenolike virus *in vitro* for their oncogenicity "switch-on" effect on type C particles; and (4) Continue the operation of a feline virus diagnostic laboratory.

Date Contract Initiated: June 25, 1970

UNIVERSITY OF PADUA (PH43-68-1389)

Title: Collection of Human Tissue Specimens

Contractor's Project Director: Professor Giovanni Dogo

Project Officer (NCI): Dr. Robert H. Depue, Jr.

Objectives: To procure and ship human tumor and other tissue specimens to NCI for use in research programs.

Major Findings: Skin biopsies have been obtained from inbred and isolated populations in the Dolomite Mountains in Italy and have now been grown in tissue culture. Some specimens have shown a high virus-transformation susceptibility. Genealogical information on the donors is being analyzed to determine if the susceptibility to oncogenic transformation has a genetic correlation. These cell lines will be employed in a human oncogenic virus detection program organized by NCI.

Significance to Biomedical Research and the Program of the Institute: The skin biopsies will be used in a project to detect human oncogenic viruses *in vitro* and to determine the significance of the transformation test to oncogenesis.

Proposed Course: To collect and culture human skin biopsies as previously, along with genealogies.

Date Contract Initiated: October 27, 1964

SOUTHWEST FOUNDATION FOR RESEARCH AND EDUCATION (NIH 69-93)

Title: The Production of Simian Viruses and Homologous Antisera

Contractor's Project Director: Dr. Seymour S. Kalter

Project Officer (NCI): Dr. James T. Duff

Objectives: To determine the quality of simian virus reference reagents (seed material and antisera) packaged for NCI by another contractor. In addition, the laboratory serves as a diagnostic laboratory in a limited capacity for viral isolates that may emerge from studies done by other SVCP contractors.

Major Findings: The simian foamyvirus (FV) reagents were extensively tested and an attempt was made to determine whether a relationship exists between the Mason-Pfizer monkey virus and the foamyviruses. Assistance was given NCI personnel in the identification of *Herpesvirus saimiri*, a woolly monkey virus and in the study of other viruses isolated from primate neoplastic tissues.

All seven FV types grew at least on one of a variety of cell lines indicating viability of the ampouled stocks. Secondary rabbit kidney cell cultures supported the growth of all seven

types and working pools are in preparation on these cells. The Baboon Submaxillary Lymph Node cell (SMLN) culture has proven most useful in working with all the types except 5. This is a diploid culture now in its 10th subpassage. Cytopathology on these cells is rapid (6-8 days), reaching a maximum in two weeks and is easy to read because of the uniformity in appearance of the cell sheet. Working pools of types 1, 2, 4, 6 and 7 have been prepared on SMLN cells with titers of 2.0-3.0 logs/0.1 ml. Vero cells were next most susceptible to foamyvirus infection, producing CPE with FV 1, 2, 3, 6 and 7.

Cross neutralization testing indicated an unanticipated FV 2-7 cross reaction and FV 6 and 7 appear to be "mislabelled."

There appears to be no serologic relationship between M-PMV and FV 1, 2, 3, 4, 6, 7.

Significance to Biomedical Research and the Program of the Institute: These reagents will be useful to investigators in characterizing viruses isolated from neoplastic diseases (natural or induced) that occur in primates, and for monitoring primate colonies.

Proposed Course: Certify the packaged simian virus reagents and serve as a diagnostic laboratory for viral isolates referred to SWFRE by NCI.

Date Contract Initiated: June 15, 1966

SOUTHWEST FOUNDATION FOR RESEARCH AND EDUCATION (NIH 69-2011)

Title: Housing and Maintenance of a Chimpanzee Colony

Contractor's Project Director: Dr. Seymour S. Kalter

Project Officer (NCI): Dr. Roy F. Kinard

Objectives: To supply young chimpanzees to SVCP investigators.

Major Findings: Four infants were born this year. Two were sent to Dr. J. Melnick and two await shipment until he has suitable inocula. No abortions or stillbirths occurred this year. All six proven females are pregnant or being rebred. In May 1971, the colony consisted of 3 breeding age males, 6 breeding age females, 1 juvenile male, 3 juvenile females, 2 infant males, 1 infant female--for a total of 16 animals.

Significance to Biomedical Research and the Program of the Institute: The chimpanzee now appears to be the laboratory animal most similar to humans, biochemically and immunologically. This is the only source of newborn chimpanzees for SVCP.

Proposed Course: Continuation with no significant change.

Date Contract Initiated: April 25, 1969

UNIVERSITY LABORATORIES, INC. (PH43-66-1133)

Title: Production of Oncogenic Viruses and Antisera

Contractor's Project Director: Dr. Eugene H. Bernstein

Project Officer (NCI): Dr. Robert Holdenried

Objectives: Production of leukemia and sarcoma viruses and antisera.

Major Findings:

Virus Production

MSV(M): In the report year the contractor produced slightly over 2000 mls of MSV(M) virus extract from BALB/c mouse tumors. Virus titer of the last several months' production increased following passage of virus in suckling mice prior to inoculation into the weanling mice. Production of mouse antisera to MSV(M) was completed with the harvest of 38 ml with a neutralizing titer of 3.5 log FFU/ml of MSV(M).

MLV: Ten percent cell free spleen extracts yielded 1200 ml of virus suspension. The virus is intended primarily for "helper" virus for assays. Titer of virus varies from 7.3 to 7.9 log FFU/ml. Production of mouse antisera to MLV with high titer and high specificity continues.

FeLV production was initiated the last part of May.

RSV (Carr-Zilber) production as extracts from chicken tumors was completed early September 1970. Chicken antisera to RSV (Carr-Zilber) was also produced and is available for distribution.

RSV (RAV-1) propagated in tissue culture was shipped to Dr. Spiegelman and Dr. Joklik at Duke University. RAV-1 propagated in tissue culture was supplied directly to Drs. Spiegelman, Joklik and Frankel at Life Sciences.

Significance to Biomedical Research and the Program of the Institute: The supply of highly standardized oncogenic viruses and antisera has been extensively used by the SVCP and the research community.

Proposed Course: The contractor is in the process of converting to large scale cell culture propagation of oncogenic viruses. Propagation of cat leukemia virus should commence by April 1971, and Schmidt-Ruppin RSV should be underway by July 1971. Other virus production will be adjusted to meet SVCP requirements.

Date Contract Initiated: June 4, 1962

ZOOLOGICAL SOCIETY OF SAN DIEGO (PH43-63-56)

Title: Experimental Breeding of Small Primate Species

Contractor's Project Director: Dr. Robert W. Cooper

Project Officer(s) (NCI): Drs. Roy F. Kinard and Robert Holdenried

Objectives: To breed several selected small primate species and supply offspring to SVCP investigators; to develop antilymphocyte sera for these species; to develop and use tissue cultures in selected *in vitro* virus experiments or assays.

Major Findings: Newborn animals were supplied to Drs. F. Deinhardt, Melendez, Valerio, John Moore (NIEHS), and A. Hendrick, (National Primate Center, Davis, California). Antithymocyte globulins (squirrel monkey and talapoin) were prepared and sent to Dr. Deinhardt. Extract of woolly monkey fibrosarcoma was also sent to Dr. Deinhardt, and this extract was inoculated into six squirrel monkeys, causing two nodules. A cell line from a squirrel monkey tumor induced by FSV was developed and is available. Malignant sarcomas occurred in talapoins inoculated with Rous virus and squirrel monkeys with Rous and FSV. Six galagoes inoculated intratracheally with benzpyrene developed bronchogenic carcinoma.

Significance to Biomedical Research and the Program of the Institute: These small primate species are cheaper, easier, and safer to use than rhesus, baboons, and chimpanzees, and at least squirrels and talapoins have been shown susceptible to virus cancer.

Proposed Course: Because of certain EEO requirements, this contract will be discontinued on May 31, 1971. The work may be continued at another location if contractual details can be worked out soon.

Date Contract Initiated: August 8, 1962

Ohio State University (PH43-65-1001)

Title: Biohazard Control and Containment in Oncogenic Virus Research

Contractor's Project Director: Dr. R. A. Griesemer

Project Officer (NCI): Dr. A. Hellman
Dr. A. K. Fowler

Objectives:

The purpose of this research program is to elucidate the hazards to man and experimental animals from exposure to oncogenic viral agents and nucleic acids currently used in laboratory investigations. The primary aim of research in progress during this report period is to evaluate the potential hazards from oncogenic viral nucleic acids through studies that define the transmissibility, oncogenicity and host range of viral nucleic acids. A second series of investigations in progress deal with the biohazards associated with feline leukemia and sarcoma viruses.

Significance to Biomedical Research and the Program of the Institute:

Oncogenic Viral DNAs: Thus far, three tumors have appeared, all in a group of thymectomized hamsters that had been exposed at 3 weeks of age to $10^{7.88}$ TCD50 of an aerosol of infectious nucleic acid. Three weeks after aerosol exposure, 2 hamsters developed subcutaneous tumors which histopathologically are undifferented sarcomas with minimal central necrosis and hemorrhage. Serum from the one hamster examined thus far contained T-antibody but we were unable to demonstrate T-antigen in the tumor cells. Five weeks after exposure a third hamster developed a subcutaneous tumor. During the past 3 weeks the tumor has slowly enlarged and now measures 3 cm. in diameter. Continuing efforts will be made to relate tumor production to the aerosol exposure.

Feline Leukemia Transmission: One contact-exposed gnotobiotic cat died of malignant lymphoma at 330 days of age. The cat had been housed with two littermates that were inoculated intraperitoneally with feline leukemia virus on the day of birth and died from malignant lymphoma 105 and 120 days later. Attempts to demonstrate viral and gs antigens will be performed during the next report period. We consider this presumptive evidence that horizontal transmission to neonatal cats is possible. Presently, there are 9 additional gnotobiotic cats ranging in age from 35 to 95 days which have been contact-exposed since birth to feline leukemia virus-infected cats.

Feline Serology: Standardization of FeLV and FeSV serologic tests are in progress. Hyperimmune goat and rabbit anti-FeLV serum have been prepared to purified Theilen FeLV. Serologic activity and specificity of the reagents have been confirmed by complement fixation, immunodiffusion, tanned cell hemagglutination, PRILOT, and by indirect immunofluorescence. Rabbit and goat anti-FeLV serum, after extensive

adsorptions with fetal calf serum and feline embryo cell hemoganates, were used in the CF; Immunodiffusion; Tanned cell HI; Paired Iodine Labeling and Indirect Immunofluoresence tests.

These tests will be used individually or in groups to monitor g.s.-3, for micro quantities of feline leukoviral antigen and for localization of antigen.

Immune Reactivity of the Cat: Progress is being made in the purification and characterization of feline immunoglobulins. Feline IGg has been purified from cats immunized with sheep erythrocytes. The protein was isolated by DEAE ion exchange chromotography. It has the electrophoretic properties of IgG of other species and causes hemagglutination of sheep erythrocytes, thus satisfying one functional test for specific antibody. Specific antiserum to this immunogglobulin has been produced in rabbits. IgA and IgM separation procedures using isoelectric focusing and Sephadex G200 column chromatography are presently in progress. The humoral and cellular immune responses of non-leukemic cats of various ages is under study. These efforts will be expanded during the next report period to evaluate the immune reactivity of pre-leukemic and leukemic cats and will also serve as the baseline for evaluation of anti-viral vaccines.

Proposed Course of the Project: In order to develop a model system to study the susceptibility of a host to concurrent infection and cross infection, the cat model will be developed. We plan to study this animal as one would humans on chemotherapy, in order to determine his capacity for antibody synthesis, response to chemotherapy, enhancement of malignancy by immunosuppression and look at the significance of hormonal imbalance in the development of malignancy. Both cellular and humoral immunologic responses will be investigated.

Date Contract Initiated: June 22, 1965

The Dow Chemical Company (PH43-65-1045)

Title: Research and Development of Biohazards Containment Facilities

Contractor's Project Director: Mr. Cyril B. Henke

Project Officer (NCI): Mr. W. Emmett Barkley

Objectives:

The objectives of this contract are to evaluate possible hazards to personnel conducting research in the virus-cancer field, study the state-of-the-art of agent control and containment from the standpoint of personnel protection and increasing the validity of experimental studies; assist in the planning, construction and evaluation performance of new concepts for facilities and programs involving hazardous agents.

More specifically, the current contract effort is being directed to the following program areas:

1. Applied research and development studies on biohazards control and containment.

2. The continued implementation and further development of an environmental monitoring program for Building 41.

3. The continued evaluation of the performance of environmental control features incorporated in Building 41 and into the prototype laboratory units.

4. Related operational activities in Building 41.

5. Consultation for the Special Virus Cancer Program with special emphasis on providing assistance to NCI contractors through a site visit program.

Significance to Biomedical Research and the Program of the Institute:

The data collected in this program indicates that the facility systems and operational procedures are very effective in maintaining low contamination levels and minimizing cross contamination within the facility. Dow personnel continue to provide operational engineering analysis support to the NCI virus containment facility to improve the operation and maintenance of the primary and secondary barrier systems.

A theoretical analysis has been completed in which equations were developed to describe the removal of airborne contaminants from a room utilizing various combinations of air filtration, building ventilation systems and air recirculation devices. It has been concluded that while such devices are impractical for controlling room air pressures, they are highly effective in improving room air quality. A significant

part of the work effort during this contract period has been given to the NCI safety and environmental control site visit and consultation program. Site visits have been made to the following facilities:

Bionetics, Kensington, Maryland
Microbiological Associates, Bethesda, Maryland
University Laboratories, Highland Park, New Jersey
Flow Laboratories, Rockville, Maryland
Albert Einstein College of Medicine, Bronx, New York

Proposed Course of the Project: Experiments are being prepared to: 1) obtain comparative data between theory and operation of air recirculation unit(s) within a typical research laboratory, 2) determine the unit size required to obtain an equivalent high air change rate and 3) determine how unit location and air distribution can most effectively minimize high concentrations of contaminants at specific locations within the laboratory.

Date Contract Initiated: June 25, 1965

Naval Biological Laboratory (FS-57)

Title: Studies of Environmental and Physiological Factors Influencing Virus-Host with Action

Contractor's Project Directors: Dr. R. L. Dimmick
Mr. M. A. Chatigny

Project Officers: Dr. A. Hellman
Dr. A. K. Fowler
Mr. W. E. Barkley

Objectives: This contract has four objectives, they are:

1. Virus laboratory hazards evaluation. The objective of this section of the proposal is to evaluate the extent of possible hazards involved in biochemical and biophysical procedures used in virus-tissue culture laboratories.

2. Studies on environmental effects on physical and biological characteristics of viral aerosols. The objective of this section of the proposal is to provide survival data of both "model" and oncogenic viruses as related to environmental parameters (e.g. temperature, RH, RH changes, and trace chemicals - decontaminants) for use in Section 1, and to evaluate the importance of end-spectrum (0.1 to 0.5 um) (5 to 15 um) particles on virus-host interaction considering both the hazard to humans and animals and the potential for cross contamination.

3. Host-virus interactions. The objective of this section is to evaluate the effect of selected stress situations (physiological as by hormonal imbalance, immunological as by concurrent infection or biochemical, as by exposure to injurious chemical vapors of aerosols) on induction of viral disease or cancerous trauma, and to evaluate the role airborne particle size might play in such interactions.

4. Evaluation of disinfectants and decontamination procedures for selected viruses. The objective of this section is to conduct studies of the decontamination efficiency of selected disinfectants on the viruses selected from Section 2 with the purpose of recommending optimal procedures.

Date Contract Initiated: March 1, 1971

Southwest Foundation for Research and Education (NIH-71-2348)

Title: Study of Latent Virus Infection and Transmission

Contractor's Project Director: Dr. S. S. Kalter

Project Officer (NCI): Dr. A. Hellman

Objectives:

1. Determine the inactivation by heat and ultraviolet light of selected oncogenic and non-oncogenic viruses in the cell-free and intracellular states.

2. Determine the persistence of viral activity in air dried cells under normal laboratory conditions.

3. Attempt to initiate and quantitate solid tumors in experimental animals by exposure to infected cells, tumor cells, or transformed cells via the respiratory and subcutaneous routes.

4. Cultivate various organs from nonhuman primates and test for the presence of virus by observation for cytopathology in living and stained cells. Overt virus infections will be tested for in these same organs. Sera from the animals will be tested for the presence of antibody against such viruses in order to determine what relationship this may have to the state of virus infection.

5. Investigate the latent viral flora of cell lines to be used for tumor vaccine production.

Date Contract Initiated: June 3, 1971

University of Texas (NIH-71-2135)

Title: Biohazards Information Gathering Center

Contractor's Project Directors: Dr. S. E. Sulkin
Dr. R. M. Pike

Project Officers (NCI): Dr. A. Hellman
Mr. W. E. Barkley

Objectives:

1. To gather information concerning laboratory-acquired infections and accidents by the use of questionaires and personal contact.

2. To make available to laboratory personnel thru the Office of Biohazard, VO, Et., NCI, information concerning the hazards of working with infectious agents.

3. To assemble information concerning techniques and equipment which would tend to minimize this hazard.

4. To prepare a continually updated guide for dealing with various aspects of this problem, to be published by the NCI, NIH, PHS.

Date Contract Initiated: April 6, 1971

Dr. Robert J. Huebner, VCB, Etiology Area, Chairman
Dr. James T. Duff, VCB, Etiology Area, Vice Chairman

CALIFORNIA STATE DEPARTMENT OF PUBLIC HEALTH (PH43-68-997)

Title: Role of Oncogenic Viruses in the Causation of Cancer in Man and his Domestic Animals

Contractor's Project Directors: Dr. Edwin H. Lennette
Dr. John Riggs

Project Officer (NCI): Dr. James T. Duff

Objectives: To apply the newer knowledge of the nature of RNA viruses affecting murine species to the study of neoplasms of other species, namely domestic cats, dogs, and man.

Major Findings: Utilizing the indirect fluorescent antibody procedure, the infectious titer of feline leukemia viruses (FeLV) was determined and stocks of virus prepared for use in viral genome rescue studies of non-virus producing cell cultures derived from tumors of cats, dogs, and humans. This technique has also been used to determine the neutralizing ability of serum against FeLV.

A survey was made of normal cat sera and the sera of cats with various types of malignancies for the presence of antibody against FeLV. Preliminary results indicate that approximately 5% of normal cats and 30% of cats with malignancies have antibodies to FeLV as determined by the indirect fluorescent antibody technique.

Studies with serum obtained from veterinarians utilizing the indirect fluorescent antibody procedure to detect antibodies to FeLV were completed on 698 sera obtained. Only one serum could be interpreted as positive using a human cell line infected with FeLV as the test antigen and the uninfected cell line as the normal control. A subsequent serum sample from this individual was negative in the test. It is thus evident that this "high risk" group do not produce antibodies against FeLV as determined by this technique. Samples of these sera have been submitted to NCI laboratories for complement fixation studies.

Cell cultures have been established from various histological types of tumors from dogs. Attempts are currently under way to rescue a focus-forming viral genome from these cell lines by superinfecting the cells with various strains of feline leukemia viruses. By utilizing the fluorescent antibody

technique we can determine if a productive infection has taken place. Once infection is established, tests for focus-forming ability are carried out using primary feline embryo cells and a line of beagle embryo cells in the test system. To date no foci have been produced by the cell lines that have been tested.

Rapidly passaged beagle embryo cells continue to give satisfactory results in titrations of feline sarcoma virus stocks by focus-formation with both the GA and ST strains of feline sarcoma virus. Sera from 2 dogs and 1 cat hyperimmunized with GA-FSV tumor preparations completely neutralize at least 2 log dilutions of GA virus. One "normal" cat serum obtained from the municipal animal pound and positive by the indirect FA test partially neutralized the same stock of virus.

Collaborative experiments with Stanford University laboratories (NIH 69-2053) utilizing the Stanbridge technique of treating mice with anti-thymocyte serum to suppress their immune response resulted in the demonstration of a high degree of tumorigenicity of tissue culture cells derived from 1 feline carcinoma, 1 dog osteosarcoma and 1 human rhabdomyosarcoma.

In vitro infection of normal cat cell lines with feline leukemia viruses and subsequently reacting them in the ferritin-labeled antibody technique showed that the membrane antigen noted before on established cell lines carrying C-type virus particles was infection-mediated. This excludes the possibility that the membrane-tagging observed in this study is a characteristic of transformed cells having nothing to do with viral infection.

In a series of cats examined for the presence of C-type particles; 22 of 33 cats with malignant lymphoma were positive (66%), 12 of 35 cats with various other malignancies were positive (34%), and 10 of 33 cats with no evidence of a malignancy were positive (30%).

Proposed Course: (1) To continue utilizing these techniques to determine if an immunological relationship exists in tumors of different animals and different species. (2) To continue to establish cell cultures from tumors of different animals and attempt to rescue a defective viral genome from such cultures.

Significance to Biomedical Research and the Program of the Institute: These studies are an integral part of a comprehensive field program on the etiology of cancer being carried out as a collaborative effort between the Viral Carcinogenesis Branch, NCI, the California State Department of Public Health (NIH 69-87), the University of California (Naval Biomedical Research Laboratory, PH43-63-13), the University of

Southern California (PH43-68-1030), the Los Angeles Children's Hospital and the Los Angeles County Health Department. To a large extent, the success of these programs depends on the acquisition of fresh human and animal (pet) cancer materials and tissue cultures derived from donors of these species having varying genetic backgrounds.

Date Contract Initiated: June 24, 1968

CALIFORNIA STATE DEPARTMENT OF PUBLIC HEALTH (NIH 69-87)

Title: Human-Feline Cancer Household Study

Contractor's Project Director: Dr. Robert Schneider

Project Officer (NCI): Dr. James T. Duff

Objectives: The purpose of this study is to determine if an association exists between human and feline cancer. The primary study is a retrospective one, utilizing personal interviews of case and control households initially identified in the Animal Neoplasm Registry (ANR). Case households are those in which a malignant cancer has been diagnosed in a cat. Control households are of two types: the first contains cats that have not had cancer and the second comprises non-pet-owning ones for at least 5 years prior to interview. Other related epidemiological studies also are being carried out from data available in the ANR, the Alameda County Tumor Registry (Human) and the household interviews.

Major Findings: Preliminary analyses have been completed on the 675 case and control households as to whether there was increased human, canine, or second cat cancers in households containing index cats with cancer. No evidence for transmission of feline oncogenic agents to man and dogs or between cats was found. Canine tumor numbers were 30 and 33 in case and control groups, respectively. There was no pattern of dog tumors with the feline leukemia-sarcoma complex; many different types of canine tumors occurred. Human varified tumor numbers of 75 and 57 in case and control households, respectively, did not differ significantly after adjustments were made for human population age difference in the two groups. As with the dogs, the overall human tumor numbers in the case group were proportionately distributed among the various cat cancer types. There were nine human leukemia-lymphomas of various types and one sarcoma diagnosed in association with cat cancers; however, there was no pattern with these occurrences. In 27 of the 675 case households, two or more cats had confirmed tumors during a

6 year period. Malignant lymphoma was the cancer found most often, either in both cats in the same household or in association with other tumors. The high prevalence of malignant lymphoma was found due to it being the principle cancer of cats and its occurrence in high frequency in young cats. Testing the eight observed two-cat combinations of the leukemia-lymphoma group indicated that the number was not significantly different from the 6.6 expected. Other tests carried out on this data reinforced these negative results.

Proposed Course: The epidemiological survey will continue in order to follow the occurrence of cancer in households that have been identified and to follow any leads that come from the initial study.

Significance to Biomedical Research and the Program of the Institute: This is the only population based animal tumor registry in the world, at present. It has been functioning since July 1963. All cases are histopathologically confirmed. A case record is present for each animal, which in addition to animal and tumor data, contains the owner's name and address. Thus, human contacts for specific animal cancers can be identified and sera can be obtained from such persons for serological testing. In addition the ANR obtains fresh animal tissues, animal fetuses, and live cancerous animals for the other contracts within the NCI Special Virus Cancer Program. This epidemiological study is extremely relevant to determining the relationship of the cat leukemia virus to human cancer and also for determining the role of horizontal spread of virus from cat to cat and from cat to dog.

Date Contract Initiated: June 19, 1969

CALIFORNIA, UNIVERSITY OF (PH43-63-13 and NCI-FS-8)

Title: Development and Evaluation of Cell Substrates for the Study of Cancer Viruses

Contractor's Project Directors: Dr. Stewart Madin
Dr. Neylan Vedros
Dr. Adeline Hackett
Dr. Walter Nelson-Rees

Project Officer (NCI): Dr. James T. Duff

Objectives: The Cell Culture Laboratory (CCL) is physically located at the Naval Biomedical Research Laboratory (NBRL), in Oakland. The program of the CCL is funded by a contract (43-63-13) between the University of California and the NCI.

In addition, maintenance and operating expenses generated by the CCL are repaid to NBRL by an interagency transfer of funds (FS-8) between NCI and NBRL. The research studies include the development and evaluation of cell substrates for the study of cancer viruses, development of large quantities of specific cell substrates, karotyping of cell cultures, and performing biophysical, virological, and cytogenetic research.

Major Findings: From June 1, 1970, to May 31, 1971, 818 human and other animal-derived cell cultures from normal and tumor tissues were distributed to 81 recipients primarily within the Special Virus Cancer Program.

The latest catalogue (June 1, 1971) lists 1254 cell substrates initiated or propagated and stored in this laboratory for distribution following antibiotic-free cultivation, characterization and assurance of species specificity and freedom from microbial contamination.

Survey for presence of C-type virus in cell culture continues. Two hundred eighty-two cultures were examined by electron microscopy (EM) and 685 by isotope labeling (DIPIC). Particularly, a strain of KB cells in long-term cultivation, as well as one of a harbor seal, gave positive results and are being analyzed extensively.

Several human breast tumor cultures have revealed herpes-like virus by EM and/or RNA "packaging" by DIPIC.

Feline whole embryo cultures examined by DIPIC indicate high incidence of C-type virus. The virus appears to be concentrated in the thymus. Murine (NIH Swiss) cell lines from embryos and mothers of multiparous birth reveal C-type virus in embryos, but not in the maternal reproductive system.

Newer methods of fixation of cultured cells for EM is revealing definitive differences between A, B and C type virus particles.

The characterization and transmission studies of the M-PM virus continues utilizing EM and biophysical techniques as well as a newly developed chimpanzee cell line.

Mouse mammary tumor-derived cell lines continue to be studied for their production of A, B and C type virus particles.

A variety of human and other animal-derived cell lines are in long-term cultivation with and without hormones.

Effects of rifampicin and its derivatives on virus-infected mouse cells has revealed inhibition of transformation with

dose levels which do not reduce cell replication.

