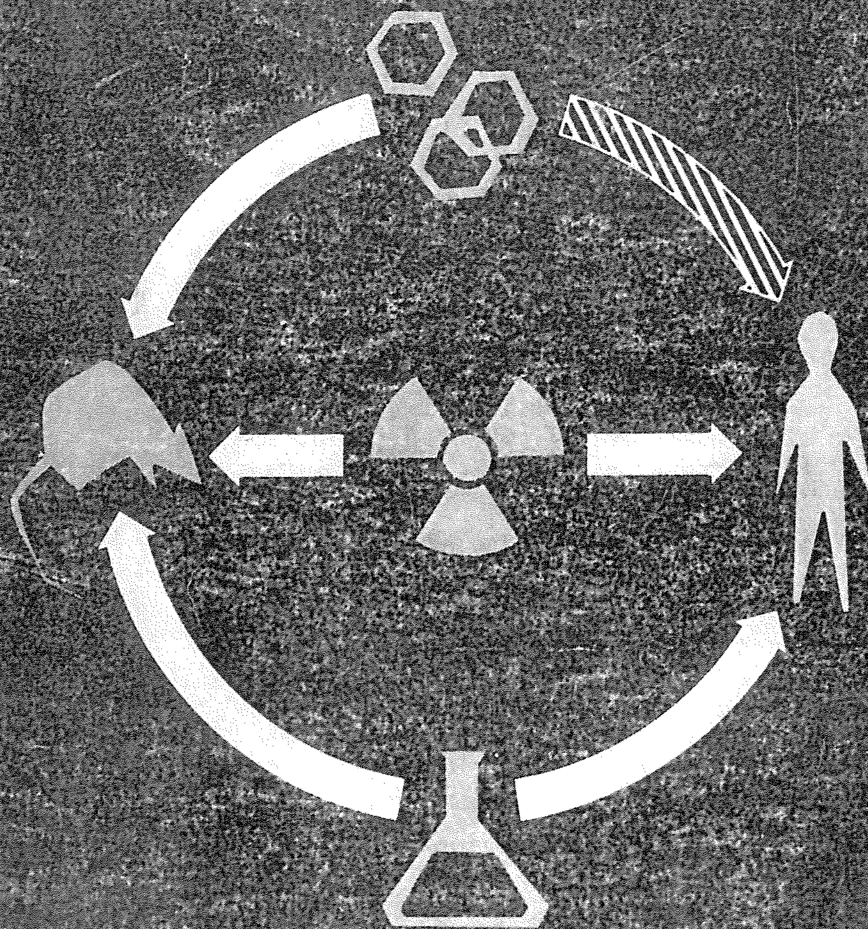
the virus-cancer program

August 1974



Division of Cancer Cause and Prevention
National Cancer Institute

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

Public Health Service

National Institutes of Health

THE VIRUS-CANCER PROGRAM

PROGRESS REPORT #11

August, 1974

This report was prepared by the senior staff of Viral Oncology Area,
Division of Cancer Cause and Prevention, NCI

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PROGRESS REPORT

THE VIRUS CANCER PROGRAM

July 1, 1973 - June 30, 1974

J. B. Moloney, Ph.D.

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I. VIRAL ONCOLOGY AREA - THE VIRUS CANCER PROGRAM (VCP)

A. Introduction

The Viral Oncology Area is responsible for planning and conducting the Institute's program of coordinated research on viruses as etiological agents of cancer. Scientists within this Area not only provide the broad operational management for intramural and collaborative research but also conduct investigations on oncogenic viruses and their interaction with host cells. Any information obtained from these coordinated studies is rapidly applied (1) to search for viruses or virus genetic information which may be etiologically related to the initiation of human cancer and (2) to develop therapeutic and preventive measures for control of human cancers when such causative agents are found. This program, as it is now structured, contributes to six of the seven goal-oriented and key objectives set forth in the National Cancer Plan, the ultimate goal of which is control of all human cancers.

Contract supported research is conducted within the Viral Oncology Program under The Virus Cancer Program (VCP). The yearly budget from the inception of this Program in 1964 to the present and the number of professional positions are given in a separate section of this report. The Program has succeeded in marshaling many of the nation's finest virologists, biochemists, immunologists, molecular biologists, epidemiologists and physicians for this strongly goal-oriented effort. From the beginning, it was clear that an understanding of the suspected relationship between tumor viruses and human neoplasia would not only require an interaction among these groups of scientists but sound and constructive administrative support as well.

The Research Logic for the VCP, based on the premise that a virus or viral genetic information persists in the host and is an indispensable element for the induction of certain kinds of human cancer, is continually updated. The plan is reviewed regularly by the Director, NCI; the Director, Division of Cancer Cause and Prevention, NCI; the National Cancer Advisory Board, NCI; the Executive Committee, NCI; and the Cause and Prevention Executive Staff, NCI. Last year at the request of the Viral Oncology Area, the National Cancer Advisory Board appointed an *ad hoc* committee to review the research efforts of the VCP and suggest any relevant changes.

B. Organization (Viral Oncology Area - The Virus Cancer Program)

From its inception, the Viral Oncology Area and The Virus Cancer Program were organized into an integrated structure which manages both intramural and collaborative research studies. All efforts are presently coordinated by the Office of the Associate Director with its three Offices and three Branches, each with several sections (and units) and program segments. Each segment is assisted by a working group with members outside the federal government.

Within the Office of the Associate Director are:

Office of Biohazards and Environmental Control, with
    Biohazards Research Section
    Environmental Control Section

Office of the Coordinator for Ultrastructural Studies, with
    Viral Studies Section

Office of Program Resources and Logistics

The responsibilities for program data and analysis and for scientific information exchange, formerly in the Office of Program Analysis and Communications, have been transferred to the Office of Program Resources and Logistics and the Office of the Associate Director, respectively.

The Viral Biology Branch is composed of five research sections:

Cell Biology Section
Microbiology Section
Experimental Pathology Section
Human Tumor Studies Section
Virus and Disease Modification Section

The responsibilities of the Electron Microscopy Section have been transferred to the Office of the Coordinator for Ultrastructural Studies within the Office of the Associate Director.