Chromosome monitoring of cell cultures continues for in-house production and experimentation, and in collaborative efforts, as well as for karyotype reference.

Proposed Course: Continue to develop cell reagents as substrates for human carcinogenesis; attempt to isolate and characterize viral agents from human tumor cells; continue a reference laboratory karology of cells in culture; study oncogenic viral antigens during embryogenesis--and continue basic research in the biology of tumor viruses.

Significance to Biomedical Research and the Program of the Institute: The contractor has developed an excellent tissue culture facility and is supplying cell cultures for cancer research studies to NCI investigators and SVCP contract laboratories. These studies are oriented toward a study of the fundamental biology of tumor cells, and the interaction between tumor cells and viruses of oncogenic importance.

Date Contract Initiated: October 1, 1962

CALIFORNIA SCHOOL OF MEDICINE, UNIVERSITY OF (NIH 71-2147)

Title: Role of Virion-associated DNA Polymerase in Malignant Transformation by Avian Tumor Viruses

Contractor's Project Director: Dr. J. Michael Bishop

Project Officer (NCI): Dr. James T. Duff

Objectives: Conduct investigations on the molecular biology of the avian leukosis viruses, particularly with respect to the virion-associated DNA polymerase and characterization of the enzyme itself.

Major Findings: This is a new contract and major findings have not been reported.

Significance to Biomedical Research and the Program of the Institute: These studies may provide an important insight into the mechanism by which RNA tumor viruses bring about malignant transformation, and perhaps will lead to significant advances in the understanding of the causation and control of human neoplastic disease.

Date Contract Initiated: June 1971

CALIFORNIA, UNIVERSITY OF (NIH 71-2173)

Title: Studies on the Structure and Replication of Viruses and Mechanism of Regulation

Contractor's Project Directors: Dr. Howard K. Schachman
Dr. Peter Duesberg

Project Officers (NCI): Dr. Robert J. Huebner
Dr. James T. Duff

Objectives: Research on the structure of viruses includes studies on the type specific antigens, nucleoid structure and viral subunits of RNA tumor viruses, and the nucleic acids of various mutant viruses such as the radiation or chemically induced variants of Rous virus. Research on the replication of RNA tumor viruses includes studies on the RNA-dependent DNA polymerase and other enzymes of these viruses, and the analysis of temperature sensitive mutants of Rous sarcoma virus. Research on the mechanisms of regulation include transcriptional control by a satellite virus and factors controlling the growth of mammalian cells in culture.

Major Findings: This is a new contract and major findings have not been reported.

Significance to Biomedical Research and the Program of the Institute: These studies may provide important insight into the mechanism by which RNA tumor viruses bring about malignant transformation, and perhaps will lead to significant advances in the understanding of the causation and control of human neoplastic disease.

Date Contract Initiated: June 1971

FLOW LABORATORIES, INC. (NIH 71-2097)

Title: Virus Laboratory for Cancer Research

Contractor's Project Director: Dr. Raymond V. Gilden

Project Officer (NCI): Dr. Robert J. Huebner

Objectives: In addition to the gs-1 and gs-3 antigenic specificities localized on the major polypeptide of mammalian RNA tumor viruses, an additional "gs" reactivity has been identified using sera from rats immunized with MSV transplant tumors. This antigen is localized in electrofocus separations clearly distinct from the major gs antigen. Guinea pig antisera to the MSV (RaLV) gs antigen have been prepared. These appear species specific in gel diffusion but are not yet suitable for CF purposes. Sensitive radioimmunoassays are being developed for gs antigen and antibody using both agar gel and test tube methodology. Such tests will be available for routine use relatively soon. In addition, fluorescent antibody (FA) and other cytological methods for gs antigen detection are being developed for application to tissue sections. Studies using the FA technique have confirmed the species specificity of gs antisera prepared in guinea pigs and the cross-reactivity of sera from rats immunized with MSV tumors. Antibody to the DNA dependent DNA polymerase from cat C-type virus was prepared in a single rat. This serum inhibited activity of the mammalian C-type polymerases but not MTV or viper virus. RNA dependent activity was not inhibited suggesting separation of the two enzyme activities. Preliminary attempts to purify envelope antigen based on glucosamine labeling have allowed the recovery of labeled material on acrylamide gels; however, the recovered peaks did not possess serum blocking activity.

The 3' OH terminus of 70S RNA from several mammalian and the viper virus was determined by periodate oxidation and reductive tritiation with sodium borohydride. Uridine was found to be the predominant 3' terminal base in each virus. Molecular weights of about 2.5×10^6 were calculated for the viral RNA based on the tritiation reaction.

Using autoradiographic techniques, synthesis of cytoplasmic DNA was demonstrated in mouse cells infected with several murine RNA tumor viruses (in collaboration with T. Kakefuda). This new DNA presumably represents the product of virion polymerases and is thus the previously hypothetical DNA intermediate. Chronically infected cells did not show cytoplasmic DNA synthesis.

Experiments using cell hybridization techniques have revealed the following: Hybrids of mouse cells infected with Rauscher leukemia virus and the hamster cell line (HT-1) carrying the defective MSV genome yield initially sarcoma virus, however, on passage differences in yields from clonal lines became apparent and even one completely negative line has been obtained. Fusion of HT-1 with normal mouse or human leukemic cells has not yet resulted in activation of potential latent leukemia virus.

Immunological studies by immunoferritin of the herpes-like virus (HLV) associated with Burkitt lymphomas have been augmented by the development of a method for obtaining high yields of HLV positive cells. This technique will allow more definitive identification of anti-virion antibodies in sera from tumor (Burkitt and post-nasal carcinoma) and non-tumor patients.

Contracts NIH 70-2015, PH43-67-1396, and NIH 69-97 were combined into a single contract on February 1, 1971.

Proposed Course: (1) Structural analysis of the gs proteins from viruses of mouse, cat, hamster, and rat origin.
(2) Search for similar proteins in other species.
(3) Purification and characterization of virion polymerases.
(4) Characterization of polymerase activity in tumor cells.
(5) Extension of hybrid cell techniques to human cell systems.
(6) Continued definition of Herpesviruses (especially HLV) reagents.

Significance to Biomedical Research and the Program of the Institute: This project bears importantly on a main problem associated with determining whether viruses recovered from man, and which may be oncogenic for laboratory animals, are also oncogenic in man. A main emphasis of the Viral Oncology Program is to detect, isolate and identify viruses from human cancer patients. Since man will not be used as an experimental recipient, it is necessary to gain proof of oncogenicity by other means including seroepidemiological surveys for virus, virus antigen, and/or specific antibody. The detection of a common antigen among known mammalian C-type viruses would provide a powerful incentive to look for similar antigens in human material.

Date Contract Initiated: February 1, 1971

MAIMONIDES HOSPITAL (NIH 71-2046)

Title: Viral Transformation and Chromosome Abnormalities in Human Tumors

Contractor's Project Director: Dr. Harvey Dosik

Project Officer (NCI): Dr. George J. Todaro

Objectives: Conduct systematic investigations including clinical, epidemiologic and cytogenetic studies on individuals possessing various disorders. These studies are to include patients and relatives of patients with

chromosome abnormalities, patients with a known increased risk of malignancy and patients with established malignant disorders before and after treatment with anti-neoplastic agents.

Major Findings: Skin fibroblast cultures have been initiated from over 40 individuals with chromosome abnormalities and/or genetic diseases associated with a high risk of cancer. Age and sex match culture biopsies for each sample have also been obtained. These are being studied in collaboration with Dr. Todaro to determine the extent to which genetic and chromosomal factors contribute to cellular susceptibility to transformation by both oncogenic RNA and oncogenic DNA viruses. An additional 12 families with chromosomal abnormalities have been identified and cell lines are being initiated from them.

Normal and leukemic spleens and normal and tumor tissues of various organs have been provided to the Special Virus Cancer Program for comparative study of the polymerases and other enzymes found in these organs. Chromosome analysis is performed on these tissues as well as on the skin fibroblasts described above.

Proposed Course: Continue to supply normal and neoplastic tissues from individuals with chromosome abnormalities as requested by NCI for on-going cancer virus studies within the SVCP.

Significance to Biomedical Research and the Program of the Institute: These studies are enabling the SVCP to determine, on a broader scale, the relationship between chromosome anomalies (particularly those which involve an excess of genetic material), susceptibility to cellular transformation by oncogenic agents and an increased incidence of malignancy.

Date Contract Initiated: October 7, 1970

THE JACKSON LABORATORY (PH43-67-744)

Title: Natural Occurrence of RNA Tumor Viruses (Genomes) and Host-Gene Control of Their Expressions

Contractor's Project Director: Dr. Hans Meier

Project Officer (NCI): Dr. Robert J. Huebner

Objectives: To determine the natural occurrence of leukemia and sarcoma viruses and viral genomes utilizing inbred, hybrid

and recombinant mouse strains to more precisely define the genetic mechanisms underlying mutations, differential leukemogenic responses, and regulation of cell turnover in vivo. Studies are designed to determine the relationship between the C-type RNA virus and host genes associated with spontaneous and carcinogen-induced tumors, and the role of the C-type genome in embryo development and definition of the kinetics of growth of embryonic cultures.

Major Findings: The program to date has played a fundamental role in defining the natural history of the C-type RNA virus and viral genome, contributing significantly to the development of new concepts and approaches in the Viral Carcinogenesis Branch program effort. Specifically, in large measure as a result of this contract effort, it has been established that (1) the C-type virus genome is universal in all mice and very likely in rabbits as well; (2) the presence of infectious leukemogenic or sarcomagenic viruses is a rare event in most mouse strains, and probably appears only as the result of intense inbreeding reflecting a "throwback" to its early genesis; (3) that when it is present, infectious virus is most frequently symbiotic and does not threaten species survival. In addition, there is no apparent horizontal spread of virus.

Dr. Meier and his group have defined a number of genes associated with enhance oncogenicity and have evidence that host susceptibility to neoplasia is very likely attributable to combination of particular alleles at several loci.

Proposed Course: In addition to ongoing studies, efforts are now being focused on converting high cancer susceptible mouse strains to resistant strains, as well as the reverse, through genetic manipulation. Experiments are being designed to determine the number, mode of inheritance (dominant or recessive) linkage relationships and location of host genes which serve as oncogenic determinants.

Significance to Biomedical Research and the Program of the Institute: This program has contributed many of the important keys and much of the basic data on the genetic determinants of oncogenesis and the natural expressions of the endogenous C-type RNA viruses and viral genomes. It has pointed up the overwhelming influence of genetic predisposition in the development of natural cancer and in susceptibility to environmental carcinogens. Contractor is well on the way to defining and locating some of the genes and loci involved in oncogenesis and viral replication. Information derived from this research bears direct relevance to the human cancer problem and, in addition, may well provide important leads to the autoimmune phenomenon and the role of the C-type genome in normal mammalian growth and development.

Date Contract Initiated: May 2, 1967

MICROBIOLOGICAL ASSOCIATES, INC. (PH43-67-697)

Title: Detection, Characterization and Assay of Animal Tumor Viruses

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

Project Officer (NCI): Dr. W. Ray Bryan
Dr. Robert J. Huebner

Objectives: The contract provides essential service type activities, including in vivo bioassays of leukemogenic and other RNA tumor viruses, histopathology, inventory and storage of human and animal specimen materials, and other services involved in various intramural and extramural collaborative SVCP activities. In addition, in vitro methods are utilized for detection and assay of the murine C-type RNA virus and viral genomes in spontaneously occurring tumors and leukemias as well as in normal and embryonic tissues of mice, to determine the possible role of the C-type genome in normal growth and development as well as its association with the neoplastic process.

Major Findings: Original spleen antigen test (SPAT) isolates of MuLV tested in vitro were Bc-E tropic regardless of whether the original donor specimen was normal or neoplastic. The original specimen yielded both B/c-E and NIH-E tropic virus in CoMuL tests. Although viruses of both tropisms were present in the original tissue, there was preferential growth of the B/c-E tropic virus over the NIH-E tropic virus in the spleen of BALB/c mice.

The reactivity of gs antigen in the uterus was examined in ovariectomized and non-ovariectomized mice at various stages of sexual maturity. In young mice, prior to breeding, ovariectomy decreased the amount of antigenic expression, an effect not noted in older, multiparous mice. This was possibly due to extra-ovarian sources of estrogen or infectious virus. Study of the effect of ether treatment on uterine gs antigen following ovariectomy disclosed relative resistance of gs antigen to ether treatment, especially when tested vs MSV 25.

Attempts to cultivate spontaneous carcinomas from Fischer rats and BALB/c mice are being made. The use of hydrocortisone and insulin to elicit selective isolation and growth of epithelial cells is being attempted. Neutralizing antibody was produced against a C-type virus isolated from a

transplantable spontaneous rat leukemia.

Large scale production of MSV antibody using transplantable MSV rat tumors was initiated. Timed pregnancies in BALB/c mice using hormonally stimulated females were supplied to different laboratories.

All human tumor specimens in refrigerated storage were physically inventoried and an updated list of specimens furnished to PAC-NCI.

Inhibitors isolated from cell culture and normal tissues were assayed both in vivo and in vitro. These inhibitors will be further characterized.

Levels of neutralizing antibody to MuLV in various strains of mice are being studied. No neutralizing antibody was found in 3MC tumored or non-tumored C57BL/6 Cum mice.

Sera from NZB mice with low levels of proteinuria exhibited more neutralizing antibody against AKR-LV than did mice with elevated levels of proteinuria. A relatively high percentage (59.4%) of retired NZB breeders had sera with neutralizing antibody.

Proposed Course: Long-term longitudinal studies will continue with stress on the role of the C-type RNA genome in normal growth and in naturally occurring cancer. In addition, more definitive studies on the influence of gonadotropic hormones on genome expression will be undertaken.

This facility will also provide the animals and experienced personnel to help isolate and test postulated natural inhibitors and/or repressors of the RNAvirus oncogene, and also of such synthetic or exogenous anti-neoplastic agents that may become available.

Significance to Biomedical Research and the Program of the Institute: This contract represents an extension of the intramural laboratory facilities for groups who need the services of bioassay systems for oncogenic RNA viruses and histopathology, thus saving an enormous amount of time, space, and job positions. In addition, in vitro detection and assay systems applicable to the human cancer problem are being developed. The demonstration of a C-type virus genome present in all cells with an apparent role in the developing embryo reveals the cancer process to be a natural biological event. The ability of the host to repress expression of the genome during most of its lifetime and the factors influencing expression and repression represents a promising approach for the control of cancer, and the BALB/c strain would appear to be a particularly appropriate model for such studies.

Date Contract Initiated: November 15, 1961

MICROBIOLOGICAL ASSOCIATES, INC. (NIH 70-2068)

Title: The Roles of Viruses and Chemicals in the Etiology of Cancer

Contractor's Project Director: Dr. Riley Housewright

Project Officer (NCI): Dr. Robert J. Huebner

Objectives: Project A - Dr. Johng S. Rhim and Project B - Dr. Aaron E. Freeman. These projects are concerned with the development, evaluation, standardization and application of *in vitro* assay systems for studies of carcinogenic agents found in the environment. Test systems include rat, mouse, hamster, and human tissue cultures at advanced levels of subculture, both uninfected and infected with various C-type RNA viruses. Materials under study will include known and suspected environmental carcinogens, and their noncarcinogenic analogs, and DNA tumor viruses.

The focus of Project B is to define the mechanisms of chemical and viral carcinogenesis *in vitro*, and to correlate these findings with *in vivo* studies of related programs. The ultimate objective is to develop sensitive *in vitro* systems for environmental carcinogens.

Project C - Dr. Padman S. Sarma. Development and utilization of sensitive *in vitro* assay systems for field studies of the prevalence and behavior of the feline leukemia and sarcoma viruses in cat, dog, and human cells, including the focus-forming, neutralization and complement fixation (COCAL) tests. Emphasis is focused on trans-species rescue of known and postulated defective sarcoma virus genomes from murine, avian, canine, and human tumor, using the feline leukemia virus as helper; and on isolation and characterization of endogenous avian, feline, and rat C-type RNA viruses/genomes.

Project D - Dr. Carrie E. Whitmire. To a large extent, this project parallels and complements Projects A and B. Studies are designed to test RNA virus genome derepression by carcinogens *in vivo*, leading to gs antigen, infecticus virus or tumor induction. The animal systems used include a variety of high and low leukemic incidence strains of mice, hamsters, and rats, including those strains from which tissue cultures were derived for Projects A and B.

Project E - Dr. M. Lee Vernon. This project is a continuing one designed to apply electron microscopy of high resolution capability to a search for the presence of C-type virus particles and other viruses and virus-like structures in normal and cancerous tissues and cultured cells. Candidate specimens eminate largely from contract laboratories at Microbiological Associates (NIH 70-2068 and PH43-67-697), and from the Project Officer.

Project F - Dr. John C. Parker. This laboratory provides the serological support essential to the contract and related (PH43-67-697) programs.

Major Findings: Project A. Several in vitro systems were developed for sensitive quantitative assay of chemical carcinogens. Findings: Rauscher leukemia virus (RLV)-infected rat embryo cells rapidly transformed by as little as 0.01 ug of DMBA produced tumors in newborn homologous hosts; control cells failed to show evidence of transformation and produced no tumors.

One of the more promising in vitro methods developed for assaying the relative effects of various environmental carcinogens was the AKR-infected NIH Swiss mouse embryo cell system. This system proved even more sensitive than rat and hamster systems for assaying various experimental carcinogens, and is now being used to test smog and tobacco fractions.

Uninfected and untreated normal rat embryo cell lines exhibited morphological alteration after 65 subcultures and produced tumors in homologous hosts. Concomitantly, a new antigen having the properties of gs antigen, was switched on. Since the antigen, which is one shared with the MSV-induced rat tumor system, was switched on much earlier by cells transformed by polyoma virus, RLV-infected rat cells responded with markedly accelerated transformation when treated with polyoma virus. It therefore appears that cell transformation by polycma virus, as has been shown with SV40, may be mediated by endogenous or added C-type RNA genomes.

Project B. Dr. Freeman and his group developed several in vitro systems for assay of the transforming effects of benz(a)pyrene, 3-methylcholanthrene, cigarette smoke condensates and smog components. Transformation of a variety of rat and hamster embryo cells with diethylnitrosamine and 3-methylcholanthrene in combination with leukemia viruses, and with city smog by itself and in combination with leukemia viruses, were recently published. Other recent important observations include the following:

When uninfected hamster embryo cell cultures treated with 3-methylcholanthrene or cigarette smoke condensates were transformed and produced tumors in hamsters, 5 of 6 yielded infectious hamster leukemia virus (HaLV), thus indicating that the chemicals activated an endogenous latent HaLV.

Chemical carcinogens on human (WI-38) cells infected with feline leukemia virus (FeLV) have not led to transformations. However, the FeLV-infected, chemically treated WI-38 cells have continued to replicate at normal rates for many subcultures beyond the terminal stages of the untreated control cultures.

Project C. Several new tests for assay of feline tumor viruses were developed by Dr. Sarma and his group, as follows:

(1) A quantitative assay for feline leukemia infectious virus and viral antigen, the COCAL test, modeled after the COFAL test for avian leukosis; and (2) a viral interference test, analogous to the RIF test of Rubin's (avian system), and to a previous test developed by Dr. Sarma for murine leukemia viruses. Preliminary observations have revealed differential interference patterns analogous to those described by Vogt and Ishizaki for the avian leukosis-sarcoma complex. Feline leukemia virus (FeLV) strains and FeLV pseudotypes of MSV derived from these strains can now provisionally be placed in three subgroups: A, B, and C. Unlike the COCAL test, the FeLV viral interference test is based on the detection of type-specific rather than group-specific antigens; its usefulness therefore will probably be confined to studies of viral envelope antigens.

Utilizing the new methodology it has been ascertained that the three distinct strains of the feline sarcoma virus fall under subgroup B of the feline leukemia-sarcoma complex.

Dr. Sarma and his group recently isolated a non-cytopathogenic rat-cell-specific C-type virus from stocks of MSV(O). It appears from studies by Dr. Sarma and Dr. Aaronson (VLLB) that this isolate is a rat leukemia virus and that MSV(O), described by Ting in 1968, can be characterized as a rat pseudotype of the Moloney murine sarcoma virus.

A new technique for demonstrating avian leukosis viral genome was also developed during the contract year. A serial tissue culture line of hamster tumor cells derived from a Rous sarcoma virus-induced hamster tumor was found to contain avian C-type virus which proved to be defective. Upon co-cultivation of the hamster tumor cells with cultures of avian embryos positive for avian leukosis gs antigen, the hamster-derived virus acquired the property of oncogenicity for quails. These preliminary studies suggest that the

hamster virus is defective requiring a helper virus, supplied in this instance by the vertically transmitted avian leukosis viral genome present in SPAFAS and "clean" University of Connecticut chickens.

Previous studies established and described the prevalence of a vertically transmitted avian C-type viral genome in normal chicken embryos expressed in the form of gs antigen. Follow up studies revealed this antigen to be demonstrable in virtually all embryos at all stages of development, implying not only natural inheritance of the C-type genome, but also that the genome and the gs antigen may play a critical role in avian growth and development.

Project D. Studies initiated in 17 strains of mice during the past year to determine the effects of endogenous C-type RNA viral genome on tumor induction with 3-methylcholanthrene were completed. Histological studies showed that most of the tumors induced subcutaneous fibrosarcomas at the site of infection. Of most interest was the finding that the majority of tumors were positive for gs antigen in the complement fixation test, furnishing evidence that the carcinogenic chemicals "switched on" on the RNA tumor virus genome in the induced tumors. In addition to pointing up differences in tumor/virus/antigen expression, determined by the genotypes of the inbred mice studied, it is not suprising to note that variations in response between the inbred strains was greater than in non-inbred mouse lines, thus reflecting the comparatively greater influence of gene segregations in the former which are not observed in the presumably more stable gene pools of continuously outbred strains. (An interesting but not unexpected disparity was noted in the high leukemic strains which are programmed for thymic lymphoma and hence are resistant to induction of fibrosarcomas by hydrocarbons. The mutual exclusiveness between lymphomas and sarcomas induced by chemicals in these studies was similar to that observed in the HRS/J strain studied by Drs. Meier and Myers at the Jackson Laboratory).

The results in these studies have established baselines for developing sensitive in vivo assay systems for measuring the carcinogenic potential of the environmental carcinogens also under test in vitro in Projects A and B.

Project E. More than 1,000 specimens were received for electron microscopic study from the programs on this contract and related collaborating groups (Viral Carcinogenesis Branch, PH43-67-697, and PH43-67-744). The most important controlled study done consisted of a systematic survey for C-type and other "viral" particles in embryo tissues from a number of strains of mice at various gestational periods. The results indicated widespread distributions of budding

forms of C-type RNA viruses in prenatal tissues, findings which correlate with results in complement fixation. The particles were associated principally with rapidly growing tissues such as the hematopoietic organs. These data confirmed previous evidence that the C-type RNA viral genome is part of natural inheritance in mice and may play a regulatory role during embryogenesis.

Parallel studies of uterine tissue to determine the influence age, gestational stage, number of pregnancies, coitis and conception on the distribution of C-type particles have shown a widespread distribution, again suggesting a regulatory role in rapidly growing tissues.

Recently initiated studies of mouse ova and the preimplantation stages of mouse embryos by complement fixation and electron microscopy represent an area of increasing effort. In addition, studies patterned after those conducted in the mouse are being extended to selected strains of other species, including the chicken, guinea pig, rabbit, and hamster.

Project F. The serodiagnostic laboratory has been in operation for almost a year. This laboratory primarily services Project D of this contract and contract PH43-67-697 and has a capacity of approximately 2,000 tests per week.

Proposed Course: Projects A and B. Studies will be continued on the mechanism of carcinogenesis in vitro, particularly (1) activation of endogenous C-type viral genomes; (2) chemical activation of viral oncogenes; and (3) the effect of a variety of DNA oncogenic viruses in transforming cells uninfected and infected with RNA tumor viruses. The ultimate objective is to develop reliable, sensitive and rapid assay systems for environmental carcinogens.

Project C. The development and refinement of tests for detection of endogenous mammalian oncogenes for eventual application in the human system will be continued.

Project D. With the establishment of baselines of C-type RNA virus and viral genome expression in selected mouse strains, large scale in vivo assays of a variety of known and suspected environmental carcinogens will be undertaken; these tests will be run in parallel with materials to be tested and cell lines utilized in Projects A and B.

Project E. Electron microscopy studies of various tissues associated with rapid cell replication (embryos, ova, etc.) will be expanded; preliminary studies of human cancer tissue will be continued at an increased level.

Project F. The serodiagnostic laboratory will be utilized to full capacity, and will likely service additional contract and inhouse operations in the near future.

This contract program is an integral part of the Viral Carcinogenesis Branch research effort.

Date Contract Initiated: February 1, 1970
(Note: On February 1, 1970, the following contracts were combined into the existing contract: PH43-64-941, PH43-68-705, and PH43-68-706).

PRINCETON UNIVERSITY (NIH 71-2372)

Title: Studies on Surface Alterations in RNA Tumor Virus Transformed Cells

Contractor's Project Director: Dr. Max M. Burger

Project Officer (NCI): Dr. James T. Duff

Objectives: Using a phytagglutin isolated and purified from wheat germ, it has been possible to show that DNA virus transformed cells reacted either exclusively or to a much higher degree with the agglutinin as compared with untransformed or normal cells. Several RNA virus transformed tumor cell lines will be screened for agglutinin ability with pure wheat germ agglutinin, concanavalin A and soy bean agglutinin (semiquantitative). For any line that has been found to interact, exact titers will be determined (quantitative). For any line found to interact, hapten inhibition studies will be carried out, which should answer the question whether the immunologic determinant for the agglutinin is similar or identical for the particular agglutinin (qualitative).

Major Findings: This is a new contract and major findings have not been reported.

Significance to Biomedical Research and the Program of the Institute: These studies may be useful in establishing new techniques and in understanding the changes that occur in the molecular architecture of the cell surface after transformation. Preferential loss of surface contact in transformed cells after exposure to plant agglutinins could be used as a general method for detection or quantitation of transformed cells. The surface alterations of the virus transformed cells could be related to physical changes of the cell surface or to specific chemical changes induced by the viral genome. These studies may shed light on these phenomena, particularly from

the comparison of the chemical structures of the agglutinin receptor sites of the cell lines before and after transformation.

Date Contract Initiated: June 1971

SAINT LOUIS UNIVERSITY (PH43-67-692)

Title: Search for Viral-Specific Genetic Material in Human Cancers and Studies on the Mechanism of Oncogenesis by RNA and DNA Tumor Viruses

Contractor's Project Director: Dr. Maurice Green

Project Officer (NCI): Dr. Robert J. Huebner

Objectives: To conduct studies of the basic mechanisms of viral oncogenesis with both DNA and RNA tumor viruses and of the relationship between these viruses and human cancer.

Major Findings: The RNA dependent DNA polymerase (RDDP) of murine sarcoma virus (MSV) and the avian myeloblastosis virus (AMV) transcribed the whole viral genome in vitro. The virion enzymes have been purified and their templates specificity studied in order to distinguish between RDDP and DNA polymerase (DDDP) activities in cells. A detailed study was made of the DNA polymerase activities of mammalian cells. Five enzyme fractions were isolated from 18 different normal, human, rat, mouse, virus infected, transformed and cancer cells. These fractions have been partially characterized and analyzed with regard to RDDP and DDDP activities. RDDP was only found consistently in cells replicating RNA tumor viruses.

The mechanism of viral RNA synthesis in cells transformed by RNA tumor viruses was investigated. The whole viral genome is transcribed in transformed cells which produce MSV, not as a 70 S viral RNA molecule, but in the form of viral RNA subunits. In contrast, cryptically transformed hamster cells are missing one RNA subunit.

Specific inhibitors of RDDP and DDDP activities of RNA tumor viruses and cells were investigated. Of 141 inhibitors analyzed thus far, seven were very active against purified RDDP, disrupted virions, and focus formation by MSV in mouse and rat cells. These derivatives also show specific inhibition of some cell DNA polymerases. Based on our increasing knowledge of gene replication and gene expression in normal, virus infected, and transformed mammalian cells, we propose that a rational approach to the chemotherapy of viral diseases

and cancer may be based on the design of inhibitors specific for different cell and viral polymerases.

Over 200 human cancer RNA specimens were analyzed and found to contain less than 1,000 viral mRNA molecules specific for Ad 2, 7, and 12.

The summer adenocarcinoma of the frog, which contains no detectable herpes-like particles transcribes herpesvirus specific RNA, thus providing strong evidence for the viral etiology of this tumor.

Proposed Course: A comprehensive program studying RNA tumor viruses will focus on four major areas: (1) properties and biological function of the RNA dependent DNA polymerase of the leukemia-sarcoma virus. (2) The mechanism of RNA tumor virus replication and cell transformation. (3) The properties and function of gs antigen in virus infected, transformed, and embryo cells, and (4) the application of basic information on RNA tumor virus replication and cell transformation to the analysis of human cancers. Specific inhibitors of the RNA tumor virus RNA dependent DNA polymerase will be studied and evaluated as to their usefulness for a rational chemotherapy of virus diseases and cancer. In addition temperature sensitive mutants of MSV and adenoviruses will be used to analyze viral gene functions involved in cell transformation.

Significance to Biomedical Research and the Program of the Institute: Within the Special Virus Cancer Program sequential scientific activities, which must be conducted prior to the development of a means for the prevention of virus-induced neoplasia in man, include (a) detection of the virus or virus product in human materials, (b) identification of the virus as a known or new agent, (c) selected biochemical characterization of the agent, and (d) verification of oncogenicity for man. The basic research on the molecular biology of normal and virus infected cells may provide the basis for understanding the mechanism of animal virus infection and carcinogenesis. These studies provide the basis for developing a rational chemotherapy for viral diseases and cancer.

Date Contract Initiated: March 20, 1967

SALK INSTITUTE (PH43-67-1147)

Title: Characterization of Temperature-Sensitive Mutants of Polyoma Virus, and Interaction Between Polyoma Virus and C-type RNA Viruses

Contractor's Project Director: Dr. Walter Eckhart

Project Officer (NCI): Dr. George J. Todaro

Objectives: To conduct studies on the characterization of temperature-sensitive mutants of polyoma virus that are defective in functions required for cell transformation. Interactions between polyoma virus and C-type RNA viruses are being studied to determine whether any polyoma virus function can activate the expression of a C-type RNA virus genome. Initiate studies on the isolation of cells transformed by murine sarcoma virus that are temperature-sensitive in their transformed phenotype.

Major Findings: Studies have continued on temperature-sensitive mutants of polyoma virus to identify the viral functions required for transformation. Studies with these mutants have identified two viral gene functions required for transformation. One is required transiently to initiate or stabilize the transformation, the other is required continuously to maintain certain aspects of the transformed phenotype, particularly, cell surface alterations that affect cell growth control.

The majority these studies are concerned with the ts3 mutant, which is defective in a gene function required for induction of cellular DNA synthesis in resting Balb/3T3 cells, and for initiating and maintaining surface alterations detected by agglutination of infected or transformed cells by wheat germ agglutinin or concanavalin A. It was found that the ts3 mutant neither relieves the cells of a serum factor requirement, nor alters the surface in such a way as to break contact between cells to allow expression of virus induced DNA synthesis. It is possible that the ts3 gene function may act directly on the DNA synthesizing machinery of the cell. Several kinds of ts3 transformed cells have been selected to test specific ideas about the chemistry of surface changes and stabilization of viral genes in transformed cells. Attempts are being made to clarify the genetic relation between mutants defective in functions required for transformation and other mutants of polyoma, such as host range mutants.

Proposed Course: (1) Determine whether more than two viral genes are involved in transformation. (2) Identify the surface alterations of transformed cells that are important for cell growth control. (3) Characterize the "activation" function of polyoma that is responsible for induction of cellular DNA synthesis after infection and that appears to be involved somehow in the cell surface changes and cell growth alterations that take place after infection. (4) Determine whether any of the ts mutants of polyoma virus affects the expression of antigens associated with the C-type RNA viruses and attempt to relate the functions of the two different kinds of viruses.

Significance to Biomedical Research and the Program of the Institute: Polyoma virus can infect certain cells and cause them to be stably transformed so that their phenotype resembles, in many respects, that of tumor cells. It should be possible to define the genome of these viruses, including those genes responsible for the establishment and maintenance of transformation, by isolating temperature-sensitive mutants. An understanding of the molecular mechanisms of virus transformation should also throw light on the essential differences between normal and cancerous cells.

Date Contract Initiated: June 5, 1967

SOUTHERN CALIFORNIA SCHOOL OF MEDICINE, UNIVERSITY OF and CHILDREN'S HOSPITAL OF LOS ANGELES (PH43-68-1030)

Title: A Comprehensive Field and Laboratory Research Program on the Etiology and Epidemiology of Human Cancer

Contractor's Project Directors: Dr. Murray B. Gardner (USC) and Dr. Robert M. McAllister (Children's Hospital)

Project Officer (NCI): Dr. Robert J. Huebner

Objectives: A multifaceted, highly interrelated effort to determine the actuating and contributing roles of viruses, physical and chemical carcinogens, as well as other factors associated with cancer development, both individually and in concert, to the etiology of human and animal cancer in a natural urban ecology.

Area I - Project A (USC): The collection, distribution, growth, and study of specified human and animal cancer and control materials, as well as tissues derived from genetically defective individuals and spontaneous and therapeutic abortions.

Area I - Project B (Children's Hospital): In vitro studies involving materials derived primarily from cancer patients, as well as those whose genetic deficiencies are associated with a high incidence of cancer. Primary emphasis is focused on transformation of human cultures in an effort to rescue the human C-type virus genome.

Area II - Epidemiology: Studies of the incidence and prevalence of contemporary cancers in relation to (a) exposure to environmental pollutants; (b) possible genetic or ethnic influences; (c) dietary and other cultural and individual patterns, which are suspected to have possible cancer-inducing properties (coffee and tea, smoking, incense, hormone therapy (the pill), immunosuppressants, etc); (d) development and organization of hospital based registries; and (e) computerization of data for USC and NCI use.

Area III - Environmental and Ecological Studies: Studies to measure relative exposures to environmental pollutants on a residential and occupational basis, and as determined by leads developed in Areas I and II of the program. Materials collected are characterized and disseminated for study in animal and tissue culture systems at the USC and other collaborating laboratories (VCB, NIH 70-2068).

Area IV - Co-carcinogenesis Studies: Studies to determine effects of naturally occurring environmental carcinogens in various animal species, with particular emphasis on free-living animals (wild mice, cats, etc.), which share man's ecology.

Major Findings: Area I - Projects A and B. Resources. Two hundred twenty-eight human tumors and 68 human fetuses (from spontaneously and therapeutic abortions) were obtained and processed in the period July 1, 1970, to March 15, 1971 Tumors included 24 sarcomas, 5 lymphomas, 155 carcinomas and 44 benign conditions. Five non-tumor specimens of interest (i.e., rejected transplanted kidney) were processed for study as well. (Thirteen specimens, not included in the totals, comprised muscle and nerve biopsies performed for diagnostic purposes.) Sera was obtained on 91 patients (38%) and normal tissue from 79 (33%).

In other categories 83 field cats and four dogs, carrying a variety of tumors, were referred by agreement with local veterinarians.

In addition to on-site serological, immunological, biochemical, and electron microscopy studies, human and feline tissue extracts were sent to the Viral Carcinogenesis Branch, NCI for serology; and selected tissues were sent for culture and study to the Naval Biomedical Research Laboratory (PH43-63-13),

the California State Department of Public Health (PH43-68-997), Flow Laboratories (NIH 71-2097), and other contract and collaborating laboratories, as appropriate.

Serology and Immunology. Positive complement fixation reactions in a large number of human tumor and fetal tissue extracts with several MSV rat sera pools suggest the presence in these tissues of an interspecies cross-reacting gs antigenic component. Comparable CF reaction with one human cancer sera suggest a possible break in immunologic tolerance in this patient to this particular antigen.

Several human tumor culture supernatants showed H^3 uridine uptake at 1.16 density in a sucrose gradient. Evidence of virus particles or virus activity in the primary tumors or gradient bands has, however, not yet been demonstrated. Study of C-type virions shed from human RD sarcoma cells following in vitro infection with FeLV and, on occasion, following transplantation into fetal cats, continues in an effort to demonstrate possible rescue or activation of a human sarcoma genome, either by producing sarcoma in cat fetuses, producing transformed foci in cat, dog, and human cell cultures, or showing different electrophoretic mobilities of the denatured virion RNA compared to the input FeLV RNA dependent DNA polymerase activity was demonstrated in the virions shed from the RD cells and the DNA product of the viral genome was hybridized with specific RNA from the FeLV infected human RD sarcoma cells.

Broadly reactive MSV rat sera pool 21 and FSV dog antisera generally react in CF with tissues from those feline conditions most commonly associated with C-type viruses, namely spontaneous lymphoma, infectious peritonitis and anemia, and experimental FSV sarcoma. We have been unsuccessful, so far, in isolating additional feline RNA tumor viruses by inoculation of cat fetuses with several naturally occurring feline sarcomas and carcinomas, some containing demonstrable C-type particles. The DNA polymerase activities of FeLV and FSV using natural and synthetic templates were studied. The DNA directed enzyme activity of FSV with a synthetic duplex required simultaneous copying of both template strands of a homopolymeric duplex. Nearest neighbor analyses of DNA products of representative feline, avian and murine RNA tumor viruses suggest interspecies variation in the nucleotide sequence of the RNA genome copied in vitro.

Feline Studies: Studies of spontaneously occurring cancer in the cat have revealed an unusual predilection for oral cancer, which in view of feline grooming habits, raises the question of possible carcinogen induction (i.e., smog particulates collected on the coat and transported via

licking to the mouth and tongue).

Serological tests indicate there is good correlation between tissues containing C-type particles and positive reactions in the complement fixation test.

Studies to determine possible horizontal transmission of feline cancer cells or leukemia viruses by the common cat flea have proved negative, although it has been ascertained that cells could technically be transferred from one cat host to another. In addition examination of two of seven modified live vaccine products grown in feline embryo tissue cultures for possible contamination with the feline leukemia virus proved negative for infectious virus and in the electron microscope

A cat breeding program at a nearby reformatory has been very successful and promises to provide the bulk of cats needed by the program.

Area II - Epidemiology and Cancer Surveillance Program: The Cancer surveillance program is being developed to utilize a rapid reporting system from hospital pathology and hematology laboratories. Pilot efforts are now under way in three large hospital centers--University of Southern California Medical Center, UCLA Medical Center, and the Queen of Angels Hospital. Cases being referred will be utilized initially to study the realtionship of several factors including (a) maternal and paternal age of cancer patient, (b) birth order, (c) family history of cancer, allergic conditions, diabetes, and congenital abnormalities, and (d) ethnic background to the risk of cancer. A questionnaire is being devised and will be field tested shortly.

Area III - Environmental Studies: Four sampling trailers, located at defined high and low smog areas, are now in full operation and a comprehensive fractionation has been started on an annual composite of organic extracts of airborne particulate matter. When separations are complete, fractions will be distributed for biological testing. Crude fractions have already been tested *in vitro* under another contract (NIH 70-2068) and were found to produce cell transformation.

Area IV - Co-carcinogenesis (in vivo): Using broadly reactive MSV rat sera pool 21, gs antigenic expression has been commonly detected in different tissues from freshly trapped untreated wild house mice never housed in the laboratory. C-type and intracisternal A-type particles are also seen on occasion in many of these tissues. This indicates that the C- and A-type viral genomes must, indeed, be ubiquitous in this natural feral species. However, infectious C-type virus has not yet been isolated from any

source despite all the more recent virus isolation procedures. B-type virus particles were seen in breast tissue from two normal lactating wild mice never housed in the laboratory. A polyoma virus infected ecology of wild mice has been defined and will be studied for RNA tumor virus expression with aging in comparison with polyoma free mice. Attempts to activate infectious virus by UV irradiation from a Ki-MSV transformed non-productive rat cell line and monocellular clones and from spontaneous transformed foci of rat cells were unsuccessful.

Proposed Course: Area I - Projects A and B. In addition to ongoing studies, Area I, contract 68-1030 (projects A and B) has been expanded to include two biochemical units, which will focus on the following problems: (1) the role of the C-type RNA viral genome and the RNA-dependent DNA polymerase in normal and cancer cell replication, as well as in normal embryonic cell growth and differentiation, (2) to determine the presence or absence of double-stranded viral RNA and the RNA-dependent DNA polymerase or their products in human and animal cancer cell lines, (3) attempt to isolate and characterize possible inhibitors or repressors of RNA tumor virus expressions, (4) attempts to characterize virus derepressing mechanisms of chemical carcinogens, and (5) determine whether components of a human sarcoma virus genome are incorporated into human sarcoma cell lines infected with animal C-type viruses and utilize animal model system cell hybridization techniques in efforts to detect and rescue components of the human C-type RNA genome and its associated polymerase.

The serological unit is being expanded to accommodate the increased specimen load. An immunology unit has been established; its initial focus will be directed to isolating human C-type RNA particles or viral genomes utilizing immunological procedures, which proved successful in unmasking covert animal cancer viruses. Methods to be employed include (1) screening of a variety of tissues from genetically susceptible individuals with leukemia, stimulating the leukemic lymphocytes with phytohemagglutinin (PHA) in efforts to detect the human C-type genome, (2) performing parallel studies in human and animal tissues from a variety of age groups, (3) combining hydrocarbon carcinogens with PHA in efforts to "activate" viruses and transform lymphocytes, (4) establishing long-term lymphocyte cultures from selected patients, and (5) culturing animal tumor viruses in long-term and PHA-treated human lymphocyte cultures.

Area II - Epidemiology: The epidemiology program, designed to gain information on the contemporary occurrence of cancer within several defined subenvironments of Los Angeles County is being expanded to provide back-up service and training for a proposed county-wide cancer surveillance registry. When

fully implemented, it is estimated that Project Directors will have access to most of the major hospitals in the Los Angeles area, covering 70-80% of all cancer patients including their physicians and families and their medical, residential, and occupational histories.

Area III - Environmental Studies: At the present time facilities for sampling four reasonably distinct areas of Los Angeles County in terms of air pollution are in full operation. Smog specimens are collected, concentrated, and disseminated for in vivo and in vitro studies at USC and in related contract (NIH 70-2068) and NCI laboratories. Characterization of components has been expanded from benzopyrene and various gases to a number of additional hydrocarbon and tar constituents.

Area IV - Co-carcinogenesis: Proposed studies are in line with original objectives to explore the interaction of chemical carcinogens with the C-type viral genome, particularly in the cat and feral mouse. Atmospheric residues, demonstrated by Freeman, et. al, to be carcinogenic in vitro (collected and distributed by Area III air sampling program) will be utilized to determine their effects in vivo and in vitro under a variety of host, host cell, and environmentally defined circumstances.

Significance to Biomedical Research and the Program of the Institute: This program concerns itself directly with the search for causes of human, pet, and other animal cancers in a natural diversified ecology, utilizing (1) the technology developed in experimental animal cancer systems (2) the information developed on genetic influences, cell susceptibilities, etc., (3) relevant chemical and physical carcinogenesis studies, and (4) epidemiological approaches designed to exploit Los Angeles resources, and to obtain a profile of cancer incidence in humans and animals, which share their ecology, in relation to exposure, to known and suspected environment carcinogens.

In addition the USC program continues as a prime resource for supplying human and animal materials to the SVCP in-house and contract programs, in general, but particularly to VCB contract groups (Microbiological Associates--NIH 70-2068, Flow Laboratories--NIH 71-2097, St. Louis University--PH43-67-692, Naval Biomedical Research Laboratories--PH43-63-13, and the California State Department of Public Health--PH43-68-997).

Date Contract Initiated: June 26, 1968

STANFORD UNIVERSITY (NIH 69-2053)

Title: Procurement, Processing, Storage, Distribution and Study of Human Tumor Cell Cultures and Operation of a Central Mycoplasma Diagnostic Laboratory

Contractor's Project Director: Dr. Leonard Hayflick

Project Officer (NCI): Dr. James T. Duff

Objectives: Part A is for the procurement, processing, distribution and study of human tumor cell cultures. Serum samples and skin punch biopsy material will be collected from the patient and will be available to recipients of the tumor material, if necessary. In addition to the characterization and distribution of these materials, research studies will be directed toward the detection of a viral genome in these cells. The human tumor material is obtained primarily from hospitals in the San Francisco Bay Area and other collaborating contractors in the Special Virus Cancer Program.

Part B serves as a central diagnostic facility for the detection and identification of mycoplasma contamination in virus preparations, sera cell cultures and clinical materials submitted by other SVCP contractors. Upon request, virological identification of isolates is made as to species and mycoplasma antigens are distributed to those investigators requiring these materials.

Major Findings: Thirty-eight human tumours have been cultivated. All viable cultures were photographed and from 3 to 11 ampoules of 6 tumours have been stored in liquid nitrogen. All tumour culture data, patient history and cell storage data is now being organized for deposition in our computer.

The compound cytocholasin B, which produces multinucleation and at high concentration causes enucleation of cells, is being studied for use in virus genome rescue experiments.

The use of antilymphocyte serum treated mice in assessing the malignancy of human tumour cells is continuing with very promising results.

Studies are proceeding with efforts to produce malignant transformation of normal human cells in vitro using chemical carcinogens and/or certain oncornaviruses.

Nine hundred and fifty-five samples were received (Feb. 1 - May 30) from SVCP laboratories to be tested for mycoplasma contamination. One hundred thirty-two have been found to be positive. This represents the largest number of mycoplasma

samples received in any 4-month period since the inception of the contract seven years ago.

Studies continue in an effort to understand the interaction of mycoplasmas with cells cultured in vitro and the effects of new mycoplasma inhibitors.

Collaborative studies were undertaken with Drs. Todaro and Aaronson on isotope labelling and density gradient separation of mycoplasma contaminants in cell cultures. All species, except M.salivarium, can be detected by this method. Quantitative aspects are now under study. Studies were completed on the sterol requirements of the T-mycoplasmas. Proof of a sterol requirement for the T-mycoplasmas and several other properties of these unusual micro-organisms suggests they be regarded as a new genus in the Class Mollicutes.

Proposed Course: (1) Continuation and expansion of collection, cultivation and characterization of human tumor cells. (2) Baking of human tumor cells in LN_2. (3) Attempts to transform normal human cells with RNA tumor viruses. (4) Continuation of studies on (a) effects of various chemical carcinogens on normal human cells in vitro, (b) quantitation of isotope labelling technique for mycoplasma detection and identification, and (c) discrimination by animals treated with antilymphocytic sera (ALS) between human normal and cancer cells, (5) Initiation of studies on cell fusion, hybridization and keterokaryon formation in vitro, and (6) detection of gs antigen in human embryonic tissue and tumor cells.

Significance to Biomedical Research and the Program of the Institute: The mycoplasma diagnostic facility is a testing and monitoring service available to all SVCP contractors and Viral Oncology intramural staff. All of the most important viral specimens, cell cultures, sera, etc., used in the Viral Oncology Program are sent to this facility for PPLO testing and many of the SVCP contractors are dependent upon this facility for this service or as a check on their own techniques. In addition the contractor is growing human tumor cells in vitro as a resource for other SVCP contractors and for the purpose of his own research on the detection and/or isolation of a human cancer virus or oncogene.

Date Contract Initiated: June 19, 1969

WASHINGTON, UNIVERSITY OF (NIH 71-2171)

Title: Studies on Tumor Specific Transplantation Antigen

Contractor's Project Directors: Dr. Karl Erik Hellström
Dr. Ingegerd Hellström

Project Officer: Dr. Charles W. Boone

Objectives: The objective of this contract is to detect and characterize tumor-specific antigens, and serum-mediated and cell-mediated immune responses to these tumor-specific antigens, in human tumors.

Major Findings: Two extremely significant facts were established in over 200 patients with a variety of histologic types of cancer: a) human tumors of the same histologic type have common antigens. Lymphocytes from a given cancer patient will inhibit his own tumor cells in tissue culture and also tumor cells of other patients of the same histologic type, but not of different histologic types; b) human tumors of the same histologic type have common blocking antibodies. The serum from a given patient will block the tumor-destructive effect of his own lymphocytes for his own tumor and also for tumors of the same histologic type from other patients.

Six publications occurred in the contract year on blocking and nonblocking serum effects of cellular immunity to both animal and human tumors. Cell-mediated immunity against antigens common to human colon carcinoma and fetal gut epithelium was demonstrated by the colony inhibition assay.

Proposed Course: Will continue screening human tumors for TSTA and will begin to analyze the tumor patients immune responses in relation to conventional forms of therapy.

Significance to Biomedical Research and the Program of the Institute: By analogy with the situation in animal model systems of viral carcinogenesis, those human neoplasms potentially caused by viruses should be the ones most likely to exhibit cross-reacting antigens. The discovery of cross-reacting antigens. The discovery of cross-reacting antigens specific for different morphological types of tumor by the principal investigator is of some significance in relation to possible viral etiology.

Date Contract Initiated: April 14, 1969

WEIZMANN INSTITUTE OF SCIENCE (NIH 69-2014)

Title: Study of Virus-Induced Tumor-Specific Transplantation Antigens

Contractor's Project Director: Dr. Leo Sachs

Project Officer (NCI): Dr. Charles W. Boone

Objectives: (1) To investigate the differences between the structure of the surface membranes of normal cells and of cells transformed by viral and non-viral carcinogens; (2) to determine oncogenic virus-specific changes in the structure of cell surface membrane by studying the binding of amino acid co-polymers and carbohydrate binding agglutinins.

Major Findings: It was shown that a certain plant glycoprotein agglutinates mouse, rat, and human cell lines transformed by oncogenic viruses but not normal cells from which the transformed cells were derived. The agglutination was specifically inhibited by a number of saccharides. The binding sites of the glycoprotein were found to exist in cryptic form in normal cells and could be exposed by treatment with trypsin. The size and conformation of the binding site was studied. In addition ornithine-leucine co-polymer was found to produce a specific aggregation of SV40 transformed cells.

Significance to Biomedical Research and the Program of the Institute: Current research in tumor immunology has shown that a large number of human cancers have tumor-specific antigens against which the cancer patient has mounted an immune response. This contract is aimed at understanding and characterizing virus-induced tumor-specific transplantation antigens similar in nature to those found in humans. Information derived from these studies will be useful in determining how human tumor specific antigens can be used both diagnostically and therapeutically.

Proposed Course: The research will continue to be concerned with the use of purified carbohydrate-binding proteins and glycoproteins and synthetic polyamino acids in order to investigate the structural difference in the surface membrane between normal cells and transformed cells, with particular reference to virus-induced transformation.

Date Contract Initiated: April 22, 1969

WISTAR INSTITUTE OF ANATOMY AND BIOLOGY (NIH 71-2092)

Title: Extraction and Characterization of Virus-Induced Transplantation Antigen from Sarcomas and Leukemias

Contractor's Project Director: Dr. Anthony J. Girardi

Project Officer (NCI): Dr. George J. Todaro

Objectives: To extract and characterize induced tumor-specific transplantation antigens of selected DNA and RNA tumor viruses.

Major Findings: Oncorna Virus Study. (a) The prevention of spontaneous tumors in mice. This portion of the program was initiated during the current reporting period. Five histologic types of tumors are being studied in high-incidence strains: mammary tumor, primary lung tumor, hepatoma, reticulum cell sarcoma, and lymphocytic leukemia. Immunization is proceeding along several lines, but in all studies the mice will receive vaccines during the latent period before the appearance of spontaneous tumors. Five of the 11 strains of mice required for this study are being used at present and two of the five types of malignancies are being actively investigated. (b) Immunization against neoplasia induced by known laboratory strains of oncorna viruses. The agents selected for this portion of the study include murine sarcoma virus (MSV), Rauscher leukemia virus (RLV), and more recently, Gross leukemia virus. A part of the past year was spent in obtaining and preparing necessary virus pools, reagents, etc. and immunization studies have been performed with two of the the three agents mentioned. The MSV was used in BALB/c mice previously immunized with five different cell lines (non-virus- or low-virus-shedding lines) with little evidence of immunity except with one cell line. However, by altering the immunization procedure we were able to demonstrate resistance to MSV challenge. This consisted of immunizing adult female mice with MSV, mating them, and challenging the offspring with MSV. The various cell lines will be reevaluated with this method to determine whether non-virus-shedding, MSV genome-carrying cells express MSV transplantation antigen.

Extraction of Transplantation Antigen. SV40 hamster tumor cells were treated with neuraminidase and the supernatant fluid was concentrated by different methods as a prelude to further purification and characterization. High-speed centrifugation, vacuum dialysis and Diaflo membrane concentration techniques were satisfactory for this purpose, whereas "batch-type" treatment with dry Sephadex gel was unusable. Concentrated preparations were fractionated through Sephadex G200 columns and are being tested for immunogenicity in vivo.

During the course of this study the "nonactive" supernatant fluid obtained after 145,000 x g centrifugation was found to enhance tumor formation. A recent study now in progress seems to be confirming this finding.

Significance to Biomedical Research and the Program of the Institute: Current research in tumor immunology is showing that human tumors have tumor-specific antigens which induce a cellular immune response in the host. Understanding and utilizing this immune cancer involves the isolation and characterization of tumor-specific transplantation antigens.