The Viral Leukemia and Lymphoma Branch is composed of seven research sections:

Primate Virus Section
Tumor Virus Section
Clinical Studies Section
Immunology Section
Viral Pathology Section
Genetics Section
Viral Biochemistry Section

The Viral Carcinogenesis Branch is composed of four research sections:

Molecular Biology Section
Ecology and Epizoology Section, with
    Field Studies Unit
Solid Tumor-Virus Section
Viral Genetics Section, with
    Serology Unit
    Trailer Unit

The Viral Oncology Area is planning a major reorganization of its in-house structure. Guidance is being provided by the Management Analysis Office, Office of the Director, National Cancer Institute, to permit better planning and coordination of both intramural and collaborative research programs.

Program Management Personnel

Science Management Team

Dr. J. B. Moloney, Associate Director for Viral Oncology
Dr. L. R. Sibal, Deputy Associate Director for Viral Oncology
Dr. Elke Jordan, Coordinator for Collaborative Research, Viral Oncology
Dr. H. J. Hearn, Scientific Coordinator for Viral Oncology,
Frederick Cancer Research Center

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Mr. N. A. Olimpio, Program Analyst,
Division of Cancer Cause and Prevention
Mr. Robert Velthuis, Administrative Officer,
Viral Oncology
Mrs. Linda Christoferson, Administrative Assistant,
Viral Oncology

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Mr. William Caulfield
Mr. Charles Fafard
Mr. J. Thomas Lewin
Mr. Thomas Porter
Mr. Jacques Labovitz
Mr. Sidney Jones
Mr. William Mundorf

Program Resources and Logistics Advisory Group

Dr. Jack Gruber, Chairman
Dr. David Howell, Executive Secretary
Dr. Robert Bassin
Dr. James Duff
Dr. Peter Fischinger
Dr. Maurice Guss
Dr. Robert Holdenried
Dr. Garrett Keefer
Dr. Gary Kelloff
Dr. Wade Parks
Dr. Gary Pearson
Dr. George Vande Woude

Frederick Cancer Research Center Advisory Group

Dr. Henry J. Hearn, Chairman
Dr. James Duff
Dr. Maurice Guss
Dr. Jack Gruber
Dr. Louis Sibal
Dr. Bernard Talbot
Mr. Robert Velthuis

Program Management Segment

Dr. J. B. Moloney, Chairman
Dr. L. R. Sibal, Executive Secretary
Dr. Michael Chirigos
Dr. A. J. Dalton
Dr. James Duff
Dr. Jack Gruber
Dr. Alfred Hellman
Dr. Robert Holdenried
Dr. Robert Huebner
Dr. Robert Manaker
Dr. George Todaro

PROGRAM SEGMENTS

Developmental Research Segment

Dr. Robert Manaker, Chairman
Dr. Michael Chirigos, Vice Chairman
Dr. Maurice Guss, Executive Secretary
Dr. Michael Brennan, Michigan Cancer Foundation
Dr. Arthur Brown, University of Tennessee
Dr. Bernice Eddy, retired from Bureau of Biologics, NIH
Dr. Paul Gerber, DBS, NIH
Dr. David Kohne, Scripps Clinic and Research Foundation
Dr. Mathilde Krim, Sloan-Kettering Institute
Dr. William Moloney, Peter Bent Brigham Hospital
Dr. Malcolm Pike, University of Southern California
Dr. Lise Thiry, Pasteur Institut du Brabant, Brussels

*Members of Research Contracts Branch, NCI

Solid Tumor Virus Segment

Dr. Robert Huebner, Chairman
Dr. James Duff, Vice Chairman
Ms. Harriet Streicher, Executive Secretary
- Dr. Stuart Aaronson, NCI
- Dr. Francoise Haguenau, College de France
- Dr. Janet Hartley, NIAID, NIH
- Dr. Jerard Hurwitz, Albert Einstein College of Medicine
- Dr. Henry Kunkel, Rockefeller University
- Dr. Wade Parks, NCI
- Dr. Paul Zamecnik, Massachusetts General Hospital

Tumor Virus Detection Segment

Dr. George Todaro, Chairman
Dr. Bernard Talbot, Vice Chairman
Dr. Bernard Talbot, Acting Executive Secretary
- Dr. J. Thomas August, Albert Einstein College of Medicine
- Dr. Paul Black, Massachusetts General Hospital
- Dr. Dani Bolognesi, Duke University
- Dr. Janet Butel, Baylor University
- Dr. Peter Fischinger, NCI
- Dr. Charlotte Friend, Mt. Sinai Hospital, New York
- Dr. Clarence Gibbs, NINDS, NIH
- Dr. Adeline Hackett, Naval Biological Laboratories
- Dr. Gertrude Henle, Children's Hospital of Philadelphia
- Dr. Robert McAllister, Children's Hospital of Los Angeles
- Dr. Paul Neiman, University of Washington
- Dr. Edward Scolnick, NCI

Immunology-Epidemiology Segment

Dr. Paul H. Levine, Chairman
Dr. Gary Pearson, Vice Chairman
Dr. Clarice Gaylord, Executive Secretary
- Dr. Laure Aurelian, Johns Hopkins University
- Dr. Diane Fink, DCC, NCI
- Dr. Anthony Girardi, Wistar Institute
- Dr. William Hardy, Sloan-Kettering Institute
- Dr. Eva Klein, Karolinska Institute
- Dr. M. Lieberman, Stanford University
- Dr. Richard Morrow, Harvard School of Public Health
- Dr. Ken Takemoto, NIAID, NIH

Biohazards Control and Containment Segment

Dr. Alfred Hellman, Chairman
Dr. Emmett Barkley, Vice Chairman
Dr. Garrett Keefer, Executive Secretary
- Mr. Mark Chatigny, Naval Biological Laboratories
- Dr. Michael Chirigos, NCI
- Dr. Peter Gerone, Tulane University
- Dr. Seymour Kalter, Southwest Foundation for Research and Education
- Dr. Maurice Mufson, Westside VA Hospital, Chicago
- Dr. William Payne, Frederick Cancer Research Center
- Dr. J. A. Schneider, University of California, La Jolla
- Dr. Arnold Wedum, Fort Detrick