Proposed Course: (1) Continue studies on the preparation of cell membranes and extracts from immunogenic, SV40 virus-induced tumor development, (2) continue investigations on presence of immunogenically significant transplantation antigen in RNA virus-induced malignancies, and (3) to determine whether murine and feline leukemia are related to human leukemia as determined by shared transplantation antigens.

Date Contract Initiated: February 1, 1971

THE WORCESTER FOUNDATION FOR EXPERIMENTAL BIOLOGY (NIH 69-2007)

Title: Isolation and Purification of Tumor-Specific Transplantation Antigens

Contractor's Project Director: Dr. Donald F. H. Wallach

Project Officer (NCI): Dr. Charles W. Boone

Objectives: To investigate the biochemical and antigenic changes which occur in the plasma membranes of cells during and after transformation by oncogenic viruses.

Major Findings: During the last year VERO, MA-134, and four other cell lines were tested for optimum growth of SV40 virus. Culture methods for easily growing large quantities of mammalian cells are in operation. Specialized electrophoretic techniques using both gel slabs and gradient gels were developed. Most significantly, a "neoprotein" was found to be present in the membranes of cells permissively infected with SV40 virus. Specialized methods for isolating purified plasma membranes of virus-infected cells on sucrose gradients were developed.

Significance to Biomedical Research and the Program of the Institute: The characterization of structural and functional properties of tumor cell membranes using virus-induced tumor antigens as a model, promotes the development of powerful biochemical and immunological tools for the diagnosis and treatment of human tumors.

Proposed Course: At the instigation of the Project Officer, the present technical capability which has been built up is in the process of being reorganized and will be intensively focused on the isolation and characterization of cellular antigen from AKR virus infected cells.

Dr. Grant Fairbanks will replace Dr. Wallach as Principal Investigator.

Date Contract Initiated: March 12, 1969

PFIZER INC. (PH43-67-1176)

Title: Electron Microscopy Related to Viral Studies of Human and Animal Breast Cancer

Contractor's Project Director: Dr. J. J. Oleson

Principal Investigator: Mr. George Schidlovsky

Project Officer (NCI): Dr. W. Ray Bryan

Objectives:

This is a professional services contract established to provide electron microscopic monitoring and collaborative research in support of virological research on human and animal breast cancer conducted by other investigators and contractors participating in programs of the Breast Cancer Task Force and Special Virus Cancer Program of the NCI.

A. Studies on Human Breast Cancer

The search for viruses in association with human breast cancer involves a close collaboration between this contractor and Georgetown University (Contract PH43-65-53), Dr. W. F. Feller, Principal Investigator). Clinical and pathological studies as well as procurement and processing of specimens used in the virus search are performed by the latter contractor. Preparation of materials for electron microscopy and the EM study of specimens are conducted by this contractor.

B. Studies on Animal Breast Cancer

Electron microscopic studies similar to those on human materials are also made on various animal tumors, and EM back-up is provided for virological studies of any viruses isolated, in collaboration with participants in the Special Virus Cancer Program and Breast Cancer Task Force.

Major Findings:

A. Human Studies

A cell line derived from human breast cancer (established by Dr. L. Old, Sloan-Kettering Institute) was found in collaborative studies with Dr. Feller to produce small amounts of virus-like particles resembling the C-type oncogenic viruses of animals. The particles continue to be produced by this culture at a low, constant level. Efforts to increase their level by co-cultivation with a hormone-producing human cell line, Be Wo, (in collaboration with Dr. Pattillo PH43-68-1010), or by

treatment of cultures with hormones or blastogens (in collaboration with Dr. Feller) thus far have not succeeded.

B. Animal Studies

As previously reported, efforts under the EM guidance of this contract have resulted in the isolation and successful laboratory propagation of two new C-type animal viruses: (1) the Mason-Pfizer monkey virus (M-PMV) isolated from a breast cancer of a Rhesus monkey; and (2) the R-35 rat virus, isolated from a transplant of a malignant breast tumor which arose spontaneously in a Sprague-Dawley rat.

Further studies supported under this contract and in collaboration with scientists under another contract with this same contractor (NIH 70-2080) have resulted in the following important new findings:

1. Specific antigens of the Mason-Pfizer monkey virus have been found in 2 of 5 normal Rhesus monkey embryos thus far investigated. Particles typical of the M-PMV were found by EM in one of the antigenically positive embryos. This could indicate that the M-PMV is transmitted vertically.

2. After an extensive search for a susceptible substrate cell in which the R-35 rat virus might propagate a suitable substrate was found to be primary cultures of lactating normal rat mammary glands. The R-35 virus transforms such normal cultures to produce foci of cells resembling those of the original line of rat breast cancer now carried in tissue culture as a source of the virus. Budding C-type particles are prominent in the foci. This development promises to yield in vitro methods both for more efficient propagation of the virus (for production) and for its biological assay (previously entirely lacking by any method).

Significance to Biomedical Research and the Program of the Institute:

The collaborative studies involving this and other contracts were set up under the Breast Cancer Task Force and Special Virus Cancer Program, to determine whether sufficient evidence could be found for an association of virus with human breast cancer to justify a broader program effort on the viral approach to this human disease. The studies on animal breast cancers not now known to be caused by viruses, are for the purpose of guiding the human studies. The candidate human and animal viruses found have already justified a major increase in the program effort on the viral etiology of breast cancer.

Proposed Course:

In addition to continuation of the exploratory type EM back-up studies which have led to identification and isolation of the present candidate agents, it is anticipated that this contract will be expanded to permit systematic virological studies of the 2 new animal viruses (M-PMV and R-35) already propagatable in sufficient quantities to support such research. In particular,

the important new leads reported above will be vigorously pursued.

Date Contract Initiated: 6/28/67

GEORGETOWN UNIVERSITY SCHOOL OF MEDICINE (PH43-65-53)

Title: Human Breast Cancer Studies

Contractor's Project Director: Dr. William F. Feller

Project Officer (NCI): Dr. W. Ray Bryan

Objectives:

(1) Continuation of studies to determine whether viral etiological agents are associated with human breast cancer.

(2) Tissue culture studies in attempts to propagate virus-like particles found in human milk and breast cancer cells.

Major Findings: (Joint studies in collaboration with Contract PH43-67-1176 and intramural scientists of NCI)

As previously reported, milk specimens procured from a total of 16 women who have had breast cancer (1 breast removed) were fractionated by density gradient centrifugation, and the fractions expected to contain viruses were separated and further concentrated. Examination by thin section electron microscopy revealed virus-like particles definitely resembling the known RNA tumor viruses of animals in 6 (37.5%) of the specimens. A few additional specimens showed less definite but possible virus-like entities.

Similar virus-like particles have now been observed, with low frequency, in a continuous line of cells in tissue culture (the Levine 3 line derived from human breast cancer and supplied by Dr. Old of the Sloan-Kettering Institute), and in 4 of approximately 40 explants of breast cancer tissue that could be maintained *in vitro* for 60 days or more.

Extensive efforts to increase the amount of particles being produced by the Levine 3 line through stimulation with hormones and blastogens were made during the year but were discontinued because of lack of significant progress.

In continuation of studies on milk from women of high- and low-breast-cancer-risk groups, a collaborative investigation to correlate particles detected by EM (with Dr. Chopra, NCI), and the newly discovered enzyme, reverse transcriptase (with Dr. Todaro, NCI), were initiated. Results already obtained show the enzyme to be present more frequently in milk of women with a family history of breast cancer (see Dr. Todaro's report).

Significance to Biomedical Research and the Program of the Institute:

One of the major programs of the NCI concerns the study of viruses in relation to the causation of human cancer. The detection by electron microscopy (both under this collaborative project and under another related project--see contract PH43-68-1000) of entities which could represent a virus, in frequent association with human breast cancer, led to the initiation during the year of a more comprehensive program effort on breast cancer under the SVCP.

Proposed Course:

This contract was recently supplemented to increase the tissue effort by adding a full time tissue culture virologist and research and technical assistants.

Major emphasis is now being placed on: (1) the procurement of a large number of milk specimens from women with a history of breast cancer, as well as larger volumes of milk from each; (2) attempts to establish new cell lines from breast cancer which may elaborate more of the particles spontaneously; and (3) attempts to propagate the particles from milk in tissue culture substrates.

Date Contract Initiated: 11/19/64

INSTITUTE FOR MEDICAL RESEARCH (PH43-68-1000)

Title: Studies of Human Milk and Mammary Tumors

Contractor's Project Director: Dr. Dan H. Moore

Project Officer (NCI): Dr. W. Ray Bryan

Objectives:

To explore human milk and breast cancer tissue in search of a virus that might be etiologically related to the disease.

Major Findings:

Methodology developed in studies with the mouse mammary tumor virus model system has been applied in the study of human milk specimens. Large virus particles having the size and ultrastructural characteristics of the mouse mammary tumor virus have been observed in 7 (4.5%) of 156 specimens from normal women of the general population in the Philadelphia area. When present, they were found consistently in repeated studies on different samples of the same specimens. Similar particles have also been observed in 6 (60%) of milk specimens from 10 normal women belonging to "high breast cancer" families and whose mothers and/or grandmothers had had breast cancer.

Joint studies with the Tata Memorial Cancer Center of Bombay, India, (supported under PL 480 funds) for the study of milk from women of the inbred Parsi community of Bombay, have shown similar particles to be present in 18 (39%) of 46 milk specimens from normal women of this highly inbred sect.

In collaborative studies on some of the same milk specimens (with Doctors Schlom and Spiegelman--NIH 70-2049) a perfect correlation of the presence or absence of reverse transcriptase and the presence or absence of virus-like particles was found among 13 samples tested.

Significance to Biomedical Research and the Program of the Institute:

This was one of three projects initially set up to determine whether sufficient evidence could be found for an association of virus with human breast cancer to justify a broader formal program on the virus approach to the etiology of the human disease. The results of studies under this and the other related contracts (see also PH43-65-53 and PH43-67-1176) led to an expansion of programmed activities on breast cancer during the year.

Proposed Course:

This contract was recently supplemented to create a full-scale systematic research effort on breast cancer viruses, as compared with the small "exploratory" type effort that had been supported previously. This is part of a general expansion of program activities on breast cancer now under way, and toward which this contractor has been a key contributor.

Special population groups having both high and low risk to breast cancer will be studied on a larger scale and efforts will be made to procure larger volumes of positive milk specimens to permit further characterization of the virus-like particles.

A new tissue culture laboratory has been established and extensive efforts will be made both to propagate the milk particles in tissue culture and to isolate similar particles from breast cancer explants in tissue culture.

Date Contract Initiated: 6/28/68

MASON RESEARCH INSTITUTE (NIH 70-2204)

Title: Hormonal Influences on the Induction of Breast Cancer in Specific Virus Infected Animals

Contractor's Project Director: Dr. Marcus M. Mason

Contractor's Principal Investigator: Dr. Arthur E. Bogden

Project Officer (NCI): Dr. W. Ray Bryan

Objectives:

(1) By studies of the hormone profiles of blood and urine of experimental animals during normal physiological cycles and during different regimens of hormone treatment, to determine the hormone dosages, combinations, and sequences to be tested in the virus-hormone co-carcinogenesis studies of objective (2).

(2) To determine whether candidate viruses isolated from animal breast cancers are capable of inducing breast cancers in their natural hosts and/or animals of other species, using appropriate hormone stimulation of the hormone-dependent target tissues (mammary glands). Candidate viruses currently under investigation are: (a) the Mason-Pfizer monkey virus (M-PMV) isolated from a breast cancer of a Rhesus monkey at the contractor's institution; and (b) the R-35 virus isolated from a transplant of a spontaneous breast cancer of a Sprague-Dawley rat, also at the contractor's institution.

Major Findings:

The required specimens for determining hormone profiles (objective 1) have been taken and the hormone assays are well along. The estrogen profiles have been largely completed and hormone regimens have been established for inducing lactation in non-pregnant female monkeys.

The observations on monkeys inoculated neonatally with M-PMV have detected no tumors thus far in the oldest group, now about 8 months old.

One breast tumor (a papillary adenocarcinoma) appeared at 121 days in one of the first group of rats to be inoculated neonatally with the R-35 virus. Additional breast tumors will have to follow before this preliminary result can be considered of significance with respect to tumor induction by this candidate virus.

Significance to Biomedical Research and the Program of the Institute:

At the present time, breast cancer is known to be caused by a virus in only one animal species, the mouse. This project, involving biological testing of candidate viruses for oncogenicity, is part of a broader program activity for determining whether viruses are related to breast cancer in animals other than mice. Such animal studies are necessary for developing technology and approaches to the study of the human breast cancer virus problem.

Proposed Course:

The experiments under way will be continued until the planned numbers of experimental animals have been inoculated with virus (50 monkeys, 2000 rats, and several hundred each of mice and hamsters, all inoculated as newborns). Observations for tumor induction will be continued for the lifetimes of the rodents, and for 3-5 years in the case of monkeys. If oncogenicity is demonstrated for any candidate virus, systematic follow-up studies will be introduced.

Date Contract Initiated: 6/9/70

MICHIGAN CANCER FOUNDATION (NIH 71-2421)

Title: Studies in High Breast Cancer Families: Phase I. Collection of "B" Particle Milks.

Contractor's Project Director: Dr. Michael J. Brennan

Project Officer (NCI): Dr. Harry J. Clausen

Objectives:

Phase I of this contract is a feasibility study to determine whether sufficient quantities of the "B" type particles being observed in milk of women with a high risk to breast cancer can be obtained from this natural source to support further research on, and viral characterization of the particles. The primary purpose is to increase the supply of milk specimens from lactating women of designated high risk populations, but selected controls having a negative family history for breast cancer will also be studied.

Major Findings:

This contract was negotiated very recently and is still in the early stages of implementation at this writing.

Significance to Biomedical Research and the Program of the Institute:

The "B" particles observed in human milk cannot now be grown in tissue culture and procurement from a natural source (lactating women of high-risk populations) constitutes the only procedure available for procuring sufficient quantities of the agent for further characterization and investigation of its role in causing the greater risk among members of "high-breast-cancer-families".

Proposed Course:

If this feasibility study (Phase I) proves successful a larger milk procurement operation will be set up and systematic studies will be initiated on the relation of the "B" particles to high-risk to breast cancer (Phase II).

Date Contract Initiated: 6/20/71

MEMORIAL HOSPITAL (NIH 71-2194)

Title: Procurement of Human Serum Specimens from Defined Population Groups for Immuno-epidemiological Studies.

Contractor's Project Director: Dr. Herbert F. Oettgen

Project Officer (NCI): Dr. Harry J. Clausen

Objectives:

Procurement of serum specimens from the following defined population groups as part of a collaborative effort to determine whether candidate viruses isolated from human or animal sources are related etiologically to human breast cancer.

Basic defined population: Women entering Memorial Hospital, New York City, for first diagnosis of any breast disease.

Test group: Women whose lesions prove to be malignant as determined by biopsy.

Control groups:

a. Women whose lesions are found on biopsy to be benign proliferative reactions or reactions suspected as being pre-neoplastic in nature.

b. Women whose lesions are considered to be unrelated to neoplasia.

Major Findings:

This recently negotiated contract is still in the early stages of implementation at this writing.

Significance to Biomedical Research and the Program of the Institute:

Since viral agents suspected of causing cancer in man cannot be tested directly in human subjects, it is necessary to establish etiological relationship indirectly through immuno-epidemiological studies. This contract is for procuring the epidemiologically defined bank of serum specimens essential to the determination of whether antibodies against suspect viruses occur with higher frequency, and in larger amounts, in sera of women with breast cancer as compared with appropriate controls.

Proposed Course:

It is anticipated that a sufficiently large bank of specimens for the proposed sero-epidemiological studies can be acquired under this contract within a period of 2 to 3 years.

Date Contract Initiated: 6/28/67

MELOY LABORATORIES (PH 43-66-458) formerly Mel-Labs, Inc.

Title: Murine Mammary Tumor Virus Studies

Contractor's Project Director: Dr. John E. Verna

Project Officer (NCI): Dr. Louis R. Sibal

Objectives: Part A is a continuation of the present contract to propagate, purify and provide murine mammary tumor virus (MTV) suitable for collaborating investigators; to perform immunological and biological assays for the detection and quantitation of MTV; to develop improved methods for the propagation and detection of MTV and MTV antigens; to conduct studies on the control of neoplasia in the susceptible murine host by vaccination with inactivated virus. The ultimate objective of this part is to apply information to the study of the possible viral etiology of breast cancer in man.

Part B. Increased production of MTV positive-mouse milk to prepare purified virus for inhouse and contract laboratories.

Major Findings: C_3H milk production has been increased significantly. The use of programmed sucrose density gradients in the rate-zonal step of virus purification has resulted in better separation of the virus band from high and low density protein bands. Greater yields of purified virus have resulted from the use of these new gradients.

Hemagglutination, hemagglutination inhibition, immunoduffusion and indirect fluorescent antibody assays have become reliable, reproducible, and sensitive testing procedures for viral antigens and/or antibodies to MTV.

A series of experiments to determine whether vaccination of C_3H with purified, formalin-inactivated MTV has been initiated. Immunized mouse groups showed humoral antibodies to MTV; milks collected from various females will be assayed for viral antigen. Data relating to protection from tumor development are expected soon.

Investigations to establish in culture, a cell line which produces significant amounts of MTV *in vitro* and to develop an *in vivo* assay system for MTV have been initiated. To date, many tumor cell suspensions have been established in tissue culture. Of these, 10 lines were free of mycoplasma and bacteria and are positive for MTV antigens by immunofluorescence. Cells growing in the absence of hormonal stimulation are almost entirely fibroblastic; cells growing in the presence of insulin hydrocortisone and prolactin at a final concentration of 10 ug/ml are epithelial in nature. On the basis of serological and

electron microscopic tests, it is very likely that MTV or MTV antigens were synthesized in vitro by hormone stimulated cells.

Significance to Biomedical Research and the Program of the Institute: Breast cancer is a leading cause of death from cancer among women. The recent finding of a virus, resembling a Type B RNA oncogenic virus of mice, in the milk of a significant number of women from high-risk breast cancer families strongly suggests a possible viral etiology for this disease. A major effort of the Special Virus Cancer Program will be directed to determine the relationship of viruses to human breast cancer. This contract was established for the purpose of obtaining correlative information on the detection, isolation, and propagation of a murine mammary tumor virus, because this is the only available animal model system in which approaches to the study of viruses as a cause of breast cancer in man may be developed.

Proposed Course: The biological and immunological methods developed in this laboratory will be used in systematic studies to develop further the mouse MTV system as a laboratory model for breast cancer virus studies in man. Greater emphasis will be placed on propagating this virus in tissue culture and on in vivo infection of cells in cultures. It is anticipated that the information gained from these studies will be applied to human breast cancer studies within the newly-created Breast Cancer Virus Segment.

Date Contract Initiated: December 30, 1965

PROGRESS REPORT ON INVESTIGATION OF CARCINOGENESIS WITH SELECTED VIRUS PREPARATIONS IN THE NEWBORN MONKEY

SUBMITTED BY

BIONETICS RESEARCH LABORATORIES, INC.

A DIVISION OF LITTON INDUSTRIES

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BETHESDA, MARYLAND 20014

CONTENTS

SUMMARY OF THE INOCULATION PROGRAM

The following review summarizes simian studies at Bionetics Research Laboratories under contracts PH 43-62-42, PH 43-67-661 and NIH 71-2025. The report period extends from May, 1962 through June, 1971. Inoculation of simians and their general protocols are reviewed.

Since 1962, a total of 2,274 primates have been inoculated at Bionetics. The majority of these have been of the Macaca sp. (90%), while Papio sp., Pan sp., Galago crassicaudatus, Aotus trivirgatus, Cercopithecus aethiops, and Saguinus sp. have also been utilized in the program. Over 70 investigators in 50 different laboratories throughout the world have provided inocula. These inocula are accepted with the understanding that they contain specific viruses, virus-like particles or viral expression that may have an association with the neoplastic process. The most numerous inocula fall into two categories--viruses and tissues or cells. The two categories are further defined as follows.

1. Viruses: Rous sarcoma virus, Moloney sarcoma virus, Herpes type 1 and 2, Herpesvirus saimiri, EBV, Adeno 12, SV-40, Echo 9, Reo 1 and 3 and rubella.

2. Tissues or cells from patients with myelogenous leukemia, lymphocytic leukemia, Hodgkin's disease, Burkitt's lymphoma, polycythemia, rhabdomyosarcoma, epidermoid carcinoma, papilloma and infectious mononucleosis.

The number of inoculated animals currently being held (as of July 1, 1971) is 1,190. At least 60% of these animals are maintained as part of our long-term holding procedures for expression of the neoplastic process; approximately 40% are involved in short-term, more intensive studies. The remainder of the inoculated animals (1,084) are dead or have been transferred. Animals are transferred upon request by and/or consent of the Project Officer and the Principal Investigator, to various institutions or primate centers. Studies utilizing these animals are complete at the time of their shipment and are considered negative for tumor growth.

At Bionetics, complete necropsies are performed by veterinary board-qualified pathologists and a histopathologic examination is made as required by the investigator or as dictated by suspicious lesions. Complete histopathology reports are sent to the investigator for review. Duplicate records of pathological findings are maintained on file.

Since the major portion of this program has been devoted to collaborative studies, the results of experiments must be interpreted by individual investigators and associated study groups, primarily those outside Bionetics. Implementation of protocols designated by the investigator and collection of data thus generated are handled by Bionetics. Upon release to the investigator, details concerning evaluation of these data become privileged information and are within the province of the investigator rather than the contractor.

Although we are not in a position to scientifically evaluate the program's results in their entirety, several studies have yielded positive results in the form of neoplasia. These findings are related below. All other animals within this program should be considered negative for neoplasia to date.

One of the initial attempts to produce neoplasia in monkeys was accomplished using Rous sarcoma virus (Schmidt-Ruppin strain). A fibrosarcoma was induced in rhesus and cynomolgus monkeys which proved regressive in nature. The feline fibrosarcoma virus (Snyder-Theilen) has also been utilized to induce tumors in the macaque. Significantly, it has been this single strain of the agent which yields positive results.

In a study by Innes, Stewart and Landon a hamster passaged cell-free extract of Burkitt's lymphoma induced a panencephalitis in monkeys. This type of encephalitis is pathologically identical to the herpetic type of necrotizing encephalitis found in humans. The herpes-type virus which is related to Burkitt's lymphoma has been linked to the panencephalitis in monkeys.

In another study, an animal inoculated with a Burkitt tissue culture preparation and treated with a co-carcinogen (benzo[a]pyrene) developed a myxofibrosarcoma. Pathological findings indicated a more direct causal relationship with the co-carcinogen than with the Burkitt preparation. This animal is part of an intensive study (MK-SVLP).

More recently, Herpesvirus saimiri, a virus isolated from the squirrel monkey, has caused the induction of malignant lymphoma with a type of lymphocytic leukemia in the owl monkey. To date, attempts to induce neoplasms in Old World monkeys other than those mentioned above have proven unsuccessful.

Various techniques have been employed as adjuncts to viral inoculation. Immunosuppression, co-carcinogens, and surgical manipulation (e.g., thymectomies, splenectomies, in utero inoculation) are some of the considerations. Serological studies are performed to determine the immunological status of the animal prior to inoculation. More recently, stringent procedures for hematological monitoring have been introduced to evaluate the effects of the inoculum. Within the past several years, the trend has changed from one of basic tumor induction to studying the underlying mechanisms which may accompany the process. The materials inoculated are being more closely evaluated for their oncogenic potential, a consideration that has developed with the state of the art. Other positive results within the experimental aspects of the program include: establishment of effective immunosuppressive regimens; maintenance of chronic malaria in simians; and use of marker antigens to elicit predictable responses in the simian immune system.

FORMAT OF THE REPORT

This review is divided into five types of studies plus an Addendum. The studies are:

A. Major Studies
B. Special Studies
C. Other Active Studies
D. Long-term Holding Studies
E. Terminated Studies

A major study is the product of an ad hoc committee formed within the Special Virus Leukemia Program to investigate areas of significance. These are major group or collaborative efforts with emphasis on inoculation of human material and subsequent long-term holding. These studies extend from August 1964 to May 1967.

The special studies program was formally initiated in June 1969, although procedures of this type had been employed since September 1968. With the shift in emphasis from gross tumor development to more sophisticated procedures involving inoculation and detection, a new type of program was developed. The objectives were to provide for experimental manipulation, close observation and monitoring of a limited number of selected animals. These studies proceed according to more formal protocols which involve greater varieties of inoculation procedures, possible animal preconditioning such as immunosuppression, or surgical manipulation, delayed hypersensitivity and more extensive and diverse monitoring.

Section C consists of current studies not of a special nature. These are programs with specified time limits for review, evaluation and subsequent implementing of decisions. Many of these may be considered preliminary investigations into previously undefined areas.

Section D includes those animals being maintained for extended time periods. The rationale is based on known long latent periods in primary animal tumor systems. In most of these, the inocula were human leukemic or tumor materials inoculated between 1962 and 1965.

Section E lists all completed studies.

The Addendum contains reports on two uninoculated groups:

1. Spontaneous neoplasia in the primate breeding colony;
2. Incidence of neoplasia in animals experimentally manipulated elsewhere and held at Bionetics.

Under Sections A through E, the studies are arranged alphabetically by investigator. Various codes are used to make the tables containing the information more meaningful. Origin of material is a capital letter (key 1.a) and is associated with the disease type, which is also coded (key 1.b). Information relative to source--the type of material used--is coded by numeral (key 1.c). The number inoculated and the number dead or

transferred are real numbers. The dates present in the tabulations refer to the time the animals were placed on study.

1. Material inoculated

a. Origin

A	avian
B	bovine
C	chemical
E	equine
F	feline
G	guinea pig
H	human
M	murine
O	ovine
R	rabbit
S	simian

b. Diagnosis

A12S40	Adenovirus 12 + SV-40
A2S40	Adenovirus 2 + SV-40
Ad2P	Adenovirus 2 + parainfluenza
Ad 7	Adenovirus 7
AL	Acute leukemia
ALL	Acute lymphocytic leukemia
ALL I	Acute lymphocytic leukemia + influenza
ALL PI	Acute lymphocytic leukemia + parainfluenza
AM BL	American Burkitt's lymphoma
AML	Acute myelogenous leukemia
AM MOL	Acute myelogenous leukemia + monocytic leukemia
AMOL	Acute monocytic leukemia
Arbo	Arthropod-borne virus
AT MON	Atypical monocytosis
Au Ag	Australia antigen
Bac Agt	Bacterial agent
BL	Burkitt's lymphoma
BOL	Bovine leukemia
CA	Condyloma acuminatum
CCHy	Congenital cerebral hyperplasia
CF	Control familial
C-H	Chediak-Higashi
Chondr	Chondrosarcoma
CLL	Chronic lymphocytic leukemia
CML	Chronic myelogenous leukemia
CMV	Cytomegalovirus
CSCL	Congenital stem cell leukemia
DC	Disease control
D Enc	Dawson's encephalitis
Echo 9	Echovirus 9
EL	Erythroid leukemia

Eosinp	Eosinophilia
Fibro	Fibrosarcoma
GB	Glioblastoma
H-1	H-1 virus
Herp/G	H. genitalis
Herp/S	H. simplex
HD	Hodgkin's disease
HV	Herpesvirus
I	Influenza
IM	Infectious mononucleosis
Kuru	Kuru
L	Leukemia
Liposar	Liposarcoma
L lymph	Lymphocytic leukemia
LRL	Leukemoid reaction of the liver
LS	Lymphosarcoma
Lymph	Lymphoma
Mamm T	Mammary tumor
Mening	Meningitis
MH	Malignant histiocytosis
Misc L	Miscellaneous leukemia
Misc V	Miscellaneous virus
ML	Malignant lymphoma
MM	Multiple myeloma
MSV	Moloney sarcoma virus
MSV AV	Moloney sarcoma virus + arbovirus
MSV L	Moloney sarcoma virus + leukemia
MSV MT	Moloney sarcoma virus + monkey tumor
Osteo S	Osteosarcoma
P	Papilloma
PI	Parainfluenza
PIA C	Pia mater control cell culture
Plyctm	Polycythemia
PPLO	Mycoplasma
R	Rubella
Rau Vi	Rauscher virus
RCS	Reticulum cell sarcoma
Reo 1	Reovirus 1
Reo 3	Reovirus 3
Rhabd L	Rhabdomyosarcoma + leukemia
Rhabdo	Rhabdomyosarcoma
RTC	Rous transformed cells
S	Sarcoma
S20S40	SV-20 + SV-40
SA 7	Simian agent 7
SCL	Stem cell leukemia
Sq S	Squamous cell sarcoma
SV-5	Simian virus 5
SV-20	Simian virus 20
SV-40	Simian virus 40
T	Thrombocytopenia

Trn Ce	Transformed cells
Undiag	Undiagnosed
W Tumr	Wilm's tumor
Yaba	Yabavirus

c. Source--coded as follows.

1	tissue culture
2	blood
3	plasma/serum
4	tissue mince
5	buffy coat
6	chemical
7	ascites
8	milk
9	spinal fluid
10	bone marrow
11	culture

2. Number of animals inoculated.
3. Number of animals dead or transferred.

SUMMARY OF STUDIES*

A. Major Studies

1. BFKMRS, 10/65-3/66

This study was established under the Special Virus Leukemia Program of the NCI to investigate human leukemic materials, human papilloma and infectious mononucleosis in conjunction with the co-carcinogens benzo[a]pyrene and benzanthracene. The program was a product of the study group that included Drs. Bryan, Falk, Kotin, Manaker, Rauscher and Stevenson. This study has recently been terminated and the remaining animals are in the process of being transferred.

Inoculum	Source	No. inoculated	Dead or transferred
H P	1	11	6
H L	1	15	3
H IM	1	11	3
Control		9	2

*Some studies summarized are in the process of being terminated and this may not be reflected in the total numbers. This is due to lag times in finalization of reports on selected studies.

2. Fink-Malmgren-Rauscher, 8/64 - 9/65

This program was designed to investigate the ability of fresh human leukemic materials, as well as cultured and manipulated cell products, to induce similar neoplasia in the newborn monkey.

Inoculum	Source	No. inoculated	Dead or transferred
H AML	2	6	5
H ALL	2	11	9
H EL	2	5	5
H CML	2	2	1

3. Irradiation Study, 2/67 - 5/67

The major emphasis of this study was to determine the suppressive effects of radiation upon primates and the subsequent enhancement or creation of a more favorable environment for neoplastic alteration. The program was under the direction of Drs. Reisinger and Bowser, with Drs. Rauscher, Landon, Stewart, Moloney, Perry and Hart collaborating.

Inoculum	Source	No. inoculated	Dead or transferred
H BL+Irr.*	1	16	2
H BL+Irr.+ BSA		8	5
H L+Irr.	2	8	2
H S+Irr.	3	4	3
M S+Irr.	4	3	2
S LE1,2+Irr.	1	4	2
A S-R+Irr.	1	5	1
Irr.		35	12
Irr.+BSA		17	3

*Irradiation

4. MK-SVLP, 2/66 - 3/67

This study was initiated by Drs. Manaker and Kotin in collaboration with an ad hoc committee of the SVLP. The prime objective was the induction of neoplasia in primates using Burkitt and other lymphoma material in conjunction with the co-carcinogens benzanthracene and benzo[a]pyrene.

Inoculum	Source	No. inoculated	Dead or transferred
H BL	1	105	11
H Lymph	1	33	5
H L	1	18	3
H Sq S	1	13	0
Control		6	1

5. Perry-Rauscher, 7/66-10/68

This program was initiated by Dr. Rauscher in collaboration with Dr. Perry of NCI and Dr. Landon of Bionetics. Fresh whole blood from leukemic patients was inoculated directly into monkeys using multiple sites and volumes as large as possible.

Inoculum	Source	No. inoculated	Dead or transferred
H CML	2	26	6
H AML	2	12	0
H LS	2	4	0
H ALL	2	18	0
H CLL	2	6	0
H RCS	2	1	0
H HD	2	3	1
H AT MON	2	2	1
H ML	2	2	0
H C-H	2	4	2
H BL	2	1	0
H Lymph	2	1	0

6. PSG-Melnick, 9/65-1/68

This study was a product of the Primate Study Group headed by Dr. J. Melnick. The primary objective was the investigation of the possible oncogenicity of selected human prototype viruses in primates in conjunction with the use of co-carcinogens. This study is in the process of being terminated with the remaining animals being transferred to Dr. Melnick.

Inoculum	Source	No. inoculated	Dead or transferred
H Reo 1	1	9	6
H Reo 3 + BL	1	15	15
H CMV	1	12	5
H P	1	1	1
H Herp/S	1	12	7
H Ad2P	1	12	1
H&S A2S40	1	12	1
H R	1	12	2
H Echo 9	1	12	5
C	1	1	0

B. Special Studies (active)

		Inoculum	Source	No. Inoc.	Dead or Transferred
1.	Ablashi, 10/70	S; H. saimiri	1	22	9
2.	Bryan-Jensen, 1/69	S; Mamm T	1	20	8
3.	Levine, 6/69	H; BL	1	38	8
4.	Melendez, 3/70	S; H. saimiri	1	16	5
5.	Pearson, 3/71	H; BL	1	16	0
6.	Rickard, 6/69	F; Lymph	1	11	3
7.	Theilen, 6/69	F. Fibro	1	13	0
8.	Witter, 7/70	A; HV of turkeys	1	8	0
		A; Marek's disease	1	8	0

Special Studies (terminated)

1.	Dunkel, 7/70	H; BL	1	37	37
2.	Landon, 7/70	S&H; Misc V	1	26	10
3.	Landon-LaFontaine, 7/70	F; S	1	3	3
4.	Sibinovic-Ulland, 8/70	C; Dilantin	6	6	6

C. Other Active Studies

1.	Adamson, 5/68	C; MCA-Cu chelate	6	37	26
2.	Blumberg-London, 3/68	H; Au Ag	3	11	7
3.	Gerber, 4/64	H; BL	3,5	16	16
		H; BL	7	2	0
		O&S; Misc V	2	12	4

D. Long-term Holding Studies

1.	Feller, 7/66-11/66	H; Mamm T	8	5	0
2.	Fischinger-O'Connor, 8/69	F; Lymph	1	6	0
3.	Kelly, 6/67-4/68	C; MCA	6	3	3
		C; Benzo[a]pyrene	6	10	7
4.	Landon-O'Gara, 6/70-7/70	S; Misc L	4	5	0
5.	Landon-Rauscher-BRAF, 12/67	S&M; Rhab L	4	17	7
6.	Manaker, 8/64-10/69	H; BL	1	16	13
		H; L	2	1	1
		H; S	1	8	1
		H; Rau Vi	1	8	0
		S&M; Misc L	1	9	2
7.	Manaker-Landon, 5/67	H; ALL	1	1	0
8.	Manaker-Landon-Rauscher, 2/67	H; BL	1	8	7

		Inoculum	Source	No. Inoc.	Dead or Transferred
9.	Manaker-Rauscher, 6/66-7/67	H; RCS	1	1	0
		H; BL	1	3	0
10.	Manaker-Stevens, 6/67-12/68	H; BL	1	11	2
		H&S; BL + Malaria	1,2	12	0
		H&S; BL + Reo + Malaria	2	3	0
		S; Malaria	2	19	14
11.	Melnick, 12/63-6/68	H; AL	3	6	6
		H; IM	1	13	9
		H; ALL	1	21	15
		H; P	4	5	5
		H; Herp/G	1	44	1
		H; Reo 1	1	3	3
		H; Ad2P	1	4	1
		H; Herp/S	1	4	1
		H&S; Adeno+SV-40	1	3	2
12.	Moloney, 4/62-2/69	M; L lymph	1	12	8
		H; ALL	1	23	21
		H; CLL	3	5	5
		H; AMOL	3	1	1
		Control		23	13
		H; CSCL	3	3	2
		H; AML	3	20	15
		H; LS	1	4	1
		H; HD	3	1	0
		H; SCL	1	7	4
		H; CF	3	5	2
		H; CML	1	20	16
		H; MM	3	1	1
		H; P	1	1	1
		H; BL	7	14	5
		M; S	4	2	2
		H; RCS	3	2	2
		M; MSV	4	1	1
13.	Moloney-Reisinger-Bowser, 5/67	M; MSV	4	4	2
14.	Moore, 9/69	H; BL	1	2	0
15.	O'Connor, 4/65-9/67	H&M; ALL PI	1	3	3
		H&M; ALL I	1	3	3
		H; Rhabdo	1	3	0
16.	Old, 9/67-12/69	H; Osteo S	1	3	0
		S; Osteo S	1	2	0
17.	Rauscher-Landon, 12/66-2/70	S; Rhabdo	1	2	2
		H; ML	1	4	3
		H; BL	7	13	0
		S; Mening	4	2	2
		M; AL	3	4	3
		H; HD	1	1	0

		Inoculum	Source	No. Inoc.	Dead or Transferred
18.	Rauscher-Reisinger-Bowser, 4/67-5/67	H; BL	1	14	6
		Irradiation		1	1
		H; CML	2	1	0
19.	Sarma-Huebner, 9/69	F; Fibro	1	3	0
20.	Shachat-Moloney, 8/65-11/66	M&S; MSV MT	1	2	2
		M; Rhabdo	1	10	8
21.	Stewart, 4/62-6/68	H; ALL	1	56	24
		H; AML	1	6	6
		A; S	4	32	23
		H; GB	1	2	2
		H; BL	1	51	31
		H; CML	1	2	2
		H; HD	1	9	9
		H; Liposar	1	4	4
		H; D Enc	1	3	3
		H; Undiag	1	2	2
		S; SV-5	1	3	3

E. Terminated Studies

1.	Aisenberg-Zamecnik, 5/64-6/64	H; HD	2	8	5
2.	Blumberg-Moloney, 6/66-10/66	M; Rhabdo	4	1	1
		M; MSV L	1	2	2
3.	Chirigos, 5/66-3/69	C; pI:C	6	8	7
		M; S	1	6	6
		M; Arbo	1	2	2
		M; MSV AV	1	2	2
		M; MSV	1	2	1
4.	Cohen, 3/68-1/69	H; AL	2	6	5
		H; BL	1	3	3
		R; ALS	3	7	6
		Control		2	2
		H; AML	2	2	0
		H; CLL	2	2	0
		H; EL	2	2	0
5.	Dreyer, 9/64	H; ML	1	2	2
6.	Gajdusek, 1/67	H; D Enc	4	4	3
7.	Gajdusek-Gibbs, 4/66-5/66	H; Kuru	4	8	4
8.	Gazdar-Moloney, 7/69-8/69	F; Fibro	4	3	0
9.	Grace, 2/64-8/64	H; MyL	1	1	1
		H; AML	1	10	8
		H; ALL	1	2	1
10.	Grace-Horoscewicz, 5/67	H; BL	1	6	6
11.	Gross, 5/62-4/63	M; L lymph	4	12	7

		Inoculum	Source	No. Inoc.	Dead or Transferred
12.	Howard-Notkins, 5/67-1/68	H; Gamma globulin	3	16	16
13.	Huebner-Coates, 8/64-3/68	H; Ad 7	1	3	3
		H; Ad 12	1	6	4
		A; S	1	3	2
		M; S	1	4	3
		S&H; A12S40	1	2	0
14.	Johnson-Hull, 8/65-3/67	S; SV-20	1	4	3
		S; SA 7	1	10	10
15.	Kinard-Rauscher, 3/67-9/67	A; S	3	18	18
16.	Koprowski-Jensen, 4/66-6/66	H; Ad 7	1	2	2
17.	Landon, 7/65-5/70	B; Non-inf	2	14	9
		Control		16	14
		S; Rhabdo	1	7	7
		S; Rhabdo	4	18	9
18.	Landon-Darrow-Stewart, 1/68	S; CCHy	9	2	2
19.	Landon-Rauscher, 2/68-7/68	S; Plyctm	2	8	6
20.	Landon-Valerio, 8/67	S; LRL	4	2	2
21.	Manaker-Landon-Darrow, 6/68-7/68	Skin graft		6	6
22.	Manaker-O'Connor, 3/66	H; BL	1	2	2
23.	Moloney-Herbert, 4/67	M; L	4	2	2
		M; L lymph	4	3	3
24.	Moloney-Manaker, 4/67	CF		2	2
		H; BL	1	2	0
25.	Moloney-Stewart, 5/63-7/63	H; DC	1	2	2
		H; ALL	1	1	1
		H; CLL	1	1	1
26.	Morgan, 3/65	H; CA	4	3	3
27.	Morris, 9/65-5/66	S; SV-20	1	7	7
		S; S20S40	1	1	1
28.	Morton, 6/68-9/69	H; Osteo S	1	12	6
		H; Liposar	1	15	9
		Control		8	6
		H; Trn Ce	1	4	2
		H; Chondr	1	1	0
		E; ALS	3	4	3
		S; Bact Agt	4	1	0
29.	Nadel-Rauscher, 1/65-2/65	G; SCL	5	3	3

		Inoculum	Source	No. Inoc.	Dead or Transferred
30.	Rabotti, 6/64-4/66	A; RSV	1	25	25
		H; AL	2	2	1
		S; RTC	1	10	10
		S; PIA C		2	2
31.	Rauscher, 4/62-1/69	M; L lymph	1	14	14
		H; CML	3	1	1
		H; ALL	1	1	1
		H; AM MOL	5	1	1
		H; AML	5	2	2
		C; Immunosuppression		2	2
		H; BL	1	1	0
		H; AM BL	10	2	2
		Control		3	3
32.	Rauscher-Davenport-Jensen, 11/65-1/66	H; BL	1	2	1
		B; PPLO	11	2	2
33.	Rauscher-Azarowicz, 8/63	H; P	1	3	3
34.	Rauscher-Landon-Darrow, 1/69	S; Eosinp	2	4	4
35.	Rauscher-Moore-Jensen, 1/63	H; AML	10	2	2
36.	Rauscher-Reisinger, 5/67	H; BL	1	2	1
37.	Rauscher-Switzer, 3/67	S; T	2	2	2
38.	Sinkovics-Rauscher, 6/64-7/65	H; ALL	1	3	3
		H; AM MOL	1	2	2
		H; AMOL	1	2	2
		H; HD	1	1	1
		H; MH	1	2	2
39.	K. Smith, 1/68-4/69	H; W Tumr	1	8	1
40.	Smith, 10/65-1/66	S; PPLO	11	3	3
41.	Stevens, 11/68	H; BL	1	3	3
42.	Stewart-Rabson, 2/63	S; SV-40	1	9	5
43.	Stewart-Reisinger-Bowser, 4/67	H; S	1	4	3
44.	Theilen, 11/64-10/69	B; BOL	8	13	13
		B; LS	3	2	2
45.	Toolan, 3/64-5/66	H; H-1	1	21	21
46.	Trentin, 2/63-8/66	H; Ad 12	1	14	9
47.	Viola, 4/68-5/68	H; CLL	4	5	2
48.	Yohn, 11/63	S; Yaba	1	2	1
49.	Zeve, 2/68-5/68	S; AL	4	9	9

1. Spontaneous neoplasia

The following are cases of spontaneous neoplasia in members of the primate breeding colony.

a. 1967 - granulosa cell tumor, ovary, rhesus monkey, with recurrence in 3-1/2 years
b. 1969 - pleochromocytoma of adrenal gland, benign cystic teratoma, ovary, rhesus monkey
c. 1970 - granulosa theca cell tumor, ovary, with coelomic spread, rhesus monkey
d. 1971 - basal cell tumor, skin, rhesus monkey
e. 1971 - fibroleiomyoma, cervix, rhesus monkey
f. 1971 - papillary cystadenoma of possible mammary origin, Galago crassicaudatus

2. Animals manipulated elsewhere

In 1965, twelve rhesus monkeys were transferred from Brooks Air Force Base to Bionetics for continued maintenance and observation. These animals had undergone irradiation treatments from 1957 to 1960. In 1966, one animal developed sarcoma of the femur, basal cell carcinoma of the skin, and two kidney tumors. In another member of this group, a seminoma of one testicle, a meningioma, and a kidney tumor were found.

F. Addendum

1. Spontaneous neoplasia

The following are cases of spontaneous neoplasia in members of the primate breeding colony.

a. 1967 - granulosa cell tumor, ovary, rhesus monkey, with recurrence in 3-1/2 years
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c. 1970 - granulosa theca cell tumor, ovary, with coelomic spread, rhesus monkey
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SPECIAL VIRUS CANCER PROGRAM

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COVERING PERIOD FROM

July 1, 1970 - October 1, 1971

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VIRAL ONCOLOGY
CONTRACTOR DIRECTORY

As of October 1, 1971

The purpose of this directory is to facilitate and expedite communications between the VO staff members and VO contractors.

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CNTRCT TITLE: Virus Rescue Studies in Human Leukemia/Lymphoma Cell Lines
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SEG CHAIRMAN: Dr. Robert Manaker, Bldg. 37, Rm. 1B-16, x-63323
CNTRCT OFCR : Mr. J. Thomas Lewin, Bldg. 37, Rm. 1A-03, x-65025

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CNTRCT TITLE: Research on Genetic and Immunological Factors in Susceptibility to Murine Leukemia Viruses
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SEGMENT : Special Animal Leukemia Ecology Studies
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SEG CHAIRMAN: Dr. Robert Manaker, Bldg. 37, Rm. 1B-16, x-63323
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DATES : 9/1/71 - 8/31/72
PRINC INVEST: Dr. Norman G. Anderson
PROJ OFFICER: Dr. Charles Boone, Bldg. 37, Rm. 1C-06, x-65141
SEGMENT : Program Management
SEG CHAIRMAN: Dr. John Moloney, Bldg. 37, Rm. 1A-13, x-61038
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CNTRCT TITLE: Studies in Viral and Chemical Co-Carcinogenesis
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*Responsible for Carcinogenesis-supported portion of this contract

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PROJ OFFICER: Dr. Jack Gruber, Bldg. 37, Rm. 1B-14, x-63323
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PRINC INVEST: Dr. Robert Ting
PROJ OFFICER: Dr. George Todaro, Federal Bldg., Rm. 502, x-66135
Dr. Michael Chirigos, Bldg. 37, Rm. 1D-19, x-61478
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SEGMENT : Program Management
SEG CHAIRMAN: Dr. John Moloney, Bldg. 37, Rm. 1A-13, x-61038
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CONTRACTOR : Bionetics Research Laboratory (NIH-71-2025)
ADDRESS : 5510 Nicholson Lane, Kensington, Maryland 20795
PHONE : 881-5600
CNTRCT TITLE: Investigation of the Carcinogenic Activity of Selected Virus Preparation in the Newborn Monkey
DATES : 9/1/71 - 8/31/72
PRINC INVEST: Dr. John Landon
Dr. David Valerio
PROJ OFFICER: Dr. Roy Kinard, Federal Bldg., Rm. 504, x-66085
Dr. Jack Gruber, Bldg. 37, Rm. 1B-14, x-63323
SEGMENT : Developmental Research
SEG CHAIRMAN: Dr. Robert Manaker, Bldg. 37, Rm. 1B-16, x-63323
CNTRCT OFCR: Mr. J. Thomas Lewin, Bldg. 37, Rm. 1A-03, x-65025

CONTRACTOR : California, University of (PH-43-63-13)
ADDRESS : Naval Biomedical Research Labs, School of Public Health, Oakland, California 94625
PHONE : AC-415, Phone 832-5217
CNTRCT TITLE: Development and Evaluation of Cell Substrates for the Study of Cancer Viruses
DATES : 10/1/71 - 9/30/72
PRINC INVEST: Dr. Stewart H. Madin
PROJ OFFICER: Dr. James Duff, Bldg. 37, Rm. 1B-22, x-65967
Dr. George Todaro, Federal Bldg., Rm. 502, x-66135
SEGMENT : Solid Tumor-Virus
SEG CHAIRMAN: Dr. Robert Huebner, Bldg. 37, Rm. 2D-24, x-63301
CNTRCT OFCR : Mr. Thomas Porter, Bldg. 37, Rm. 1A-03, x-65025

CONTRACTOR : California, University of (NIH-70-2048)
ADDRESS : School of Veterinary Medicine, Davis, California 95616
PHONE : AC-916, Phone 752-1341
CNTRCT TITLE: Comparative Leukemia and Sarcoma Virus Studies
DATES : 1/1/71 - 12/31/71
PRINC INVEST: Dr. Leo K. Bustad
PROJ OFFICER: Dr. Michael Chirigos, Bldg. 37, Rm. 1D-15, x-61478
Dr. Gary Pearson, Bldg. 41, Suite 100, x-66080
SEGMENT : Special Animal Leukemia Ecology Studies
SEG CHAIRMAN: Dr. Michael Chirigos
CNTRCT OFCR : Mr. J. Thomas Lewin, Bldg. 37, Rm. 1A-03, x-65025

CONTRACTOR : California, University of (NIH-70-2202)
ADDRESS : San Diego, California
PHONE : AC-714, Phone 453-2000, Ext. 2503
CNTRCT TITLE: Development and Operation of a Breeding Colony of Domestic Cats
DATES : 6/23/71 - 2/5/72
PRINC INVEST: Dr. Alexis Kniazeff
PROJ OFFICER: Dr. Robert Holdenried, Federal Bldg., Rm. 5A-14, x-66085
Miss Marie Purdy, Federal Bldg., Rm. 508, x-66085
SEGMENT : Program Resources and Logistics
SEG CHAIRMAN: Dr. Robert Holdenried
CNTRCT OFCR : Mr. Fred Shaw, Federal Bldg., Rm. 512, x-61282

CONTRACTOR : California, University of (NIH-71-2147)
ADDRESS : 1326 3rd Avenue, San Francisco, California 94122
PHONE : AC-415, Phone 666-9000
CNTRCT TITLE: Study on the Role of Virion-Associated DNA Polymerase
DATES : 6/2/71 - 5/2/72
PRINC INVEST: Dr. J. Michael Bishop
PROJ OFFICER: Dr. James T. Duff, Bldg. 37, Rm. 1B-22, x-65967
SEGMENT : Solid Tumor-Virus
SEG CHAIRMAN: Dr. Robert Huebner, Bldg. 37, Rm. 2D-24, x-63301
CNTRCT OFCR : Mr. Thomas Porter, Bldg. 37, Rm. 1A-03, x-65025

CONTRACTOR : California, University of (NIH-71-2173)
ADDRESS : 118 California Hall, Berkeley, California 94720
PHONE : AC-415, Phone 642-0942
CNTRCT TITLE: Studies on the Structure and Replication of Viruses and Mechanisms of Regulation
DATES : 6/29/71 - 6/28/72
PRINC INVEST: Dr. Howard K. Schachman
PROJ OFFICER: Dr. Robert Huebner, Bldg. 37, Rm. 2D-24, x-63301
SEGMENT : Solid Tumor-Virus
SEG CHAIRMAN: Dr. Robert Huebner
CNTRCT OFCR : Mr. Thomas Porter, Bldg. 37, Rm. 1A-03, x-65025

CONTRACTOR : California, University of (NIH-72-2008)
ADDRESS : 405 Hilgard Avenue, Los Angeles, California 90024
PHONE : AC-213, Phone 825-7651
CNTRCT TITLE: Cellular Immunity to Tumor Antigens
DATES : 7/12/71 - 7/11/72
PRINC INVEST: Dr. Paul I. Terasaki
PROJ OFFICER: Dr. Ernest Plata, Bldg. 41, Rm. B-306, x-62120
Dr. Ronald Herberman, Bldg. 10, Rm. 3N-119, x-61366
SEGMENT : Immunology Group
SEG CHAIRMAN: Dr. Paul Levine, Federal Bldg., Rm. 504, x-66135
CNTRCT OFCR : Mr. J. Thomas Lewin, Bldg. 37, Rm. 1A-03, x-65025

CONTRACTOR : California State Department of Public Health (PH-43-68-997)
ADDRESS : 2151 Berkeley Way, Berkeley, California 94704
PHONE : AC-415, Phone 843-7900, Ext. 208
CNTRCT TITLE: Studies on the Possible Role of Oncogenic Viruses in the Causation of Cancer in Man and His Domestic Animals
DATES : 6/24/71 - 6/23/72
PRINC INVEST: Dr. Edwin Lennette
PROJ OFFICER: Dr. James Duff, Bldg. 37, Rm. 1B-22, x-65967
Dr. Padman Sarma, Bldg. 37, Rm. 2D-20, x-63301
Dr. Paul Arnstein, Calif. State Dept. of Public Health
SEGMENT : Solid Tumor-Virus
SEG CHAIRMAN: Dr. Robert Huebner, Bldg. 37, Rm. 2D-24, x-63301
CNTRCT OFCR : Mr. Thomas Porter, Bldg. 37, Rm. 1A-03, x-65025