Breast Cancer Virus Segment

Dr. Jeffrey Schlom, Chairman
Dr. Wade Parks, Vice Chairman
Dr. Ernest J. Plata, Executive Secretary
- Dr. Robert Bassin, NCI
- Dr. Robert Gallo, NCI
- Dr. Raymond Gilden, Flow Laboratories
- Dr. Frank Lilly, Albert Einstein College of Medicine
- Dr. Hans Meier, Jackson Memorial Laboratory
- Dr. Max Myers, NCI
- Dr. Manuel Ochoa, Sloan-Kettering Institute
- Dr. William Rawls, McMaster University, Canada
- Dr. Harold Varmus, University of California
- Dr. Erwin Vollmer, NCI

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Dr. Paul Black, Massachusetts General Hospital
Dr. Barry Bloom, Albert Einstein College of Medicine
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Dr. Michael Brennan, Michigan Cancer Foundation
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Dr. Raymond Gilden, Flow Laboratories
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Dr. Seymour Kalter, Southwest Foundation for Research and Education
Dr. Albert S. Kaplan, Vanderbilt University School of Medicine
Dr. Henry Kaplan, Stanford University School of Medicine
Dr. Edmund Klein, Roswell Park Memorial Hospital
Dr. David Kohne, Scripps Clinic and Research Foundation
Dr. Pavel Koldovsky, Children's Hospital of Philadelphia
Dr. Hilary Koprowski, Wistar Institute
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Dr. Henry Kunkel, Rockefeller University
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Dr. Henry F. McFarland, Johns Hopkins University
Dr. William McGuire, University of Texas Medical School
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Dr. Thomas Merigan, Stanford Medical Center
Professor George S. Michaelson, University of Minnesota
Dr. Richard Morrow, Harvard School of Public Health
Dr. Paul E. Neiman, University of Washington School of Medicine
Dr. Robert Nowinski, University of Wisconsin
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Dr. Joseph Pagano, University of North Carolina
Dr. Theodore Pincus, Sloan-Kettering Institute
Dr. Keith Porter, University of Colorado

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Dr. Marvin Reitz, Litton-Bionetics, Inc.
Dr. Marvin Rich, Michigan Cancer Foundation
Dr. Albert Sabin, Weizmann Institute
Dr. Robert Schwartz, Tufts University School of Medicine
Dr. Aaron Shatkin, Roche Institute
Dr. Patricia Spear, University of Chicago
Dr. Chandler Stetson, University of Florida
Dr. Lise Thiry, Institut Pasteur du Brabant, Brussels
Dr. Lewis Thomas, Sloan-Kettering Institute
Dr. Robert C. Y. Ting, Bio-Tech Research Labs, Inc.
Dr. Harold Varmus, University of California, San Francisco
Dr. Samuel Wells, Jr., Duke University
Dr. Roger Wilsnack, Huntingdon Research Center
Dr. Lauren Wolfe, Rush Presbyterian-St. Luke's Medical Center

Administrative Structure of VCP

Segment Chairmen and Vice Chairmen. The segment chairmen are responsible for planning the projects in each working group. As senior scientists, they must review, analyze and integrate studies which fulfill the objectives of their working group and the total Program.

Executive Secretaries. Executive Secretaries assist the chairmen in managerial duties of contract operation. They are responsible for optimal review and complete documentation of each project within the working group.

Project Officers. Project Officers are the direct extension of program segment chairmen. To assure progress in accomplishing the goals set forth in the workscope of a project, they are called upon to advise principal investigators on scientific matters and coordinate segment and program decisions.

Review Committees (Working Groups) The program segment working groups are the peer review units for the Program. The committees review individual contracts or proposals for scientific excellence and technical competence within a given funding level. Their recommendations provide the Segment Chairmen, Associate Director for Viral Oncology, and the Director, DCCP, with a basis for program decisions.

Contract Specialists. Contract specialists are responsible for negotiating research contracts. They provide valuable advice on fiscal and legal matters to the project officers, executive secretaries, segment chairmen and Associate Director. Some specialists are well conversant with the scientific aspects of the Program. They are assigned to Program Areas by the Research Contracts Branch, NCI.

Contract Review. The projects within the total Program are reviewed at many levels:

(1) Each contract is reviewed for relevance, priority, and need to total Program by the Program Segment Chairmen.

(2) Each contract is reviewed for scientific excellence and technical competence by the Program Segment Working Group.

(3) Each contract is continually monitored for performance by the Project Officer.

(4) Each contract above the annual funding level of $1 million and with multifaceted workscope undergoes a third review by an ad hoc committee appointed by the Associate Director for Viral Oncology.

(5) Each contract is reviewed by the Associate Director for Viral Oncology; the Director, Division of Cancer Cause and Prevention; the Chief, Research Contracts Branch, NCI; and the Director, NCI.

As an aid to the review processes, key staff members receive progress reports on all contracts on a biannual basis. Collection and distribution of these reports is the responsibility of the Information Unit in the Office of the Associate Director. A single comprehensive report is prepared annually by the Associate Director.

C. Administrative Highlights. For most of the fiscal year, there were no major organizational changes in management of the Virus Cancer Program. Under the Chairman, the Associate Director for Viral Oncology, the following segments were maintained: Biohazard Control and Containment, Breast Cancer Virus, Developmental Research, Immuno-Epidemiology, Solid Tumor Virus, and Tumor Virus Detection. Contracts were reviewed for scientific excellence and technical competence by each of the six working groups composed of both intramural and extramural scientists and chaired by National Cancer Institute senior scientists.

Plans for restructuring these Working Groups are nearly complete. To meet directives of the Department of Health, Education and Welfare, the Virus Cancer Program has reduced the number of its review committees from six to two. Chartered in March, 1974 and known as Virus Cancer Program Scientific Review Committee "A" and "B", these two groups will review all individual proposals for scientific excellence and technical merit. These groups, each with 25 non-NCI scientists, will operate in conformance to the new rules set forth by the Director, National Cancer Institute: no scientist within the Viral Oncology Area will serve as the chairman of the review group; no Viral Oncology Staff will participate as voting members; no member may be appointed to or serve on the group that reviews his project proposal. As a result, many changes in the membership rosters of the committees are anticipated in the coming year. Henceforth, contracts under the Breast Cancer Virus Segment, Developmental Research Segment, and Immuno-Epidemiology Segment will be reviewed by selected members of Committee "A"; contracts under the Solid Tumor Virus Segment and Tumor Virus Detection Segment by selected members of Committee "B". Those contracts relating to biohazard safety and currently reviewed by the Biohazard Control and Containment Segment will be reviewed by the Advisory Group of the Office of Program Resources and Logistics.

It is anticipated that a third committee--the Virus Cancer Program Advisory Committee--will be chartered before the end of this fiscal year. This committee, to be composed of non-program, non-government scientists, will provide the Chairman, Virus Cancer Program with broad directions on: (1) allocation of resources, (2) areas of expansion or reduction of research; (3) development of new leads and opportunities; and (4) application of research findings to the control of cancer in man.

Viral Oncology received a significant increase in funds for the contract program in FY 74. The regular appropriation provided an increase of $4.5 million. In addition, court action brought about the release of funds which had been impounded by the Administration last year. This provided an added amount of $1.7 million for contracts for a total increase of $6.2 million. The only increase in the in-house program was to fund the pay raise of last October, approximately $100,000. The funding history for VCP contracts from its inception is given below:

Funding History - VCP Contracts
(in thousands)

Fiscal Year	Positions	VO	SVLP	VCP	Totals
64	30	4,926	-	-	4,926
65	90	5,433	8,723	-	14,156
66	95	3,064	13,556	-	16,620
67	138	3,137	13,505	-	16,642
68	140	-	-	17,241	17,241
69	157	-	-	17,985	17,985
70	167 (59)*	-	-	17,340	17,340
71	187 (68)	-	-	34,091	34,091
72	226 (79)	-	-	41,889	41,889
73	217 (83)	-	-	42,511	42,511
74	221 (84)	-	-	47,563	47,563

*Figures in parentheses represent professional staff, GS-13 and above.

D. Scientific Activities

1. Narrative.

At a time when many investigators are making a concerted effort to determine whether certain human cancers are virus induced, it is necessary to put into perspective some of the major developments in the field of tumor virology. For this reason, there is included in this report a brief history of viral oncology with emphasis on contributions made by in-house and collaborative scientists working within the Virus Cancer Program.

Although Ellerman and Bang identified the first cancer virus in 1908 in leukemias of chickens, it was not until 1964 that the concern of investigators was focused on the possibility that viruses similar to those which caused cancers in animals might also cause human cancers. Evidence suggested a viral cause of leukemia in cattle, and NCI scientists received reports that leukemic dairy cows were being killed by Danish farmers who feared transmission of the disease through milk or other dairy products. Reports on the isolation of an RNA tumor virus from leukemic cats, on finding particles in human leukemias and lymphomas which resembled RNA viruses known to cause animal leukemias, and on the isolation of a DNA virus from patients with Burkitt's lymphoma followed. Operating on a special $10 million appropriation from the U.S. Congress, the National Cancer Institute began an intensified research program on cancer viruses. With the advice of leading non-government scientists, the NCI defined its major research objectives and outlined the specific projects and resources necessary to accomplish the objectives. In addition to determining whether viruses cause human cancers and if suitable measures could be developed to control virus-caused cancers, the Institute targeted four major research areas under the program: (1) the nature and cause of animal leukemias and their relationships to man; (2) the hazards of working with viral agents; (3) development of animal and cell culture systems, quantities of viruses and other materials needed for research; and (4) improved treatment for the disease in humans.

Vast evidence accumulated in the program (and in grant-supported research) has shown that RNA viruses cause many kinds of cancer in a wide variety of animals, e.g., in rodents, chickens, cats, and more recently in sub-human primates. In the extensively studied animal models (chicken, mouse, and cat), it is clear that these viruses can cause natural disease; similarly, DNA herpes-type viruses were found in association with cancers of animals and man.

Although candidate human cancer viruses have been isolated more frequently in recent years, none has met the rigorous criteria necessary for firm identification as a cause of human cancer. Research findings and the nature of the disease in humans have led to extensive modification of the virus program. During this period scientists continued to develop techniques and experimental systems to identify and characterize viruses in animal model systems and apply the knowledge to the study of human cancer. They succeeded in demonstrating that tumor viruses could be studied <u>as viruses</u> by virologists, immunologists, and molecular biologists.

Many of the early projects concentrated on developing experimental methods and materials necessary to advance virus research. By 1967 new methods to grow the mouse leukemia virus in large quantities necessary for definitive study had been developed. This accomplishment led to development of techniques for producing other needed viruses. For example, cultured Burkitt's lymphoma cells containing the human herpesvirus (Epstein-Barr virus) were selected, grown in quantity, and made available to scientists. This was the first time that a standardized culture of Epstein-Barr virus was available for scientists to compare research findings. Until then, individual scientists had used various virus cultures, which made it difficult and sometimes impossible to compare research results. Nine techniques were developed for detecting antibody to EBV in human populations. Blood samples were collected from patients and normal controls around the world, coded, and sent to contract laboratories for tests using the various techniques. Results were compared, and three of the nine techniques were selected because they were uniformly reproducible, sensitive and least expensive for large-scale population studies.

The Virus Cancer Program currently supplies research materials of general interest from six local and regional repositories. Materials are sent to scientists under contract and within NCI, and as supply permits, to qualified cancer virus investigators throughout the world. A centralized computerized inventory lists more than 100,000 vials of blood samples from over 15,000 individuals and 3000 normal and cancerous tissue specimens from 1000 individuals. Uniformly prepared viruses, cell cultures and viral reagents are also available so that research findings may be compared. The Program has developed necessary safety standards for laboratory workers handling viral and chemical agents. These standards are being developed into an offical policy on safety. The Program has designed special containment devices necessary for the safe large-scale production of virus materials, developed certification procedures for critical safety training courses for scientists and laboratory assistants. The Program has frequently advised institutions planning new or renovated facilities for research.

Over the years, the virus program has reported several important "negative" findings. These lengthy, often expensive, but rarely dramatic studies enabled the program to identify research approaches and biological agents that were unlikely to provide solutions to the problem of cancer in man. With this negative information it was possible for the program to shift research focus into more productive scientific areas. This quick and flexible responsiveness to a rapidly evolving science base prevented continuing expenditures on outmoded research approaches and ensured maximum scientific progress for each dollar of funding. Some examples are:

(1) As the result of several studies, scientists in the program determined that mycoplasma, an organism often found as a contaminant of cell cultures and human specimens, does not cause malignant transformation of cells.