CONTRACTOR : California State Department of Public Health (NIH-69-87)
ADDRESS : 744 P Street, Sacramento, California 95814
PHONE : AC-916, Phone 445-0813
CNTRCT TITLE: Cancer in Households: A Human-Feline Retrospective Study
DATES : 11/1/71 - 10/31/72
PRINC INVEST: Dr. Robert Schneider
PROJ OFFICER: Dr. James Duff, Bldg. 37, Rm. 1B-22, x-65967
Dr. Padman Sarma, Bldg. 37, Rm. 2D-24, x-63301
Dr. Paul Arnstein, Calif. State Dept. of Public Health
SEGMENT : Solid Tumor-Virus
SEG CHAIRMAN: Dr. Robert Huebner, Bldg. 37, Rm. 2D-24, x-63301
CNTRCT OFCR : Mr. Thomas Porter, Bldg. 37, Rm. 1A-03, x-65025

CONTRACTOR : Center for Disease Control (VCL-42)
ADDRESS : Atlanta, Georgia
PHONE : AC-404, Phone 633-3311
CNTRCT TITLE: Etiologic Studies of Leukemia and Related Disease Occurring in Unusual Epidemiological or Genetic Situations
DATES : 7/1/71 - 6/30/72
PRINC INVEST: Dr. Clark W. Heath, Jr.
PROJ OFFICER: Dr. Adi F. Gazdar, Bldg. 41, Rm. A-104, x-64835
Dr. Gary Pearson, Bldg. 41, Suite 100, x-66080
SEGMENT : Special Animal Leukemia Ecology Studies
SEG CHAIRMAN: Dr. Michael Chirigos, Bldg. 37, Rm. 1D-15, x-61478
CNTRCT OFCR : Mr. J. Thomas Lewin, Bldg. 37, Rm. 1A-03, x-65025

CONTRACTOR : Chicago Park District (Lincoln Park Zoo) (PH-43-65-1017)
ADDRESS : 100 West Webster, Chicago, Illinois 60614
PHONE : AC-312, Phone 549-3000
CNTRCT TITLE: Marmoset Breeding Colony
DATES : 10/1/71 - 9/30/72
PRINC INVEST: Dr. Lester Fisher
Dr. F. Deinhardt
PROJ OFFICER: Dr. Roy Kinard, Federal Bldg., Rm. 504, x-66085
Dr. Jack Gruber, Bldg. 37, Rm. 1B-14, x-63323
SEGMENT : Developmental Research
SEG CHAIRMAN: Dr. Robert Manaker, Bldg. 37, Rm. 1B-16, x-63323
CNTRCT OFCR : Mr. J. Thomas Lewin, Bldg. 37, Rm. 1A-03, x-65025

CONTRACTOR : Children's Hospital of Philadelphia (PH-43-66-477)
ADDRESS : 1740 Bainbridge Street, Philadelphia, Pennsylvania 19146
PHONE : AC-215, Phone 546-2700
CNTRCT TITLE: Immunofluorescence Studies of Human Leukemia, Lymphomas
DATES : 2/4/71 - 1/31/72
PRINC INVEST: Dr. Gertrude Henle
PROJ OFFICER: Dr. Virginia Dunkel, Bldg. 41, Rm. B1-06, x-66738
Dr. Jack Gruber, Bldg. 37, Rm. 1B-14, x-63323
SEGMENT : Immunology Group
SEG CHAIRMAN: Dr. Paul Levine, Federal Bldg., Rm. 504, x-66135
CNTRCT OFCR : Mr. J. Thomas Lewin, Bldg. 37, Rm. 1A-03, x-65025

CONTRACTOR : Colorado, University of (NIH-69-2080)
ADDRESS : Medical Center, 4200 East Ninth Avenue, Denver Colorado 80220
PHONE : AC-303, Phone 394-8471
CNTRCT TITLE: Collection of Pediatric Tumor Specimens
DATES : 6/18/71 - 6/17/72
PRINC INVEST: Dr. William E. Hathaway
PROJ OFFICER: Dr. Paul Levine, Federal Bldg., Rm. 504, x-66135
SEGMENT : Program Resources and Logistics
SEG CHAIRMAN: Dr. Robert Holdenried, Federal Bldg., Rm. 5A-14, x-66085
CNTRCT OFCR : Mr. Fred Shaw, Federal Bldg., Rm. 512, x-61282

CONTRACTOR : Columbia University (NIH-70-2049)
ADDRESS : Institute for Cancer Research, College of Physicians & Surgeons
99 Ft. Washington Avenue, New York, N.Y. 10032
PHONE : AC-212, Phone 579-8582
CNTRCT TITLE: RNA and RNA Replicases in Tumor Cells Associated with RNA Oncogenic Viruses
DATES : 3/1/71 - 1/31/72
PRINC INVEST: Dr. Sol Spiegelman
PROJ OFFICER: Dr. Timothy O'Connor, Bldg. 41, Rm. A-107, x-63647
Dr. Robert Manaker, Bldg. 37, Rm. 1B-16, x-63323
Dr. Jack Gruber, Bldg. 37, Rm. 1B-14, x-63323
SEGMENT : Developmental Research
SEG CHAIRMAN: Dr. Robert Manaker
CNTRCT OFCR : Mr. J. Thomas Lewin, Bldg. 37, Rm. 1A-03, x-65025

CONTRACTOR : Connecticut, University of (NIH-69-52)
ADDRESS : Department of Animal Diseases, College of Agriculture, Storrs, Connecticut 06268
PHONE : AC-203, Phone 429-3311
CNTRCT TITLE: Establishment of a Specific Pathogen Free Flock of White Leghorn Chickens
DATES : 10/1/71 - 9/30/72
PRINC INVEST: Dr. Roy E. Luginbuhl
PROJ OFFICER: Dr. Roy Kinard, Federal Bldg., Rm. 504, x-66085
Dr. Robert Holdenried, Federal Bldg., Rm. 5A-14, x-66085
SEGMENT : Program Resources and Logistics
SEG CHAIRMAN: Dr. Robert Holdenried
CNTRCT OFCR : Mr. Fred Shaw, Federal Bldg., Rm. 512, x-61282

CONTRACTOR : Cornell University, N.Y. State Vet. Coll. (NIH-70-2224)
ADDRESS : Ithaca, New York 14850
PHONE : AC-607, Phone 256-2034
CNTRCT TITLE: Feline Tumor Viral Diagnostic Laboratory
DATES : 6/25/71 - 6/24/72
PRINC INVEST: Dr. James Gillespie
PROJ OFFICER: Dr. James T. Duff, Bldg. 37, Rm. 1B-22, x-65967
SEGMENT : Program Resources and Logistics
SEG CHAIRMAN: Dr. Robert Holdenried, Federal Bldg., Rm. 5A-14, x-66085
CNTRCT OFCR : Mr. Fred Shaw, Federal Bldg., Rm. 512, x-61282

CONTRACTOR : Cornell University, N.Y. State Vet. Coll. (NIH-71-2508)
ADDRESS : Department of Pathology, Ithaca, New York 14850
PHONE : AC-607, Phone 256-5014
CNTRCT TITLE: Leukemia Studies in the Cat
DATES : 6/23/71 - 6/22/72
PRINC INVEST: Dr. Charles Rickard
PROJ OFFICER: Dr. Michael Chirigos, Bldg. 37, Rm. 1D-15, x-61478
Dr. Wilna Woods, Bldg. 37, Rm. 1D-15, x-61478
SEGMENT : Special Animal Leukemia Ecology Studies
SEG CHAIRMAN: Dr. Michael Chirigos
CNTRCT OFCR : Mr. J. Thomas Lewin, Bldg. 37, Rm. 1A-03, x-65025

CONTRACTOR : Dow Chemical Company (Pittman-Moore) (PH-43-65-1045)
ADDRESS : P.O. Box 10, Zionsville, Indiana 46077
PHONE : AC-317, Phone 638-2521
CNTRCT TITLE: Research and Development of Biohazards Control and Containment Facilities
DATES : 2/1/71 - 1/31/72
PRINC INVEST: Mr. Cyril Henke
Dr. Paul Maupin
PROJ OFFICER: Mr. W. Emmitt Barkley, Bldg. 41, Rm. A-118, x-64421
Dr. Alfred Hellman, Bldg. 41, Rm. A-103, x-66758
SEGMENT : Biohazards Control and Containment
SEG CHAIRMAN: Dr. Alfred Hellman
CNTRCT OFCR : Mr. J. Thomas Lewin, Bldg. 37, Rm. 1A-03, x-65025

CONTRACTOR : Duke University (NIH-71-2132)
ADDRESS : Durham, North Carolina 27706
PHONE : AC-919, Phone 684-6468
CNTRCT TITLE: Study and Production of Avian Leukosis Viruses
DATES : 4/19/71 - 4/18/72
PRINC INVEST: Dr. Joseph W. Beard
PROJ OFFICER: Dr. Michael Chirigos, Bldg. 37, Rm. 1D-15, x-61478
Dr. John Pearson, Bldg. 37, Rm. 1D-15, x-61478
SEGMENT : Special Animal Leukemia Ecology Studies
SEG CHAIRMAN: Dr. Michael Chirigos
CNTRCT OFCR : Mr. J. Thomas Lewin, Bldg. 37, Rm. 1A-03, x-65025

CONTRACTOR : Electro -Nucleonics (NIH-71-2253)
ADDRESS : 4921 Auburn Avenue, Bethesda, Maryland 20014
PHONE : 652-7164
CNTRCT TITLE: Development of Propagation Procedures, Purification and Characterization of Viruses
DATES : 5/28/71 - 5/27/72
PRINC INVEST: Dr. Irving Toplin
PROJ OFFICER: Dr. George Todaro, Federal Bldg., Rm. 502, x-66135
SEGMENT : Program Resources and Logistics
SEG CHAIRMAN: Dr. Robert Holdenried, Federal Bldg., Rm. 5A-14, x-66085
CNTRCT OFCR : Mr. Fred Shaw, Federal Bldg., Rm. 512, x-61282

CONTRACTOR : Emory University (NIH-71-2256)
ADDRESS : Yerkes Regional Primate Research Center, Atlanta, Georgia 30322
PHONE : AC-404, Phone 377-2411, Ext. 7974
CNTRCT TITLE: Maintenance of Colony of Irradiated Rhesus Monkeys
DATES : 5/1/71 - 4/30/72
PRINC INVEST: Dr. Harold M. McClure
PROJ OFFICER: Dr. Roy Kinard, Federal Bldg., Rm. 504, x-66085
SEGMENT : Program Resources and Logistics
SEG CHAIRMAN: Dr. Robert Holdenried, Federal Bldg., Rm. 5A-14, x-66085
CNTRCT OFCR : Mr. Fred Shaw, Federal Bldg., Rm. 512, x-61282

CONTRACTOR : Flow Laboratories (PH-43-65-1012)
ADDRESS : 1710 Chapman Avenue, Rockville, Maryland 20852
PHONE : 881-2900
CNTRCT TITLE: Maintenance of a Repository for Storage and Distribution of Reagents and Tissue Specimens
DATES : 7/1/71 - 6/30/72
PRINC INVEST: Mr. Jack W. Walker
PROJ OFFICER: Miss Marie E. Purdy, Federal Bldg., Rm. 508, x-66085
SEGMENT : Program Resources and Logistics
SEG CHAIRMAN: Dr. Robert Holdenried, Federal Bldg., Rm. 5A-14, x-66085
CNTRCT OFCR : Mr. Fred Shaw, Federal Bldg., Rm. 512, x-61282

CONTRACTOR : Flow Laboratories (NIH-71-2097)
ADDRESS : 1710 Chapman Avenue, Rockville, Maryland 20852
PHONE : 881-2900
CNTRCT TITLE: Studies of Herpes Viruses and C-Type Viruses in Relation to Oncogenic Potential
DATES : 2/1/71 - 1/31/72
PRINC INVEST: Dr. Raymond V. Gilden
PROJ OFFICER: Dr. Robert Huebner, Bldg. 37, Rm. 2D-24, x-63301
SEGMENT : Solid Tumor-Virus
SEG CHAIRMAN: Dr. Robert Huebner
CNTRCT OFCR : Mr. Thomas Porter, Bldg. 37, Rm. 1A-03, x-65025

CONTRACTOR : Flow Laboratories (NIH-71-2341)
ADDRESS : 1710 Chapman Avenue, Rockville, Maryland 20852
PHONE : 881-2900
CNTRCT TITLE: The Provision of an Animal Holding Facility
DATES : 6/18/71 - 6/17/72
PRINC INVEST: Dr. W. Knapp
PROJ OFFICER: Dr. John Pearson, Bldg. 37, Rm. 1D-15, x-61478
Dr. Adi Gazdar, Bldg. 41, Rm. A-104, x-64835
SEGMENT : Special Animal Leukemia Ecology Studies
SEG CHAIRMAN: Dr. Michael Chirigos, Bldg. 37, Rm. 1D-15, x-61478
CNTRCT OFCR : Mr. J. Thomas Lewin, Bldg. 37, Rm. 1A-03, x-65025

CONTRACTOR : Georgetown University (PH-43-65-53)
ADDRESS : 3800 Reservoir Road, Washington, D.C. 20007
PHONE : 625-0100
CNTRCT TITLE: Human Breast Cancer Virus Studies
DATES : 9/1/71 - 11/30/72
PRINC INVEST: Dr. William F. Feller
PROJ OFFICER: Dr. W. Ray Bryan, Federal Bldg., Rm. 4C-08, x-64533
Mr. John Kvedar, Bldg. 41, Rm. C-503, x-65334
Dr. Louis Sibal, Bldg. 37, Rm. 1A-15, x-62796
SEGMENT : Breast Tumor Task Force
SEG CHAIRMAN: Dr. W. Ray Bryan
CNTRCT OFCR : Mr. Fred Shaw, Federal Bldg., Rm. 512, x-61282

CONTRACTOR : Germfree Life Research Center (PH-43-65-95)
ADDRESS : 3301 College Avenue, Fort Lauderdale, Florida 33312
PHONE : AC-305, Phone 587-6660, Ext. 235
CNTRCT TITLE: Research on Low Dose Virus-Induced Tumors and Other Viral Carcinogenesis Studies in Germfree Animals (Germfree Life and Oncogenesis)
DATES : 1/1/71 - 12/31/71
PRINC INVEST: Dr. Joel Warren
PROJ OFFICER: Dr. Michael Chirigos, Bldg. 37, Rm. 1D-15, x-61478
Dr. W. Ray Bryan, Federal Bldg., Rm. 4C-08, x-64533
Dr. John Pearson, Bldg. 37, Rm. 1D-15, x-61478
SEGMENT : Special Animal Leukemia Ecology Studies
SEG CHAIRMAN: Dr. Michael Chirigos, Bldg. 37, Rm. 1D-15, x-61478
CNTRCT OFCR : Mr. J. Thomas Lewin, Bldg. 37, Rm. 1A-03, x-65025

CONTRACTOR : Hazleton Labs., Inc. (NIH-69-2079)
ADDRESS : A Subsidiary of TRW, Inc., 9200 Leesburg Turnpike, Vienna, Virginia 22180
PHONE : AC-703, Phone 893-5400
CNTRCT TITLE: Etiology of Cancer in Dogs
DATES : 9/1/71 - 8/31/72
PRINC INVEST: Dr. Erling M. Jensen
PROJ OFFICER: Dr. Wilna Woods, Bldg. 37, Rm. 1D-15, x-61478
Dr. John Pearson, Bldg. 37, Rm. 1D-15, x-61478
Dr. Thomas Cameron, Bldg. 37, Rm. 5E-12B, x-61323
SEGMENT : Special Animal Leukemia Ecology Studies
SEG CHAIRMAN: Dr. Michael Chirigos, Bldg. 37, Rm. 1D-15, x-61478
CNTRCT OFCR : Mr. J. Thomas Lewin, Bldg. 37, Rm. 1A-03, x-65025

CONTRACTOR : Health Research Inc., Roswell Park Division (PH-43-63-593)
ADDRESS : 666 Elm Street, Buffalo, New York 14203
PHONE : AC-716, Phone 845-2300
CNTRCT TITLE: Biological and Electron Microscopic Studies of Viruses from Leukemia Cells, Tissues and Plasma
DATES : 7/1/71 - 12/13/71
PRINC INVEST: Dr. Edwin Mirand
PROJ OFFICER: Dr. Jack Gruber, Bldg. 37, Rm. 1B-14, x-63323
Dr. Roy Kinard, Federal Bldg., Rm. 504, x-66085
SEGMENT : Developmental Research
SEG CHAIRMAN: Dr. Robert Manaker, Bldg. 37, Rm. 1B-16, x-63323
CNTRCT OFCR : Mr. J. Thomas Lewin, Bldg. 37, Rm. 1A-03, x-65025

CONTRACTOR : Health Research, Inc. (NIH-72-2014)
ADDRESS : Roswell Park Division, 666 Elm Street, Buffalo, New York 14203
PHONE : AC-716, Phone 845-2300
CNTRCT TITLE: Stimulation of Immunity Against Tumor by Enzymatically Treated Autochthonous Tumor Cells
DATES : 9/15/71 - 9/14/72
PRINC INVEST: Dr. James Holland
PROJ OFFICER: Dr. Paul Levine, Federal Bldg., Rm. 504, x-66135

SEGMENT : Immunology Group
SEG CHAIRMAN: Dr. Paul Levine, Federal Bldg., Rm. 504, x-66135
CNTRCT OFCR : Mr. J. Thomas Lewin, Bldg. 37, Rm. 1A-03, x-65025

CONTRACTOR : Hospital for Sick Children (PH-43-65-97)
ADDRESS : 555 University Avenue, Toronto 2, Ontario, Canada
PHONE : AC-416, Phone 366-7242
CNTRCT TITLE: Collection of Specimens from Human Pediatric Leukemia Patients and Non-Leukemia Controls
DATES : 6/1/71 - 5/31/72
PRINC INVEST: Dr. Peter D. McClure
PROJ OFFICER: Dr. Paul Levine, Federal Bldg., Rm. 504, x-66135
Dr. Charles W. Boone, Bldg. 37, Rm. 1C-06, x-65141
SEGMENT : Program Resources and Logistics
SEG CHAIRMAN: Dr. Robert Holdenried, Federal Bldg., Rm. 5A-14, x-66085
CNTRCT OFCR : Mr. Fred Shaw, Federal Bldg., Rm. 512, x-61282

CONTRACTOR : Howard University (NIH-70-2178)
ADDRESS : 2400 Sixth Street, Washington, D.C.
PHONE : 265-0832
CNTRCT TITLE: Immunological Studies on Human Mammary Tumors and other Neoplasms
DATES : 5/1/71 - 4/30/72
PRINC INVEST: Dr. Michael Viola
PROJ OFFICER: Dr. Willie Turner, Bldg. 37, Rm. 1B-13, x-62600
Dr. Dan Rubin, Bldg. 37, Rm. 1C-09, x-62760
SEGMENT : Breast Tumor Task Force
SEG CHAIRMAN: Dr. W. Ray Bryan, Federal Bldg., Rm. 4C-08, x-64533
CNTRCT OFCR : Mr. Fred Shaw, Federal Bldg., Rm. 512, x-61282

CONTRACTOR : Huntingdon Research Center (NIH-69-54)
ADDRESS : P.O. Box 6857, Baltimore, Maryland 21204
PHONE : AC-301, Phone 825-3484
CNTRCT TITLE: Fluorescent Antibody Studies of Indigenous Mouse Viruses
DATES : 10/1/71 - 9/30/72
PRINC INVEST: Dr. Roger Wilsnack
PROJ OFFICER: Dr. Robert Holdenried, Federal Bldg., Rm. 5A-14, x-66085
Dr. Wallace P. Rowe, Bldg. 7, Rm. 304, x-62613
Dr. Dan Rubin, Bldg. 37, Rm. 1C-09, x-62760
SEGMENT : Program Resources and Logistics
SEG CHAIRMAN: Dr. Robert Holdenried
CNTRCT OFCR : Mr. Fred Shaw, Federal Bldg., Rm. 512, x-61282

CONTRACTOR : Indiana State University (NIH-69-2048)
ADDRESS : Center for Medical Education, 135 Holmstedt Hall, Terre Haute, Indiana 57809
PHONE : AC-812, Phone 232-6311, Ext. 2777
CNTRCT TITLE: Characteristics of Twiehaus Agent of Avian Reticuloendotheliosis
DATES : 11/8/71 - 11/7/72
PRINC INVEST: Dr. Alvin Levine
PROJ OFFICER: Dr. Michael Chirigos, Bldg. 37, Rm. 1D-15, x-61478
Dr. John Pearson, Bldg. 37, Rm. 1D-15, x-61478
SEGMENT : Special Animal Leukemia Ecology Studies
SEG CHAIRMAN: Dr. Michael Chirigos
CNTRCT OFCR : Mr. J. Thomas Lewin, Bldg. 37, Rm. 1A-03, x-65025

CONTRACTOR : Institute for Medical Research (PH-43-68-1000)
ADDRESS : Copewood Street, Camden, New Jersey 08103
PHONE : AC-609, Phone 966-7377
CNTRCT TITLE: Studies of Human Milk and Mammary Tumors
DATES : 6/28/71 - 4/30/72
PRINC INVEST: Dr. Dan Moore
PROJ OFFICER: Dr. W. Ray Bryan, Federal Bldg., Rm. 4C-08, x-64533
SEGMENT : Breast Tumor Task Force
SEG CHAIRMAN: Dr. W. Ray Bryan
CNTRCT OFCR : Mr. Fred Shaw, Federal Bldg., Rm. 512, x-61282

CONTRACTOR : International Agency for Research on Cancer (NIH-70-2076)
ADDRESS : 16 Avenue Marechal Foch, 69, Lyon, France
PHONE : AC-78, Phone 52-32-40
CNTRCT TITLE: Prospective Sero-Epidemiological Study of Burkitt's Lymphoma in East Africa
DATES : 1/1/71 - 12/31/71
PRINC INVEST: Dr. G. Blaudin de Thé
PROJ OFFICER: Dr. Robert Depue, Bldg. 31, Rm. 11A-11, x-66271
Dr. Virginia Dunkel, Bldg. 41, Rm. B1-06, x-66738
SEGMENT : Immunology Group
SEG CHAIRMAN: Dr. Paul Levine, Federal Bldg., Rm. 504, x-66135
CNTRCT OFCR : Mr. J. Thomas Lewin, Bldg. 37, Rm. 1A-03, x-65025

CONTRACTOR : Jackson Laboratory (PH-43-67-744)
ADDRESS : Bar Harbor, Maine 04609
PHONE : AC-207, Phone 288-3373
CNTRCT TITLE: Murine Leukemia-Sarcoma Complex: Natural Occurrence of Leukemia Virus and the Sarcoma Genome in Mice
DATES : 5/1/71 - 4/30/72
PRINC INVEST: Dr. Hans Meier
PROJ OFFICER: Dr. Robert Huebner, Bldg. 37, Rm. 2D-24, x-63301
SEGMENT : Solid Tumor-Virus
SEG CHAIRMAN: Dr. Robert Huebner
CNTRCT OFCR : Mr. Thomas Porter, Bldg. 37, Rm. 1A-03, x-65025

CONTRACTOR : Jewish Hospital & Medical Center of Brooklyn (NIH-71-2046)
ADDRESS : 555 Prospect Place, Brooklyn, New York 11238
PHONE : AC-212, Phone Ulster 7-8700
CNTRCT TITLE: Viral Transformation and Chromosome Abnormalities in Human Tumors
DATES : 10/1/71 - 9/30/72
PRINC INVEST: Dr. Harvey Dosik
PROJ OFFICER: Dr. George Todaro, Federal Bldg., Rm. 502, x-66135
SEGMENT : Program Resources and Logistics
SEG CHAIRMAN: Dr. Robert Holdenried, Federal Bldg., Rm. 5A-14, x-66085
CNTRCT OFCR : Mr. Fred Shaw, Federal Bldg., Rm. 512, x-61282

CONTRACTOR : Johns Hopkins University (NIH-69-2008)
ADDRESS : School of Hygiene & Public Health, 615 N. Wolfe Street, Baltimore, Maryland 21205
PHONE : AC-301, Phone 955-3457
CNTRCT TITLE: Maintenance of a Foundation Colony of Leukosis-Free Chickens
DATES : 3/24/71 - 3/23/72
PRINC INVEST: Dr. Frederick B. Bang
PROJ OFFICER: Dr. W. Ray Bryan, Federal Bldg., Rm. 4C-08, x-64533
Mr. John Kvedar, Bldg. 41, Rm. C-503, x-65334
SEGMENT : Program Resources and Logistics
SEG CHAIRMAN: Dr. Robert Holdenried, Federal Bldg., Rm. 5A-14, x-66085
CNTRCT OFCR : Mr. Fred Shaw, Federal Bldg., Rm. 512, x-61282

CONTRACTOR : Johns Hopkins University (NIH-71-2109)
ADDRESS : Charles & 34th Street, Baltimore, Maryland 21218
PHONE : AC-301, Phone 366-3300
CNTRCT TITLE: Antitumor Reactivity in Patients with Leukemia/Lymphoma
DATES : 5/1/71 - 4/30/72
PRINC INVEST: Dr. George W. Santos
PROJ OFFICER: Dr. Ronald Herberman, Bldg. 10, Rm. 3N-119, x-61366
Dr. Dan Rubin, Bldg. 37, Rm. 1C-09, x-62760
SEGMENT : Immunology Group
SEG CHAIRMAN: Dr. Paul Levine, Federal Bldg., Rm. 504, x-66135
CNTRCT OFCR : Mr. J. Thomas Lewin, Bldg. 37, Rm. 1A-03, x-65025

CONTRACTOR : Johns Hopkins University (NIH-71-2121)
ADDRESS : Charles & 34th Streets, Baltimore, Maryland 21218
PHONE : AC-301, Phone 366-3300
CNTRCT TITLE: Studies on Herpes Virus Antigens and Virions in Neoplastic Cells from Cervical Carcinoma
DATES : 5/5/71 - 5/4/72
PRINC INVEST: Dr. Laure Aurelian
PROJ OFFICER: Dr. Jack Gruber, Bldg. 37, Rm. 1B-14, x-63323
Dr. Robert Manaker, Bldg. 37, Rm. 1B-16, x-63323
SEGMENT : Developmental Research
SEG CHAIRMAN: Dr. Robert Manaker
CNTRCT OFCR : Mr. J. Thomas Lewin, Bldg. 37, Rm. 1A-03, x-65025

CONTRACTOR : Karolinska Institutet (NIH-69-2005)
ADDRESS : Torsplan 7, S-10401, Stockholm, Sweden
PHONE :
CNTRCT TITLE: Studies of the Significance of Herpes-Type Virus in the Etiology of Some Human Cancers
DATES : 4/9/71 - 4/8/72
PRINC INVEST: Dr. George Klein
PROJ OFFICER: Dr. Virginia Dunkel, Bldg. 41, Rm. B1-06, x-66738
Dr. Jack Gruber, Bldg. 37, Rm. 1B-14, x-63323
SEGMENT : Developmental Research
SEG CHAIRMAN: Dr. Robert Manaker, Bldg. 37, Rm. 1B-16, x-63323
CNTRCT OFCR : Mr. J. Thomas Lewin, Bldg. 37, Rm. 1A-03, x-65025