(2) Several human adenoviruses, which cause mild respiratory disease in humans, were found to cause tumors when inoculated into newborn rodents. Animals bearing these tumors reacted to the virus by producing

antibodies to a viral T antigen, or tumor protein. Scientists in the program purified the protein and tested 6000 human blood samples for antibodies to it. The results were completely negative. In addition, other scientists, applying a technique known as molecular hybridization, found no similarity between the mRNA of the virus and that found in human tumors. Although adenoviruses subsequently were eliminated as causative agents in human cancer, the concept developed was that tumorigenesis by DNA viruses was accompanied by integration of the viral genome into the cell, the viral DNA becoming a heritable cellular component.

(3) Program scientists were able to verify the presence of viral "particles" in approximately 30 percent of human leukemic samples studied under the electron microscope. However, they were unable to induce leukemia in approximately 700 sub-human primates inoculated as newborns with human leukemia specimens. Because of their complete failure to transmit leukemia or its "virus" to monkeys by these techniques, scientists in the program were compelled to find new ways to detect virus and its activity in human cells. Techniques were developed to find antibodies to a virus or its products; cell culture techniques were perfected; and biochemical and immunologic techniques were developed for the study of sub-viral components.

(4) Program scientists confirmed the isolation in 1964 of a cat leukemia virus. They demonstrated the ability of the virus to cause cancer in a variety of animals including dogs, cats, and monkeys and showed that the virus could infect human cells grown in culture. Because cats are closely associated with humans, a population study was begun in California in the late 1960's to determine whether cat owners were at higher risk to leukemia than the general population. The study concluded that cat owners have no increased risk of developing leukemia.

(5) Because of suggestive evidence that leukemia in cows might be caused by a virus present in the milk, the virus program sponsored studies to determine the relationship of milk "particles" to bovine leukemia. By 1968, one of these studies showed that the usual pasteurization methods kill animal RNA and DNA viruses deliberately added to cow's milk. This finding demonstrated that children and adults appear to be at no risk to developing leukemia from milk and milk products which have been pasteurized.

Studies on DNA viruses have concentrated on the known herpestype viruses--the Epstein-Barr virus strongly associated with Burkitt's lymphoma and nasopharyngeal cancer, and the *Herpes simplex* viruses associated most often with cancer of the uterine cervix in women. Epidemiologic data developed within the program has strengthened the relationship between the Epstein-Barr virus and Burkitt's lymphoma, although no causal relationship has been established. Immunologic techniques demonstrated high levels of antibody to the virus in patients with Burkitt's lymphoma and nasopharyngeal cancer and we now know that certain immunologic responses to EBV antigens in treated Burkitt's patients can be correlated with the prognosis of the disease. It was also determined that the virus is the cause of infectious mononucleosis. Hereditary

information similar to that in RNA cancer viruses also has been found in Burkitt's lymphoma and nasopharyngeal cancers, suggesting that both types of viruses may be factors in causing these cancers.

It has been difficult to prove that under natural conditions herpesviruses cause human cancer. EBV and HSV persist in large segments of the human population for extended periods, causing various types of illnesses. Whether these viruses, and other human herpesviruses such as cytomegalovirus, can cause cancerous changes in humans or act in concert with other agents to produce cancer requires additional study.

The oncogene theory, advanced in 1969 by Robert Huebner and George Todaro and the protovirus theory of Howard Temin provided the impetus for major new research within the program on the natural history, immunology and genetics of RNA cancer viruses. These two theories postulated different means by which the RNA viruses could infect and be incorporated into the hereditary information of the cell to cause cancer. For example, in the so-called oncogene theory: (a) viral genetic material is present in normal cells, (b) it can be expressed as an infectious virus, or, if defective, expressed only as viral antigens, without virus production, and (c) non-infectious expression of a portion of the viral genome may be responsible for cellular alteration leading to cancer. In the protovirus hypothesis, the genetic information required for cell transformation is postulated not to exist in the germ cell. The normal process of information transfer is deranged to give rise to the formation of genes for neoplastic transformation.

Many interesting findings resulted from work generated by these theories. Following the discovery that thymidine analogs can activate RNA viruses in cultured cells, program scientists learned that such "endogenous", or native viruses, can be activated in many types of animal and human cells. These findings indicate that many cells contain the structural components of the RNA virus in an unassembled form. Evidence indicating the oncogenic activity of endogenous viruses in mouse, rat and hamster cells already exists. However, even the limited information gained about this class of viruses within the program helped to identify a candidate human virus as an endogenous cat virus. Extensive efforts are underway to isolate similar "endogenous" viruses from human tissues.

Substantial evidence has been uncovered that, in animals at least, type C RNA viruses are transmitted as genes from parent to offspring. Within the past months scientists have isolated the first native type C RNA virus from normal baboon placental tissues. Similar virus particles have been observed consistently in normal human placental tissues, and efforts are underway to isolate and produce them in cell culture. Using DNA probes made from the baboon virus, program scientists also are attempting to determine whether the human particles are related to the endogenous baboon virus. The findings may show that type C virus is a normal phenomenon in the development of the embryo. Its presence in the placental tissues possibly was triggered by a hormonal change and could signal hereditary transmission of the type C virus in primates.

Since the discovery of the enzyme RNA-dependent DNA polymerase (RDDP) in 1970 by Drs. Temin and Baltimore research has accelerated in the field of RNA viruses. The Virus Cancer Program rapidly determined that RDDP is present in all known RNA cancer viruses. This finding established an association of the enzyme with virus-induced cancers in animals and led to the development of sensitive techniques to assay very low levels of viral activity. While studies on "reverse transcription" of viral genomic RNA continue, molecular components related to those in RNA tumor viruses have been found in human leukemic and breast cancer cells. The viral "footprints" now identified consist of two essential replicative components of RNA tumor viruses, intact 70S, viral RNA and RDDP. These findings have important bearing on the use of virus-specific molecules in human leukemic cells as diagnostic markers.

Instead of searching for the complete virus in cells, scientists now use these new techniques to search for viral hereditary information, viral reverse transcriptases, and proteins related to known cancer virus proteins. Using chemicals and cell culture techniques, they also attempt to induce the appearance of a virus or its components in human cells. New findings in virus research have demonstrated repeatedly the complexity of human cancer. However, with techniques developed within the Virus Cancer Program, scientists can now identify the biochemical and immunologic characteristics of almost any new virus discovered in animal or human cells. The major internal proteins and the reverse transcriptases of known RNA tumor viruses have been purified and characterized, and specific viral reagents are available. The activities of the Virus Cancer Program established the National Cancer Institute as a key resource for information on cancer-causing viruses.

RNA Viruses: Relationship to Human Cancer

Oncogenic RNA viruses belong to a class of RNA viruses distinguished by having an RNA-directed DNA polymerase (RDDP). At present, two groups are recognized: (1) type C viruses, and (2) type B viruses. The two groups, differentiated by their morphology and the diseases they cause, appear to show no cross reaction by immunologic and nucleic acid hybridization techniques. Viruses of both groups (1) mature by budding through the cell membrane, (2) have a density of 1.15-1.19 in sucrose gradients, (3) have 70S RNA as their genome, and (4) contain RDDP.

Type C Viruses. These viruses are distinguished from the other groups by their morphology, the site of maturation at the cell membrane, and by immunological relatedness. Type C viruses generally fall into two classes—leukemia and sarcoma viruses. Both occur in a wide variety of species where they cause leukemias, lymphomas and related neoplasms. Type C viruses can also be induced from cells of many species by carcinogens, mutagens, nucleic acid analogues and oncornavirus infection (see endogenous viruses, below). Although all leukemia viruses can infect, replicate and mature independently, mammalian sarcoma viruses are defective. The latter can transform cells which they infect but cannot replicate except in the presence of a helper leukemia virus. The leukemia virus supplies the functions lacking in the sarcoma virus but does not itself have the ability to transform cells. Many leukemia and sarcoma stocks are probably phenotypic mixtures or recombinants

of several viruses including endogenous viruses. Recent experiments have demonstrated that the various type C viruses recombine readily and that RNA from one type can be packaged in a coat of another type. It appears plausible that a transforming virus could be formed when a non-oncogenic virus picks up "transforming information" either from its host or from an endogenous virus in the host genome.

Although infection with type C viruses readily produces cancer in animals, the frequency of malignancies and the length of the latent period are governed by the genetic makeup of the host as well as a variety of environmental factors. The genetic effects of the host have been studied with the aid of a large array of inbred mouse strains. Several host genes affecting virus reproduction and tumor formation have been identified. Additionally, the genetic makeup of the virus determines the host range of the agent.

In the past year, the isolation and characterization of the structural proteins of RNA tumor viruses have been described. Using such procedures as gel filtration in guanidine hydrochloride, polyacrylamide gel electrophoresis, and iso-electric focusing, major proteins and polypeptides of mammalian type C viruses have been obtained in a relatively pure state. These biochemically defined materials have been used to produce highly-specific antisera and to develop radioimmunoassays, which are more sensitive than standard serologic tests. These reagents have not only proven valuable for the analysis of gene expression and characterization of viral particles, but they have become increasingly important in the search for possible oncogenic viruses in human neoplasia.

Several groups of mammalian RNA tumor viruses have been delineated on the basis of the serologic specificity of an internal polypeptide of approximately 30,000 m.w. (now often referred to as p27-p30) which accounts for about 30% of the virion protein mass. The N-terminal sequences of this antigen from several species of mammals show extensive homology, suggesting that the antigens are coded for by a related gene that has evolved with the host cell genome. This protein possesses both species-specific and interspecies determinants and is an important marker for virus classification. Thus, viruses from mouse, cat, hamster, rat, and higher primates can be clearly recognized. There may be exceptions as is the case with RD-114 which arose in a human tumor cell line after passage through a fetal cat. This virus appears to be of feline origin even though it does not contain species-specific determinants. Thus, while species-specific determinants can be taken to indicate the species of origin, failure to detect them is not a decisive indicator. Furthermore, some differences in the cross-reactive, interspecies determinants do occur and it has been suggested that the term "gs-3" be modified or qualified. The importance of these results in attempts to demonstrate similar reactivity in human tumors is evident.

Studies on the internal polypeptides of two recently described primate type C viruses (woolly monkey, gibbon) have shown a high degree of relationship to each other. Despite consistent differences in the isoelectric points of these proteins from two distantly related primates, the high degree of immunologic similarity offers the possibility that other primate type C viruses may be detected by currently available reagents. Additional studies

on purified elements of the viral envelope of certain murine type C viruses have been reported--the surface glycoproteins gp69 and gp71. By competition radioimmunoassays, these antigens have been found to contain a spectrum of determinants ranging from interspecies to group-specific to type-specific. It is evident that the antigenic properties of the viral proteins are more complex than previously recognized. Since antiserum prepared against gp69-71 has strong neutralizing activity, it may be possible to answer some pressing questions about which antigens are responsible for virus neutralization and possibly even tumor cell rejection.

Another antigen, a virion polypeptide designated p12, was isolated from several type C viruses of murine origin. Competition radioimmunoassays revealed both group- and type-specific determinants on the molecule and permitted classification of mouse type C viruses into different subgroups. This antigen, as well as p30, is present at very low levels in virus-negative mouse cells indicating that at least two genes for structural polypeptides are translated in these cells in the absence of detectable virus. The method may be useful in detecting viral-related antigens from species in which levels are presently below the threshold of detection.

A protein of approximately 70,000 m.w. present in the core of the virion possesses the enzymatic activities of RDDP and ribonuclease-H. Antibodies to partially purified MuLV-RDDP that also inhibit its activity were used to demonstrate that RDDP contains both interspecies and group-specific determinants. Interspecies determinants of MuLV-RDDP are closely related to those found on the RDDP of cats and hamsters, and not related to those from avian and primate type C viruses. A polymerase isolated from human acute myeloblastic leukemia cells has been found to be immunologically related to the primate RDDP, in support of the theory that this disease is caused by a virus.

The RNA of type C tumor viruses consists of a 70S genome, 28S and 18S RNA which appears to be ribosomal, and 4S and 7S RNA also of host origin. The 70S RNA can be dissociated into smaller units of various sizes. One such unit, a 4S methionine accepting tRNA, appears to be the primer for the transcription of RNA to DNA. The actual precursors of the viral genome appear to be 35S RNAs.