CONTRACTOR : Life Sciences, Inc. (PH-43-68-711)
ADDRESS : 2950 72nd Street North, St. Petersburg, Florida 33710
PHONE : AC-813, Phone 345-9371
CNTRCT TITLE: Production and Maintenance of Germfree and Selected Reagent Grade SPF Animals
DATES : 8/1/71 - 7/31/72
PRINC INVEST: Dr. Wendall Farrow
PROJ OFFICER: Mr. John Kvedar, Bldg. 41, Rm. C-503, x-65334
Dr. W. Ray Bryan, Federal Bldg., Rm. 4C-08, x-64533
SEGMENT : Program Resources and Logistics
SEG CHAIRMAN: Dr. Robert Holdenried, Federal Bldg., Rm. 5A-14, x-66085
CNTRCT OFCR : Mr. Fred Shaw, Federal Bldg., Rm. 312, x-61282

CONTRACTOR : Life Sciences, Inc. (NIH-69-63)
ADDRESS : 2950 72nd Street North, St. Petersburg, Florida 33710
PHONE : AC-813, Phone 347-6191
CNTRCT TITLE: Studies on Marek's Disease as a Model for Herpesvirus-Associated Oncogenesis
DATES : 8/1/71 - 7/31/72
PRINC INVEST: Dr. Jack W. Frankel
PROJ OFFICER: Dr. Michael Chirigos, Bldg. 37, Rm. 1D-15, x-61478
Dr. W. Ray Bryan, Federal Bldg., Rm. 4C-08, x-64533
Dr. Gary Pearson, Bldg. 41, Suite 100, x-66080
SEGMENT : Special Animal Leukemia Ecology Studies
SEG CHAIRMAN: Dr. Michael Chirigos
CNTRCT OFCR : Mr. J. Thomas Lewin, Bldg. 37, Rm. 1A-03, x-65025

CONTRACTOR : Louisville, University of (PH-43-66-902)
ADDRESS : School of Medicine, Department of Microbiology, 101 Chestnut Street, Louisville, Kentucky 40202
PHONE : AC-502, Phone 582-2211
CNTRCT TITLE: Studies on Foamy Virus Reagents and Antisera
DATES : 7/1/71 - 6/30/72
PRINC INVEST: Dr. Paul B. Johnston
PROJ OFFICER: Dr. Robert Holdenried, Federal Bldg., Rm. 5A-14, x-66085
Dr. J. Anthony Morris, Bldg. 29A, Rm. 2D-16, x-63672
SEGMENT : Program Resources and Logistics
SEG CHAIRMAN: Dr. Robert Holdenried
CNTRCT OFCR : Mr. Fred Shaw, Federal Bldg., Rm. 512, x-61282

CONTRACTOR : Makerere University Medical School (PH-43-67-47)
ADDRESS : P.O. Box 2072, Kampala, Uganda, East Africa
PHONE :
CNTRCT TITLE: Epidemiological Investigation of Burkitt Lymphoma in Uganda
DATES : 9/26/71 - 9/25/72
PRINC INVEST: Dr. George Kafuko
PROJ OFFICER: Dr. Robert Depue, Bldg. 31, Rm. 11A-11, x-66271
Dr. Charles Boone, Bldg. 37, Rm. 1C-06, x-65141
SEGMENT : Program Resources and Logistics
SEG CHAIRMAN: Dr. Robert Holdenried, Federal Bldg., Rm. 5A-14, x-66085
CNTRCT OFCR : Mr. Fred Shaw, Federal Bldg., Rm. 512, x-61282

CONTRACTOR : Mason Research Institute (NIH-70-2204)
ADDRESS : Harvard Street, Worcester, Massachusetts 01608
PHONE : AC-617, Phone 752-4601
CNTRCT TITLE: Study on the Role of Hormonal Factors on Induction of Breast Tumors
DATES : 6/7/71 - 6/6/72
PRINC INVEST: Dr. Marcus M. Mason
PROJ OFFICER: Dr. W. Ray Bryan, Federal Bldg., Rm. 4C-08, x-64533
Dr. D. Jane Taylor, Federal Bldg., Rm. 4C-04A, x-66718
Dr. Roy Kinard, Federal Bldg., Rm. 504, x-66085
SEGMENT : Breast Tumor Task Force
SEG CHAIRMAN: Dr. W. Ray Bryan
CNTRCT OFCR : Mr. Fred Shaw, Federal Bldg., Rm. 512, x-61282

CONTRACTOR : Massachusetts General Hospital (NIH-71-2174)
ADDRESS : John Collins Warren Laboratory, Boston, Massachusetts 02114
PHONE : AC-617, Phone 726-3671
CNTRCT TITLE: Transfer RNA Studies
DATES : 6/29/71 - 6/28/72
PRINC INVEST: Dr. Paul Zamecnik
PROJ OFFICER: Dr. Timothy O'Connor, Bldg. 41, Rm. A-107, x-63647
Dr. Padman Sarma, Bldg. 37, Rm. 2D-20, x-63301
SEGMENT : Developmental Research
SEG CHAIRMAN: Dr. Robert Manaker, Bldg. 37, Rm. 1B-16, x-63323
CNTRCT OFCR : Mr. J. Thomas Lewin, Bldg. 37, Rm. 1A-03, x-65025

CONTRACTOR : Massachusetts General Hospital (NIH-72-2012)
ADDRESS : Harvard Medical School, Fruit Street, Boston, Massachusetts 02114
PHONE : AC-617, Phone 726-3812
CNTRCT TITLE: Activation of C-Particles and Induction of Cancer by Immunologic and Non-Immunologic Methods
DATES :
PRINC INVEST: Dr. Paul H. Black
PROJ OFFICER: Dr. Adi F. Gazdar, Bldg. 41, Rm. A-104, x-64835
Dr. Jack Gruber, Bldg. 37, Rm. 1B-14, x-63323
SEGMENT : Developmental Research
SEG CHAIRMAN: Dr. Robert Manaker, Bldg. 37, Rm. 1B-16, x-63323
CNTRCT OFCR : Mr. J. Thomas Lewin, Bldg. 37, Rm. 1A-03, x-65025

CONTRACTOR : Massachusetts Institute of Technology (NIH-71-2149)
ADDRESS : Division of Sponsored Research, Cambridge, Massachusetts 02139
PHONE : AC-617, Phone 846-6900, Ext. 4725
CNTRCT TITLE: Studies on RNA Dependent DNA Polymerase
DATES : 5/1/71 - 4/30/72
PRINC INVEST: Dr. David Baltimore
PROJ OFFICER: Dr. George Todaro, Federal Bldg., Rm. 502, x-66135
Dr. Edward Scolnick, Federal Bldg., Rm. 504, x-66135
SEGMENT : Program Management
SEG CHAIRMAN: Dr. John Moloney, Bldg. 37, Rm. 1A-13, x-61038
CNTRCT OFCR : Mr. Thomas Porter, Bldg. 37, Rm. 1A-03, x-65025

CONTRACTOR : Medical College of Wisconsin (PH-43-68-1010)
ADDRESS : 8700 West Wisconsin Avenue, Milwaukee, Wisconsin 63226
PHONE : AC-414, Phone 344-1000
CNTRCT TITLE: Protein and Steroid Hormone Effects on Virus Production in C-Particle Containing Cancer Cells In Vitro
DATES : 10/1/71 - 9/30/72
PRINC INVEST: Dr. Roland Patillo, Colony Hospital, AC-414, Phone 258-4774
PROJ OFFICER: Dr. Robert Depue, Bldg. 31, Rm. 11A-11, x-62271
SEGMENT : Program Resources and Logistics
SEG CHAIRMAN: Dr. Robert Holdenried, Federal Bldg., Rm. 5A-14, x-66085
CNTRCT OFCR : Mr. Fred Shaw, Federal Bldg., Rm. 512, x-61282

CONTRACTOR : Meloy Laboratories, Inc. (PH-43-66-458)
ADDRESS : 6631 Iron Place, Springfield, Virginia 22151
PHONE : 354-2600
CNTRCT TITLE: Bioassays of Mouse Mammary Tumor Virus
DATES : 1/1/71 - 12/31/71
PRINC INVEST: Dr. John Verna
PROJ OFFICER: Dr. Louis Sibal, Bldg. 37, Rm. 1A-15, x-62796
Dr. W. Ray Bryan, Federal Bldg., Rm. 4C-08, x-64533
SEGMENT : Breast Tumor Task Force
SEG CHAIRMAN: Dr. W. Ray Bryan
CNTRCT OFCR : Mr. Fred Shaw, Federal Bldg., Rm. 512, x-61282

CONTRACTOR : Meloy Laboratories, Inc. (NIH-70-2047)
ADDRESS : 6631 Iron Place, Springfield, Virginia 22151
PHONE : 354-2600
CNTRCT TITLE: Cell Biology Research Facility
DATES : 10/1/71 - 4/15/72*
PRINC INVEST: Dr. John Verna
PROJ OFFICER: Dr. Charles Boone, Bldg. 37, Rm. 1C-06, x-65141
Dr. George Todaro, Federal Bldg., Rm. 502, x-66135
SEGMENT : Program Management
SEG CHAIRMAN: Dr. John Moloney, Bldg. 37, Rm. 1A-13, x-61038
CNTRCT OFCR : Mr. Thomas Porter, Bldg. 37, Rm. 1A-03, x-65025

CONTRACTOR : Memorial Hosp. for Cancer & Allied Diseases (NIH-71-2116)
ADDRESS : 444 East 68th Street, New York, N.Y. 10021
PHONE : AC-212, Phone 879-3000
CNTRCT TITLE: Acquisition of Human Materials to be Used in the Search for Transmissible Agents in Human Tumors
DATES : 3/1/71 - 3/17/72
PRINC INVEST: Dr. Yashar Hirshaut
PROJ OFFICER: Dr. Jack Gruber, Bldg. 37, Rm. 1B-14, x-63323
Dr. Robert Manaker, Bldg. 37, Rm. 1B-16, x-63323
SEGMENT : Developmental Research
SEG CHAIRMAN: Dr. Robert Manaker
CNTRCT OFCR : Mr. J. Thomas Lewin, Bldg. 37, Rm. 1A-03, x-65025

*Contract will be renewed as two separate contracts; Dr. Boone taking Task A and Dr. Todaro taking Task B.

CONTRACTOR : Memorial Hosp. for Cancer & Allied Diseases (NIH-71-2194)
ADDRESS : 444 East 68th Street, New York, N.Y. 10021
PHONE : AC-212, Phone 879-3000
CNTRCT TITLE: Collection of Breast Cancer Specimens
DATES : 6/25/71 - 6/24/72
PRINC INVEST: Dr. Herbert F. Oettgen
PROJ OFFICER: Dr. Harry J. Clausen, Federal Bldg., Rm. 4C-05, x-64533
SEGMENT : Breast Tumor Task Force
SEG CHAIRMAN: Dr. W. Ray Bryan, Federal Bldg., Rm. 4C-08, x-64533
CNTRCT OFCR : Mr. Fred Shaw, Federal Bldg., Rm. 512, x-61282

CONTRACTOR : Merck and Company, Inc. (NIH-71-2059)
ADDRESS : West Point, Pennsylvania 19486
PHONE : AC-215, Phone 699-5311, Ext. 5532
CNTRCT TITLE: Research on Oncogenic and Potentially Oncogenic Viruses, Large-scale Virus Production and Vaccine Development
DATES : 12/1/71 - 11/30/72
PRINC INVEST: Dr. Maurice R. Hilleman
PROJ OFFICER: Dr. Robert Manaker, Bldg. 37, Rm. 1B-16, x-63323
SEGMENT : Developmental Research
SEG CHAIRMAN: Dr. Robert Manaker
CNTRCT OFCR : Mr. J. Thomas Lewin, Bldg. 37, Rm. 1A-03, x-65025

CONTRACTOR : Miami, University of (PH-43-67-1187)
ADDRESS : Coral Gables, Florida
PHONE : AC-305, Phone 284-4711
CNTRCT TITLE: Immunization Studies on Avian Leukosis and Related Problems
DATES : 6/23/71 - 6/22/72
PRINC INVEST: Dr. Michael Sigel
PROJ OFFICER: Dr. Gary Pearson, Bldg. 41, Suite 100, x-66080
Dr. George Burton, Bldg. 37, Rm. 1D-21, x-64450
SEGMENT : Special Animal Leukemia Ecology Studies
SEG CHAIRMAN: Dr. Michael Chirigos, Bldg. 37, Rm. 1D-15, x-61478
CNTRCT OFCR : Mr. J. Thomas Lewin, Bldg. 37, Rm. 1A-03, x-65025

CONTRACTOR : Miami, University of (NIH-70-2211)
ADDRESS : Department of Microbiology, School of Medicine, P.O. Box 875, Biscayne Annex, Miami, Florida 33152
PHONE : AC-305, Phone 284-2590
CNTRCT TITLE: Studies on the Rat Mammary Tumor-Derived-Virus (RMTDV or BV)
DATES : 11/1/71 - 10/31/72
PRINC INVEST: Dr. Victor V. Bergs
PROJ OFFICER: Dr. Michael Chirigos, Bldg. 37, Rm. 1D-15, x-61478
Dr. Willie Turner, Bldg. 37, Rm. 1D-15, x-61478
SEGMENT : Special Animal Leukemia Ecology Studies
SEG CHAIRMAN: Dr. Michael Chirigos
CNTRCT OFCR : Mr. J. Thomas Lewin, Bldg. 37, Rm. 1A-03, x-65025

CONTRACTOR : Michigan, University of (PH-43-65-639)
ADDRESS : Medical School, Department of Microbiology, Ann Arbor, Michigan 48105
PHONE : AC-313, Phone 764-8100
CNTRCT TITLE: Collection of Leukemia/Lymphoma Specimens; FA Studies; Viral Genome Rescue Studies
DATES : 9/1/71 - 8/31/72
PRINC INVEST: Dr. Chris J.D. Zarafonetis
Dr. William H. Murphy
PROJ OFFICER: Dr. Paul Levine, Federal Bldg., Rm. 504, x-66135
Dr. Ernest Plata, Bldg. 41, B-306, x-62120
SEGMENT : Program Resources and Logistics
SEG CHAIRMAN: Dr. Robert Holdenried, Federal Bldg., Rm. 5A-14, x-66085
CNTRCT OFCR : Mr. Fred Shaw, Federal Bldg., Rm. 512, x-61282

CONTRACTOR : Michigan Cancer Foundation (NIH-71-2421)
ADDRESS : 4811 John R. Street, Detroit, Michigan 48201
PHONE : AC-313, Phone 833-0710
CNTRCT TITLE: Studies of High Risk Breast Cancer Families
DATES : 6/20/71 - 6/19/72
PRINC INVEST: Dr. Michael J. Brennan
PROJ OFFICER: Dr. W. Ray Bryan, Federal Bldg., Rm. 4C-08, x-64533
SEGMENT : Breast Tumor Task Force
SEG CHAIRMAN: Dr. W. Ray Bryan
CNTRCT OFCR : Mr. Fred Shaw, Federal Bldg., Rm. 512, x-61282

CONTRACTOR : Microbiological Associates, Inc. (PH-43-66-914)
ADDRESS : 4733 Bethesda Avenue, Bethesda, Maryland 20014
PHONE : 654-3400, Ext. 300
CNTRCT TITLE: Operation of a Balb/c Mouse Colony
DATES : 6/16/71 - 6/15/72
PRINC INVEST: Mr. Wilbur Athey
PROJ OFFICER: Mr. Samuel Poiley, Bldg. 37, Rm. 5E-10, x-61323
Dr. Michael Chirigos, Bldg. 37, Rm. 1D-15, x-61478
Mr. Clarence Reeder, Bldg. 37, Rm. 5E-12A, x-61323
SEGMENT : Program Resources and Logistics
SEG CHAIRMAN: Dr. Robert Holdenried, Federal Bldg., Rm. 5A-14, x-66085
CNTRCT OFCR : Mr. Fred Shaw, Federal Bldg., Rm. 512, x-61282

CONTRACTOR : Microbiological Associates, Inc. (PH-43-67-697)
ADDRESS : 4733 Bethesda Avenue, Bethesda, Maryland 20014
PHONE : 654-3400
CNTRCT TITLE: Detection, Characterization and Assay of Animal Tumor Viruses
DATES : 2/1/71 - 1/31/72
PRINC INVEST: Dr. Robert M. Nims
PROJ OFFICER: Dr. W. Ray Bryan, Federal Bldg., Rm. 5A-14, x-66085
Dr. Robert J. Huebner, Bldg. 37, Rm. 2D-24, x-63301
Mr. John Kvedar, Bldg. 41, Rm. C-503, x-65334
SEGMENT : Solid-Tumor Virus
SEG CHAIRMAN: Dr. Robert Huebner
CNTRCT OFCR : Mr. Thomas Porter, Bldg. 37, Rm. 1A-03, x-65025

CONTRACTOR : Microbiological Associates, Inc. (PH-43-67-700)
ADDRESS : 4733 Bethesda Avenue, Bethesda, Maryland 20014
PHONE : 654-3400
CNTRCT TITLE: Mouse Virus Typing and Diagnostic Reagents
DATES : 2/1/71 - 1/31/72
PRINC INVEST: John C. Parker, Ph.D.
PROJ OFFICER: Dr. Robert Holdenried, Federal Bldg., Rm. 5A-14, x-66085
Dr. Wallace P. Rowe, Bldg. 7, Rm. 304, x-62613
SEGMENT : Program Resources and Logistics
SEG CHAIRMAN: Dr. Robert Holdenried
CNTRCT OFCR : Mr. Fred Shaw, Federal Bldg., Rm. 512, x-61282

CONTRACTOR : Microbiological Associates, Inc. (NIH-70-2063)
ADDRESS : 4733 Bethesda Avenue, Bethesda, Maryland 20014
PHONE : 654-3400
CNTRCT TITLE: The Roles of Viruses and Chemicals in the Etiology of Cancer
DATES : 10/23/71 - 10/22/72
PRINC INVEST: Dr. Riley Housewright
PROJ OFFICER: Dr. Robert Huebner, Bldg. 37, Rm. 2D-24, x-63301
SEGMENT : Solid Tumor-Virus
SEG CHAIRMAN: Dr. Robert Huebner
CNTRCT OFCR : Mr. Thomas Porter, Bldg. 37, Rm. 1A-03, x-65025

CONTRACTOR : Minnesota, University of (NIH-69-2061)
ADDRESS : College of Medical Science, Minneapolis, Minnesota 55455
PHONE : AC-612, Phone 373-7733
CNTRCT TITLE: Tumor-Specific Transplantation Antigens in Solid Tumors
DATES : 6/15/71 - 6/14/72
PRINC INVEST: Dr. Charles F. McKhann
PROJ OFFICER: Dr. Charles Boone, Bldg. 37, Rm. 1C-06, x-65141
SEGMENT : Immunology Group
SEG CHAIRMAN: Dr. Paul Levine, Federal Bldg., Rm. 504, x-66135
CNTRCT OFCR : Mr. J. Thomas Lewin, Bldg. 37, Rm. 1A-03, x-65025

CONTRACTOR : Minnesota, University of (NIH-71-2261)
ADDRESS : 315 Morrill Hall, Minneapolis, Minnesota 55455
PHONE : AC-612, Phone 373-7733
CNTRCT TITLE: Immunological Deficiency, Diseases and Cancer
DATES : 5/13/71 - 5/12/72
PRINC INVEST: Dr. Robert A. Good
PROJ OFFICER: Dr. George Todaro, Federal Bldg., Rm. 502, x-66135
SEGMENT : Program Resources and Logistics
SEG CHAIRMAN: Dr. Robert Holdenried, Federal Bldg., Rm. 5A-14, x-66085
CNTRCT OFCR : Mr. Fred Shaw, Federal Bldg., Rm. 512, x-61282

CONTRACTOR : Montreal Children's Hospital (PH-43-65-1020)
ADDRESS : 2300 Tupper Street, Montreal 25, Quebec, Canada
PHONE : AC-514, Phone 937-8511
CNTRCT TITLE: Procurement of Blood Plasma/Serum Specimens from Human Leukemic, Malignant Tumor, and Non-Leukemic Controls, Including Family Members, Among Patients of the Montreal Children's Hospital, and Performance of Certain Laboratory Procedures Upon Such Specimens
DATES : 6/1/71 - 5/31/72
PRINC INVEST: Dr. Ronald L. Denton
PROJ OFFICER: Dr. Paul Levine, Federal Bldg., Rm. 504, x-66135
SEGMENT : Program Resources and Logistics
SEG CHAIRMAN: Dr. Robert Holdenried, Federal Bldg., Rm. 5A-14, x-66085
CNTRCT OFCR : Mr. Fred Shaw, Federal Bldg., Rm. 512, x-61282

CONTRACTOR : Naples, University of (NIH-71-2056)
ADDRESS : Instituto di Clinica Medica Generale, (I-Cattedra), Naples, Italy
PHONE :
CNTRCT TITLE: Studies in the Role of HSV Types I and II in Human Malignancies
DATES : 4/9/71 - 4/8/72
PRINC INVEST: Dr. Guilio Tarro
PROJ OFFICER: Dr. Virginia Dunkel, Bldg. 41, Rm. B1-06, x-66738
Dr. Jack Gruber, Bldg. 37, Rm. 1B-14, x-63323
SEGMENT : Developmental Research
SEG CHAIRMAN: Dr. Robert Manaker, Bldg. 37, Rm. 1B-16, x-63323
CNTRCT OFCR : Mr. J. Thomas Lewin, Bldg. 37, Rm. 1A-03, x-65025

CONTRACTOR : National Center for Health Statistics (FS-35)
ADDRESS : 330 Independence Ave., S.W., Washington, D.C. 20025
PHONE : IDS Code 13-36134
CNTRCT TITLE: Death Certificates of Children who Died of Cancer in the U.S.
DATES : FY 1972
PRINC INVEST: Dr. Robert D. Grove
PROJ OFFICER: Dr. Robert Miller, Federal Bldg., Rm. 402, x-65785
SEGMENT : Program Management
SEG CHAIRMAN: Dr. John Moloney, Bldg. 37, Rm. 1A-13, x-61038
CNTRCT OFCR : Mr. Fred Shaw, Federal Bldg., Rm. 512, x-61282

CONTRACTOR : Naval Biomedical Research Labs (FS-8)
ADDRESS : U.S. Naval Supply Center, Oakland, California 94625
PHONE : AC-415, Phone 832-5217, Ext. 26
CNTRCT TITLE: Facility for Cell Culture Research
DATES : 7/1/71 - 6/30/72
PRINC INVEST: LCDR Warren Bowe
PROJ OFFICER: Dr. James Duff, Bldg. 37, Rm. 1B-22, x-65967
Dr. Stuart Aaronson, Federal Bldg., Rm. 502, x-66135
Dr. George Todaro, Federal Bldg., Rm. 502, x-66135
SEGMENT : Solid Tumor-Virus
SEG CHAIRMAN: Dr. Robert Huebner, Bldg. 37, Rm. 2D-24, x-63301
CNTRCT OFCR : Mr. Thomas Porter, Bldg. 37, Rm. 1A-03, x-65025

CONTRACTOR : Naval Biomedical Research Labs (FS-57)
ADDRESS : U.S. Naval Supply Center, Oakland, California 94625
PHONE : AC-415, Phone 832-5217
CNTRCT TITLE: Aerosol Properties of Potentially Oncogenic Viruses
DATES : 5/1/71 - 4/30/72
PRINC INVEST: Dr. R. L. Dimmick
Mr. M. Chatigny
PROJ OFFICER: Dr. Alfred Hellman, Bldg. 41, Rm. A-103, x-66758
Dr. Arnold Fowler, Bldg. 41, Rm. A-116, x-64561
Mr. W. Emmitt Barkley, Bldg. 41, Rm. A-118, x-64421
SEGMENT : Biohazards Control and Containment
SEG CHAIRMAN: Dr. Alfred Hellman
CNTRCT OFCR : Mr. J. Thomas Lewin, Bldg. 37, Rm. 1A-03, x-65025

CONTRACTOR : Nebraska, University of (NIH-71-2076)
ADDRESS : Eppley Institute for Research on Cancer, Lincoln, Nebraska 68508
PHONE : AC-402, Phone 551-4261
CNTRCT TITLE: Studies of Temperature Sensitive Mutants
DATES : 3/12/71 - 2/14/72
PRINC INVEST: Dr. Giampiero di Mayorca
PROJ OFFICER: Dr. Gio Gori, Bldg. 31, Rm. 11A-03, x-66616
SEGMENT : Program Management
SEG CHAIRMAN: Dr. John Moloney, Bldg. 37, Rm. 1A-13, x-61038
CNTRCT OFCR : Mr. J. Thomas Lewin, Bldg. 37, Rm. 1A-03, x-65025

CONTRACTOR : Netherlands Cancer Institute (NIH-71-678)
ADDRESS : Het Nederlands Kankerinstituut, Sarphatistraat 108,
Amsterdam C., The Nederlands
PHONE :
CNTRCT TITLE: Conference on RNA Viruses and Host Genome in Oncogenesis
DATES : 4/1/71 - 3/31/72
PRINC INVEST: L. M. Boot, Ph.D.
PROJ OFFICER: Dr. Louis Sibal, Bldg. 37, Rm. 1A15, x-62796
Mr. J. Thomas Lewin, Bldg. 37, Rm. 1A-03, x-65025
SEGMENT : Program Management
SEG CHAIRMAN: Dr. John Moloney, Bldg. 37, Rm. 1A-13, x-61038
CNTRCT OFCR : Mr. J. Thomas Lewin

CONTRACTOR : North Dakota, University of (PH-43-66-8)
ADDRESS : School of Medicine, Department of Microbiology,
Grand Forks, North Dakota 58202
PHONE : AC-701, Phone 777-2411
CNTRCT TITLE: Quantitative Studies on the Transmission of Feline Oncogenic
RNA Viruses and Selected Herpesviruses by Certain Bloodsucking
Arthropods
DATES : 1/1/71 - 12/31/71
PRINC INVEST: Dr. Robert G. Fischer
PROJ OFFICER: Dr. George Burton, Bldg. 37, Rm. 1D-21, x-64450
Dr. Willie Turner, Bldg. 37, Rm. 1D-15, x-61478
SEGMENT : Special Animal Leukemia Ecology Studies
SEG CHAIRMAN: Dr. Michael Chirigos, Bldg. 37, Rm. 1D-15, x-61478
CNTRCT OFCR : Mr. J. Thomas Lewin, Bldg. 37, Rm. 1A-03, x-65025