DNA synthesis from the RNA template requires several steps. The first product of the reaction is short pieces of DNA complexed with RNA. Later single-stranded DNA and eventually double-stranded DNA appear. Only after very long periods of incubation does DNA free of RNA appear. The primer DNA is at first covalently linked to the newly synthesized DNA. Apparently, the polymerase destroys the RNA template concomitantly with the release of double-stranded DNA; RNAse-H, found in the purified enzyme, is presumably responsible. RDDP is not the only RNA → DNA transcribing enzyme known. Cellular DNA polymerases can carry out this reaction, but only RDDP can transcribe the heteropolymeric regions of viral 70S RNA. This property thus provides an extremely specific way of identifying suspected viral RNA → DNA polymerases in tumor tissues.

It is not clear how mature viruses are produced from the DNA copies made by

the viral polymerase, but it is believed that DNA synthesis is an obligatory intermediate step. The DNA copies are necessary also for integration into the host genome. Again, the mechanism is not known, but it has been shown that transformed cells contain more viral DNA copies than untransformed cells. Therefore, viral genomes can become part of the host cell genome as well as become independent of it again.

The recent impressive progress in the understanding of the replication of type C viruses has provided a variety of new tools for searching for virus information in human cancers. The biochemical methods that have been developed fall into two categories. The first is designed to detect virus related nucleic acids, RNA or DNA, in tumor cells; the second category is used to search for RNA → DNA polymerase in tumor tissues. Both the RNA and the polymerase of type C viruses have characteristics that depend on the species of origin of the virus, factors of importance when looking for viral related material in humans. The isolation of several well defined primate type C viruses has contributed to these studies.

The DNA copies of viral RNA can be used to search for virus-related RNA in cell extracts by hybridization experiments. Several methods for identifying the resulting hybrid molecules are available, some very sensitive, some very specific. It is important to be aware of the limitations of the method employed. Another source of problems with these experiments is the nature of the DNA probe. RDDP does not copy all parts of the virus equally well so that probes are produced which may not contain all the viral information. It is not known which parts of the viral genome are important for oncogenesis. DNA/RNA hybridization experiments have been used to detect Rauscher MuLV-related sequences in human leukemias, sarcomas and lymphomas and to detect MMTV-related sequences in human mammary carcinoma.

Because the initial DNA product of the RDDP reaction is complexed with RNA template, it will cosediment with the template in sucrose gradients. This is the basis for a "simultaneous detection" test that can detect both viral RNA and viral polymerase in tumor tissues. The assumption is that the only newly synthesized DNA in a cell extract that sediments at 70S will be DNA complexed to viral 70S RNA. This test has been used to detect viral "particles" in human leukemias, Burkitt's tumors, lymphomas, brain tumors and in normal human milk. The "particles" in human milk synthesize DNA that is homologous to RNA from human breast cancer tissues.

RNA → DNA polymerases can be looked for directly in human cancer tissues. Such an enzyme has been isolated from human leukemic lymphoblasts. The enzyme has the expected template specificity and is immunologically more related to primate viral polymerases than to MuLV polymerase. The three major, normal DNA polymerases of mammalian cells can be clearly differentiated from this new enzyme.

Type B Viruses. The other major group of RNA tumor viruses is the type B which causes mammary adenocarcinoma. Research on these viruses has been limited by difficulties encountered in growing high titered stocks *in vitro*. Type B viruses can be transmitted vertically via the genome or horizontally through the mother's milk. Whether a mouse harboring type B virus will

develop cancer depends on its genetic makeup, hormonal environment, and the strain of MMTV involved. Excretion of virus in the milk is also genetically controlled.

The only type B virus isolated and well characterized is MMTV. Like type C viruses, this virus contains an RDDP which can be distinguished from the type C enzyme immunologically and by different cation requirements. The two enzymes can be separated from one another chromatographically. A virus like "particle", found in normal human milk, contains 70S RNA and reverse transcriptase. Previous reports of a correlation between these biochemical particles and particles seen in the electron microscope have not been confirmed. However, DNA synthesized by the human milk "particle" will hybridize with RNA extracted from malignant human breast tumor cells, as will DNA synthesized with MMTV RNA as template. These findings strongly suggest a viral etiology for human breast cancer.

Two major viral proteins of MMTV, designated gp52 and p27, have been purified from the virus in RIII mouse milk. Both antigens have been studied in immunoprecipitation and radioimmunoprecipitation-inhibition assays. The two proteins differ completely from each other and from major type C viral polypeptides. Of importance is the finding that gp52 (formerly S-1) expression occurs in milk of all high mammary tumor incidence strains tested; it is regularly associated with mammary adenocarcinomas. In contrast to earlier studies, this antigen, which is probably group-specific for MMTV, is located on the surface or envelope of the virion and not within the core. The findings suggest that either one strain of MMTV is common to most high mammary tumor incidence strains, or that virion surface glycoproteins may not have unique type-specific determinants. It will be necessary to isolate and characterize polypeptides from different strains of MMTV to show type-specific differences. The nature of the second protein, p27 of MMTV, has not been fully investigated.

Murine mammary tumor cells with type B DNA in their genome can be grown in tissue culture and, depending on their genetic makeup, will produce low levels of virus. Dexamethasone significantly increases type B virus yields without affecting type C virus production. The hormone appears to stimulate specifically the rate of transcription as measured by viral RNA synthesis. This system is of extreme interest not only for its virus producing capability but also for its potential for the study of hormone action. This is the only known case where a hormone has been shown to stimulate preferentially transcription of a specific RNA.

Endogenous Viruses. Although the natural induction of cancer by infectious RNA viruses does occur, it is unlikely that most spontaneous cancers are caused by a communicable, infectious virus. Spontaneous cancers are probably the result of expression of a viral genome that is an integral, heritable component of the cell. The viral genome can be present in a cell in an unexpressed state in which it does not necessarily render the cell resistant to superinfection by the same virus. Viruses which can exist in an integrated state within the cell are called endogenous viruses. Endogenous viruses have been recovered from cells of mice, rats, hamsters, cats, and chickens; all clones from apparently virus-free cultured cells of these

species can be induced to yield complete or partial expressions of type C viruses. Some endogenous viruses have difficulty in replicating efficiently in the cells of the species of origin, but preferentially replicate in cells of other animal species; thus a sub-class of endogenous viruses now called xenotropic (x-tropic) has been recognized. The fact that endogenous viruses are at least partially expressed when the cells which harbor them become malignant, indicates that their study is important and potentially exploitable for the study of the cause of human cancer. Most investigators now accept as a working hypothesis that RNA tumor agents are of endogenous origin.