CONTRACTOR : Ohio State University (NIH-69-2233)
ADDRESS : 1314 Kinnear Road, Columbus, Ohio 43212
PHONE : AC-614, Phone 293-7621
CNTRCT TITLE: Application of Radioiodine Labelled Antibody Technique to Studies of Virus-Induced Tumors and Human Neoplasms of Suspected Viral Etiology
DATES : 6/27/71 - 6/26/72
PRINC INVEST: Dr. David S. Yohn
PROJ OFFICER: Dr. Jack Gruber, Bldg. 37, Rm. 1B-14, x-63323
Dr. Virginia Dunkel, Bldg. 41, Rm. B1-06, x-66738
SEGMENT : Developmental Research
SEG CHAIRMAN: Dr. Robert Manaker, Bldg. 37, Rm. 1B-16, x-63323
CNTRCT OFCR : Mr. J. Thomas Lewin, Bldg. 37, Rm. 1A-03, x-65025

CONTRACTOR : Ohio State University Research Foundation (PH-43-65-1001)
ADDRESS : Veterinary Pathology Building, 1925 Coffey Road, Columbus, Ohio 43210
PHONE : AC-614, Phone 293-5661
CNTRCT TITLE: Biohazards Control and Containment in Oncogenic Virus Research
DATES : 7/1/71 - 6/30/72
PRINC INVEST: Dr. David S. Yohn
PROJ OFFICER: Dr. Alfred Hellman, Bldg. 41, Rm. A-103, x-66758
SEGMENT : Biohazards Control and Containment
SEG CHAIRMAN: Dr. Alfred Hellman
CNTRCT OFCR : Mr. J. Thomas Lewin, Bldg. 37, Rm. 1A-03, x-65025

CONTRACTOR : Oregon State University (NIH-71-2175)
ADDRESS : Department of Agricultural Chemistry, Corvallis, Oregon 97331
PHONE : AC-503, Phone 754-1945
CNTRCT TITLE: Studies on Oncogenic Virus Nucleic Acids
DATES : 6/28/71 - 6/27/72
PRINC INVEST: Dr. George Beaudreau
PROJ OFFICER: Dr. Albert Dalton, Bldg. 37, Rm. 1C-16, x-64311
Dr. Ursula Heine, Bldg. 37, Rm. 1C-17, x-64311
SEGMENT : Developmental Research
SEG CHAIRMAN: Dr. Robert Manaker, Bldg. 37, Rm. 1B-16, x-63323
CNTRCT OFCR : Mr. J. Thomas Lewin, Bldg. 37, Rm. 1A-03, x-65025

CONTRACTOR : Padua University Hospital (PH-43-68-1389)
ADDRESS : Ospedale di Civile Padova, Via C. Battista, Padova, Italy
PHONE :
CNTRCT TITLE: Procurement of Fibroblast Cultures from Donors with a High Degree of Homozygosity and Procurement of Human Tumors
DATES : 6/7/71 - 6/6/72
PRINC INVEST: Professori Giovanni Dogo, M.D.
PROJ OFFICER: Dr. Robert Depue, Bldg. 31, Rm. 11A-11, x-66271
Dr. Charles Boone, Bldg. 37, Rm. 1C-06, x-65141
SEGMENT : Program Resources and Logistics
SEG CHAIRMAN: Dr. Robert Holdenried, Federal Bldg., Rm. 5A-14, x-66085
CNTRCT OFCR : Mr. Fred Shaw, Federal Bldg., Rm. 512, x-61282

CONTRACTOR : Pennsylvania, University of (PH-43-65-1013)
ADDRESS : School of Veterinary Medicine, Department of Clinical Studies, New Bolton Center, Kennett Square, R.D. #1, Pennsylvania 19348
PHONE : AC-215, Phone 444-5800
CNTRCT TITLE: Experimental and Natural Transmission of Bovine Leukemia
DATES : 1/1/71 - 12/31/71
PRINC INVEST: Dr. Robert Marshak
PROJ OFFICER: Dr. Michael Chirigos, Bldg. 37, Rm. 1D-15, x-61473
SEGMENT : Special Animal Leukemia Ecology Studies
SEG CHAIRMAN: Dr. Michael Chirigos
CNTRCT OFCR : Mr. J. Thomas Lewin, Bldg. 37, Rm. 1A-03, x-65025

CONTRACTOR : Pennsylvania State University (NIH-70-2024)
ADDRESS : Milton S. Hershey Medical Center, Pennsylvania State Univ., Hershey, Pennsylvania 17033
PHONE : AC-717, Phone 534-8254
CNTRCT TITLE: Studies on the Oncogenic Potential of Defective Human Viruses
DATES : 10/1/71 - 9/30/72
PRINC INVEST: Dr. Fred Rapp
PROJ OFFICER: Dr. Virginia Dunkel, Bldg. 41, Rm. B1-06, x-66738
Dr. Jack Gruber, Bldg. 37, Rm. 1B-14, x-63323
SEGMENT : Developmental Research
SEG CHAIRMAN: Dr. Robert Manaker, Bldg. 37, Rm. 1B-16, x-63323
CNTRCT OFCR : Mr. J. Thomas Lewin, Bldg. 37, Rm. 1A-03, x-65025

CONTRACTOR : Pfizer & Co., Inc. (PH-43-67-1176)
ADDRESS : 99 Maywood Avenue, Maywood, New Jersey 07607
PHONE : AC-201, Phone 845-5665
CNTRCT TITLE: Electron Microscopy Related to Viral Studies of Human and Animal Breast Cancer
DATES : 6/28/71 - 6/27/72
PRINC INVEST: Dr. J.J. Oleson
PROJ OFFICER: Dr. W. Ray Bryan, Federal Bldg., Rm. 4C-08, x-64533
Dr. Louis Sibal, Bldg. 37, Rm. 1A-15, x-62796
SEGMENT : Breast Tumor Task Force
SEG CHAIRMAN: Dr. W. Ray Bryan
CNTRCT OFCR : Mr. Fred Shaw, Federal Bldg., Rm. 512, x-61282

CONTRACTOR : Pfizer & Co., Inc. (NIH-70-2080)
ADDRESS : 99 Maywood Avenue, Maywood, New Jersey 07607
PHONE : AC-201, Phone 845-5665
CNTRCT TITLE: Tumor Virus Research
DATES : 1/1/71 - 12/31/71
PRINC INVEST: Dr. J.J. Oleson
PROJ OFFICER: Dr. Jack Gruber, Bldg. 37, Rm. 1B-14, x-63323
Dr. W. Ray Bryan, Federal Bldg., Rm. 4C-08, x-64533
Dr. Roy Kinard, Federal Bldg., Rm. 504, x-66085
SEGMENT : Developmental Research
SEG CHAIRMAN: Dr. Robert Manaker, Bldg. 37, Rm. 1B-16, x-63323
CNTRCT OFCR : Mr. J. Thomas Lewin, Bldg. 37, Rm. 1A-03, x-65025

CONTRACTOR : Princeton University (NIH-71-2372)
ADDRESS : Princeton, New Jersey 08540
PHONE : AC-609, Phone 452-3864
CNTRCT TITLE: Studies on Surface Alterations in RNA Tumor Virus Cells
DATES : 6/28/71 - 6/27/72
PRINC INVEST: Dr. Max M. Burger
PROJ OFFICER: Dr. James Duff, Bldg. 37, Rm. 1B-22, x-65967
SEGMENT : Solid Tumor-Virus
SEG CHAIRMAN: Dr. Robert Huebner, Bldg. 37, Rm. 2D-24, x-63301
CNTRCT OFCR : Mr. Thomas Porter, Bldg. 37, Rm. 1A-03, x-65025

CONTRACTOR : Public Health Research Institute (NIH-71-2129)
ADDRESS : 455 1st Avenue, New York, N.Y. 10016
PHONE : AC-212, Phone 340-4600
CNTRCT TITLE: Evaluation of Methods for Isolation of Viruses from Human Neoplasia
DATES : 4/27/71 - 4/26/72
PRINC INVEST: Dr. Hidesaburo Hanafusa
PROJ OFFICER: Dr. Jack Gruber, Bldg. 37, Rm. 1B-14, x-63323
Dr. Robert Manaker, Bldg. 37, Rm. 1B-16, x-63323
SEGMENT : Developmental Research
SEG CHAIRMAN: Dr. Robert Manaker
CNTRCT OFCR : Mr. J. Thomas Lewin, Bldg. 37, Rm. 1A-03, x-65025

CONTRACTOR : Research Foundation of the State Univ. of N.Y. (NIH-71-2137)
ADDRESS : P.O. Box 7126, Albany, New York 12224
PHONE : AC-716, Phone 845-5876
CNTRCT TITLE: Application of Immunotherapy to Malignant Disease
DATES : 5/25/71 - 5/24/72
PRINC INVEST: Dr. Edmund Klein
PROJ OFFICER: Dr. Charles Boone, Bldg. 37, Rm. 1C-06, x-65141
SEGMENT : Immunology Group
SEG CHAIRMAN: Dr. Paul Levine, Federal Bldg., Rm. 504, x-66135
CNTRCT OFCR : Mr. J. Thomas Lewin, Bldg. 37, Rm. 1A-03, x-65025

CONTRACTOR : Robert B. Brigham Hospital (NIH-71-2172)
ADDRESS : Harvard Medical Center, 125 Parker Hill Avenue, Boston, Massachusetts 02120
PHONE : AC-617, Phone 734-5700, Ext. 243
CNTRCT TITLE: Studies on Tumor Specific Transplantation Antigen
DATES : 6/28/71 - 6/27/72
PRINC INVEST: Dr. John David
Dr. Winthrop Churchill
PROJ OFFICER: Dr. Charles Boone, Bldg. 37, Rm. 1C-06, x-65141
SEGMENT : Immunology Group
SEG CHAIRMAN: Dr. Paul Levine, Federal Bldg., Rm. 504, x-66135
CNTRCT OFCR : Mr. J. Thomas Lewin, Bldg. 37, Rm. 1A-03, x-65025

CONTRACTOR : Rush-Presbyterian-St. Luke's Hospital (NIH-71-2032)
ADDRESS : 1753 West Congress Parkway, Chicago, Illinois 60612
PHONE : AC-312, Phone 942-5442
CNTRCT TITLE: Studies of Tumor Viruses in Nonhuman Primates
DATES : 10/1/71 - 9/30/72
PRINC INVEST: Dr. Friedrich Deinhardt
PROJ OFFICER: Dr. Roy Kinard, Federal Bldg., Rm. 504, x-66085
Dr. Jack Gruber, Bldg. 37, Rm. 1B-14, x-63323
SEGMENT : Developmental Research
SEG CHAIRMAN: Dr. Robert Manaker, Bldg. 37, Rm. 1B-16, x-63323
CNTRCT OFCR : Mr. J. Thomas Lewin, Bldg. 37, Rm. 1A-03, x-65025

CONTRACTOR : Rutgers University (NIH-71-2077)
ADDRESS : New Brunswick, New Jersey 08903
PHONE : AC-201, Phone 247-1766, Ext. 2275
CNTRCT TITLE: Test for Genetic Acquisition of Oncogenic Potential and Cell Transforming Capacity by RNA Animal Viruses
DATES : 2/15/71 - 2/14/72
PRINC INVEST: Dr. Robert W. Simpson
PROJ OFFICER: Dr. Willie Turner, Bldg. 37, Rm. 1D-15, x-61478
Dr. Wilna Woods, Bldg. 37, Rm. 1D-15, x-61478
SEGMENT : Special Animal Leukemia Ecology Studies
SEG CHAIRMAN: Dr. Michael Chirigos, Bldg. 37, Rm. 1D-15, x-61478
CNTRCT OFCR : Mr. J. Thomas Lewin, Bldg. 37, Rm. 1A-03, x-65025

CONTRACTOR : Salk Institute (PH-43-67-1147)
ADDRESS : P.O. Box 1809, San Diego, California 92112
PHONE : AC-714, Phone 453-4100
CNTRCT TITLE: Characterization of Temperature-Sensitive Mutants of Polyoma Virus and Interaction Between Polyoma Virus and C-Type RNA Viruses
DATES : 6/5/71 - 6/4/72
PRINC INVEST: Dr. Walter Eckhart
PROJ OFFICER: Dr. George Todaro, Federal Bldg., Rm. 502, x-66135
Dr. Stuart Aaronson, Federal Bldg., Rm. 502, x-66135
SEGMENT : Solid Tumor-Virus
SEG CHAIRMAN: Dr. Robert Huebner, Bldg. 37, Rm. 2D-24, x-63301
CNTRCT OFCR : Mr. Thomas Porter, Bldg. 37, Rm. 1A-03, x-65025

CONTRACTOR : Southern California, University of (PH-43-68-1030)
ADDRESS : 1200 N. State Street, Los Angeles, California 90033
PHONE : AC-213, Phone 225-3131, Ext. 1287
CNTRCT TITLE: A Comprehensive Field and Laboratory Research Program on the Etiology and Epidemiology of Human Cancer
DATES : 9/1/71 - 8/31/72
PRINC INVEST: Dr. Murray Gardner
PROJ OFFICER: Dr. Robert Huebner, Bldg. 37, Rm. 2D-24, x-63301
SEGMENT : Solid Tumor-Virus
SEG CHAIRMAN: Dr. Robert Huebner
CNTRCT OFCR : Mr. Thomas Porter, Bldg. 37, Rm. 1A-03, x-65025

CONTRACTOR : Southwest Foundation for Research & Education (NIH-69-93)
ADDRESS : P.O. Box 2296, 10,000 W. Commerce St., San Antonio, Texas 78206
PHONE : AC-512, Phone 674-1410
CNTRCT TITLE: Production of Simian Viruses and Homologous Antiserum
DATES : 5/1/71 - 4/30/72
PRINC INVEST: Dr. S.S. Kalter
PROJ OFFICER: Dr. James Duff, Bldg. 37, Rm. 1B-22, x-65967
SEGMENT : Program Resources and Logistics
SEG CHAIRMAN: Dr. Robert Holdenried, Federal Bldg., Rm. 5A-14, x-66085
CNTRCT OFCR : Mr. Fred Shaw, Federal Bldg., Rm. 512, x-61282

CONTRACTOR : Southwest Foundation for Research & Education (NIH-69-2011)
ADDRESS : P.O. Box 2296, 10,000 W. Commerce St., San Antonio, Texas 78206
PHONE : AC-512, Phone 674-1410
CNTRCT TITLE: Housing and Maintenance of a Chimpanzee Breeding Colony
DATES : 4/25/71 - 4/24/72
PRINC INVEST: Dr. S.S. Kalter
PROJ OFFICER: Dr. Roy Kinard, Federal Bldg., Rm. 504, x-66085
SEGMENT : Program Resources and Logistics
SEG CHAIRMAN: Dr. Robert Holdenried, Federal Bldg., Rm. 5A-14, x-66085
CNTRCT OFCR : Mr. Fred Shaw, Federal Bldg., Rm. 512, x-61282

CONTRACTOR : Southwest Foundation for Research & Education (NIH-71-2348)
ADDRESS : P.O. Box 2296, 10,000 W. Commerce St., San Antonio, Texas 78206
PHONE : AC-512, Phone 674-1410
CNTRCT TITLE: Study of Latent Virus Infection and Transmission
DATES : 6/3/71 - 6/2/72
PRINC INVEST: Dr. S.S. Kalter
PROJ OFFICER: Dr. Alfred Hellman, Bldg. 41, Rm. A-103, x-66758
SEGMENT : Biohazards Control and Containment
SEG CHAIRMAN: Dr. Alfred Hellman
CNTRCT OFCR : Mr. J. Thomas Lewin, Bldg. 37, Rm. 1A-03, x-65025

CONTRACTOR : St. Joseph Hospital (NIH-69-2074)
ADDRESS : University of Southern Florida, School of Medicine, 3001 W. Buffalo Avenue, Tampa, Florida 33607
PHONE : AC-813, Phone 877-8161, Ext. 246
CNTRCT TITLE: Study on Human Sarcomas and Their Possible Viral Etiology
DATES : 6/24/71 - 6/23/72
PRINC INVEST: Dr. Jeno Szakacs (sawcash)
Dr. Ruttgers Szakacs
PROJ OFFICER: Dr. Albert Dalton, Bldg. 37, Rm. 1C-15, x-64311
Dr. Roy Kinard, Federal Bldg., Rm. 504, x-66085
SEGMENT : Developmental Research
SEG CHAIRMAN: Dr. Robert Manaker, Bldg. 37, Rm. 1B-16, x-63323
CNTRCT OFCR : Mr. J. Thomas Lewin, Bldg. 37, Rm. 1A-03, x-65025

CONTRACTOR : St. Jude's Children's Research Hospital (NIH-71-2134)
ADDRESS : 332 N. Lauderdale, P.O. Box 318, Memphis, Tennessee 38101
PHONE : AC-901, Phone 525-8381
CNTRCT TITLE: In Vitro Studies of the Potential Oncogenicity of the Herpes Virus Associated with the Lucke Amphibian Tumor
DATES : 5/13/71 - 5/12/72
PRINC INVEST: Dr. Allan Granoff
PROJ OFFICER: Dr. Gary Pearson, Bldg. 41, Suite 100, x-66080
Dr. Wilna Woods, Bldg. 37, Rm. 1D-15, x-61478
SEGMENT : Special Animal Leukemia Ecology Studies
SEG CHAIRMAN: Dr. Michael Chirigos, Bldg. 37, Rm. 1D-15, x-61478
CNTRCT OFCR : Mr. J. Thomas Lewin, Bldg. 37, Rm. 1A-03, x-65025

CONTRACTOR : St. Louis University (PH-43-67-692)
ADDRESS : 3681 Park Avenue, St. Louis, Missouri 63110
PHONE : AC-314, Phone 865-2288, Ext. 545
CNTRCT TITLE: Search for the Virus-Specific Genetic Material in Human Cancers - A Direct Test of the Viral Etiology of Human Cancer and Studies on the Mechanisms of Viral Oncogenesis
DATES : 5/20/71 - 3/19/72
PRINC INVEST: Dr. Maurice Green
PROJ OFFICER: Dr. Robert Huebner, Bldg. 37, Rm. 2D-24, x-63301
Dr. James Duff, Bldg. 37, Rm. 1B-22, x-65967
SEGMENT : Solid Tumor-Virus
SEG CHAIRMAN: Dr. Robert Huebner
CNTRCT OFCR : Mr. Thomas Porter, Bldg. 37, Rm. 1A-03, x-65025

CONTRACTOR : Stanford University (NIH-69-2053)
ADDRESS : Stanford, California 94305
PHONE : AC-415, Phone 321-2300
CNTRCT TITLE: Procurement, Processing, Storage, Distribution and Study of Human Tumor Cell Cultures
DATES : 10/1/71 - 9/30/72
PRINC INVEST: Dr. Leonard Hayflick
PROJ OFFICER: Dr. James Duff, Bldg. 37, Rm. 1B-22, x-65967
Dr. George Todaro, Federal Bldg., Rm. 502, x-66135
Dr. Stuart Aaronson, Federal Bldg., Rm. 502, x-66135
SEGMENT : Solid Tumor-Virus
SEG CHAIRMAN: Dr. Robert Huebner, Bldg. 37, Rm. 2D-24, x-63301
CNTRCT OFCR : Mr. Thomas Porter, Bldg. 37, Rm. 1A-03, x-65025

CONTRACTOR : Texas, University of (PH-43-65-604)
ADDRESS : M.D. Anderson Hospital & Tumor Institute, 6723 Bertner Drive, Houston, Texas 77025
PHONE : AC-713, Phone 526-5411
CNTRCT TITLE: Studies on the Relationships of Viruses to Human Leukemia, Lymphoma and Solid Tumors
DATES : 2/1/71 - 1/31/72
PRINC INVEST: Dr. Leon Dmochowski
PROJ OFFICER: Dr. Jack Gruber, Bldg. 37, Rm. 1B-14, x-63323
Dr. Roy Kinard, Federal Bldg., Rm. 504, x-66085
SEGMENT : Developmental Research
SEG CHAIRMAN: Dr. Robert Manaker, Bldg. 37, Rm. 1B-16, x-63323
CNTRCT OFCR : Mr. J. Thomas Lewin, Bldg. 37, Rm. 1A-03, x-65025

CONTRACTOR : Texas, University of (NIH-71-2135)
ADDRESS : Southwestern Medical School, 5323 Harry Hines Blvd., Dallas, Texas 75235
PHONE : AC-214, Phone 631-3220
CNTRCT TITLE: Studies on Laboratory Associated Infections
DATES : 4/6/71 - 4/5/72
PRINC INVEST: Dr. S.E. Sulkin
PROJ OFFICER: Dr. Alfred Hellman, Bldg. 41, Rm. A-103, x-66758
Mr. W. Emmitt Barkley, Bldg. 41, Rm. A-118, x-64421
SEGMENT : Biohazards Control and Containment
SEG CHAIRMAN: Dr. Alfred Hellman
CNTRCT OFCR : Mr. J. Thomas Lewin, Bldg. 37, Rm. 1A-03, x-65025

CONTRACTOR : Texas, University of (NIH-71-2178)
ADDRESS : M.D. Anderson Hospital & Tumor Institute, 6723 Bertner Drive
Houston, Texas 77025
PHONE : AC-713, Phone 526-5411
CNTRCT TITLE: Immunological Treatment of Human Neoplastic Disease
DATES : 6/29/71 - 4/30/72
PRINC INVEST: Dr. Joseph G. Sinkovics
PROJ OFFICER: Dr. Berton Zbar, Bldg. 37, Rm. 2B-09, x-66141
SEGMENT : Immunology Group
SEG CHAIRMAN: Dr. Paul Levine, Federal Bldg., Rm. 504, x-66135
CNTRCT OFCR : Mr. J. Thomas Lewin, Bldg. 37, Rm. 1A-03, x-65025

CONTRACTOR : TRW, Inc. (NIH-70-2200)
ADDRESS : One Space Park, Redondo Beach, California 90278
PHONE : AC-213, Phone 679-8711
CNTRCT TITLE: Viral Antigens and Anti-Viral Antibody: Preparation, Purification and Properties
DATES : 6/15/71 - 12/31/71
PRINC INVEST: Dr. Norman Weliky
PROJ OFFICER: Dr. Vincent Hollis, Bldg. 41, Rm. B-306, x-62120
Dr. Tibor Borsos, Bldg. 37, Rm. 2B-15, x-63428
SEGMENT : Immunology Group
SEG CHAIRMAN: Dr. Paul Levine, Federal Bldg., Rm. 504, x-66135
CNTRCT OFCR : Mr. J. Thomas Lewin, Bldg. 37, Rm. 1A-03, x-65025

CONTRACTOR : University Labs., Inc. (PH-43-66-1133)
ADDRESS : 810 North Second Avenue, Highland Park, New Jersey 08904
PHONE : AC-201, Phone 246-1145
CNTRCT TITLE: The Production of Sarcoma Viruses, Helper Viruses and Antisera to These Viruses
DATES : 10/1/71 - 9/30/72
PRINC INVEST: Dr. Eugene Bernstein
PROJ OFFICER: Dr. Robert Holdenried, Federal Bldg., Rm. 5A-14, x-66085
Dr. Robert Bassin, Bldg. 41, Rm. 400, x-66588
Dr. Gary Armstrong, Bldg. 37, Rm. 2C-12, x-62631
SEGMENT : Program Resources and Logistics
SEG CHAIRMAN: Dr. Robert Holdenried
CNTRCT OFCR : Mr. Fred Shaw, Federal Bldg., Rm. 512, x-61282

CONTRACTOR : Washington, University of (NIH-71-2171)
ADDRESS : Medical School, Seattle, Washington 98105
PHONE : AC-206, Phone 543-1448
CNTRCT TITLE: Studies on Tumor Specific Transplantation Antigen
DATES : 6/30/71 - 6/29/72
PRINC INVEST: Dr. Karl Hellstrom
Dr. Ingegerd Hellstrom
PROJ OFFICER: Dr. Charles Boone, Bldg. 37, Rm. 1C-06, x-65141
SEGMENT : Solid Tumor-Virus
SEG CHAIRMAN: Dr. Robert Huebner, Bldg. 37, Rm. 2D-24, x-63301
CNTRCT OFCR : Mr. Thomas Porter, Bldg. 37, Rm. 1A-03, x-65025

CONTRACTOR : Weizmann Institute (NIH-69-2014)
ADDRESS : Section of Genetics, Rehovot, Israel
PHONE :
CNTRCT TITLE: Study on Virus-Induced Tumor Specific Transplantation Antigens
DATES : 4/22/71 - 4/21/72
PRINC INVEST: Dr. Leo Sachs
PROJ OFFICER: Dr. Charles Boone, Bldg. 37, Rm. 1C-06, x-65141
SEGMENT : Solid Tumor-Virus
SEG CHAIRMAN: Dr. Robert Huebner, Bldg. 37, Rm. 2D-24, x-63301
CNTRCT OFCR : Mr. Thomas Porter, Bldg. 37, Rm. 1A-03, x-65025

CONTRACTOR : Wistar Institute of Anatomy and Biology (NIH-71-2092)
ADDRESS : Thirty-sixth Street at Spruce, Philadelphia, Pennsylvania 19104
PHONE : AC-215, Phone 222-6700, Ext. 226
CNTRCT TITLE: Extraction and Characterization of Virus Induced Transplantation Antigen from Sarcomas and Leukemias
DATES : 2/1/71 - 1/31/72
PRINC INVEST: Dr. Anthony Girardi
PROJ OFFICER: Dr. George Todaro, Federal Bldg., Rm. 502, x-66135
SEGMENT : Solid Tumor-Virus
SEG CHAIRMAN: Dr. Robert Huebner, Bldg. 37, Rm. 2D-24, x-63301
CNTRCT OFCR : Mr. Thomas Porter, Bldg. 37, Rm. 1A-03, x-65025

CONTRACTOR : World Committee for Comparative Leukemia Research (NIH-71-1033)
ADDRESS : c/o Leukemia Society of America, 211 E. 43rd Street, New York, New York 10017
PHONE : AC-215, Phone MU 8-4400
CNTRCT TITLE: Fifth International Leukemia Symposium
DATES : 6/10/71 - 6/9/72
PRINC INVEST: Dr. Ray Dutcher
Dr. Edward Larkin
PROJ OFFICER: Mr. J. Thomas Lewin, Bldg. 37, Rm. 1A-03, x-65025
Dr. James T. Duff, Bldg. 37, Rm. 1B-22, x-65967
SEGMENT : Program Management
SEG CHAIRMAN: Dr. John Moloney, Bldg. 37, Rm. 1A-13, x-61038
CNTRCT OFCR : Mr. J. Thomas Lewin, Bldg. 37, Rm. 1A-03, x-65025