Much of the recent evidence that RNA tumor viruses exist in unexpressed form is derived from studies in inbred mouse strains. Genetic and biochemical evidence indicates that multiple copies of the viral information are integrated within the DNA of the cell. Endogenous viruses are induced spontaneously during long-term passage of cells in tissue culture and by co-cultivation with cells of a different species. The induction rate can be increased 1000-fold by treatment with carcinogenic chemicals, x-irradiation, or infection by exogenous type C viruses. It was also found that halogenated thymidine analogs (IUdR, BUdR) and protein synthesis inhibitors (actinomycin D) are extremely efficient inducers, increasing the rate of expression by more than 1 million-fold. In animals, endogenous viruses can be induced by passage of tumor material from one animal species into another. This is especially true when the recipient species has been immunosuppressed. Indeed, an example of this method was the recovery of the RD-114 virus after injection of human sarcoma cells into the brain of a fetal cat. Although this virus had antigens distinct from those of known feline type C viruses, hybridization studies revealed that the virus was of feline origin.

Several independent investigations have yielded endogenous murine type C viruses. Each appeared to be of mouse origin as determined by the antigenicity of its major virion polypeptide, p30. At first these virus isolates were indistinguishable from each other in serologic and host range properties. Newer findings, however, have shown that they differ considerably in their immunologic type-specificity (p12) thus making it possible to distinguish different subgroups of virus from the same speciesof origin. Furthermore, some grow preferentially in cell lines of another strain. For example, an inducible virus of BALB/c cells grows to markedly higher titer in NIH Swiss cells. As studies on these inducible viruses continue, it is quite likely that many subclasses of endogenous type C viruses will be discovered.

The role of endogenous viral genes in naturally occurring malignancies has yet to be elucidated. One class of type C virus that is chemically inducible from virus-negative mouse cells in tissue culture can induce lymphatic leukemia *in vivo*. It is possible to speculate that at least some naturally occurring malignancies result from the expression of individual, naturally-integrated and biologically distinct tumor viruses. Although endogenous cancer viruses have not yet been isolated from human tumors, there are good reasons for inferring their existence.

These findings raise many questions about the nature of the factors controlling viral induction. It is important to know whether viral genomes are present in human cells and how often they are expressed. Such information

can provide the basis for a specific attack against the cancer cell by either biochemical or immunological approaches. If the host immune system were to recognize some components of an endogenous virus as foreign, antibodies might influence both its expression and its biological activity.

DNA Viruses: Relationship to Human Cancer

During the last decade, various herpesviruses have emerged as proven or suspected oncogenic agents. There is now little doubt about the causal relationship between Marek's disease virus, *Herpesvirus sylvilagus* and *Herpesvirus saimiri* and lymphoproliferative neoplasias in chickens, wild cottontail rabbits and sub-human primates, respectively. These and other experimental animal systems have been extensively studied to elucidate the basic mechanisms of DNA virus-induced neoplasia and to provide information which may be applicable to human cancers of suspected herpesvirus etiology.

Herpesviruses interact with their host cells in a productive or non-productive manner. During the productive growth cycle, the synthesis of infectious progeny is invariably accompanied by destruction of the target cells. In the non-productive cycle, stimulation of cellular DNA synthesis, acquisition of virus-induced antigens, incorporation of viral nucleic acid and transformation of normal cells into established lines capable of indefinite proliferation have been described. Activation of virus synthesis in non-productively infected cells by exposure to mutagens (BUdR, IUdR) or irradiation is usually accompanied by cell death. Herpesviruses, like the RNA tumor viruses, also establish persistent covert infections. Whether latent herpes infections are the result of low-level productive or non-productive interactions has not been determined. Although transmission of the genome in non-productive cultured cells to progeny has been recorded, there is no definitive evidence that herpesviruses are vertically transmitted *in vivo* as is the case with the RNA tumor viruses. However, horizontal transmission of various herpesviruses in animals, including man, readily occurs.

The mechanisms underlying herpes-induced oncogenesis remain obscure. The close association of these viruses with several human malignancies suggests, but does not prove, their involvement as etiological agents. The possibility remains that these DNA viruses are simply co-factors, passenger viruses, or derepressed latent agents whose expression is enhanced by the oncogenic process.

Epstein-Barr Virus. Seroepidemiological, biochemical, and biological studies suggest an association between various herpesviruses and specific malignant diseases of man. The Epstein-Barr virus is intimately associated with Burkitt's lymphoma (BL) of African children and, to a lesser extent, with nasopharyngeal carcinoma (NPC). An association between EBV and Hodgkin's disease, chronic lymphocytic leukemia, and certain other malignant and nonmalignant diseases has been questioned by recent seroepidemiological studies in which a proportion of patients in these categories were found to have no antibodies to EBV. Finally, the etiological relationship of EBV to nonmalignant infectious mononucleosis in young adults has been firmly established.

Burkitt's lymphoma is prevalent in regions of East Africa and New Guinea where cases occur in time-space clusters and undergo epidemic drift characteristic of infectious diseases. However, the incidence of BL and NPC is extremely restricted compared to the ubiquitous nature of the virus, thus suggesting that cofactors and/or predisposing conditions are probably required for expression of its oncogenicity.

Seroepidemiological data associate EBV with BL and NPC. All African BL and Chinese, African and western NPC patients examined have higher than normal levels of antibody directed against EB viral capsid antigens (VCA). Additionally, antibody levels to EBV-determined cell membrane antigens (MA) and to the diffuse (D) and restricted (R) components of the EBV-induced early antigen (EA) complex confirm the association of EBV with tumor. Recently, a new EBV-related antigen has been described--the EBV-associated nuclear antigen (EBNA). This antigen is detected by an anti-C[1] immunofluorescence technique and is present in every cell containing EB viral genomes. Patients with BL or NPC develop antibodies detectable by these various procedures, the levels of which are of diagnostic or prognostic value. For example, antibodies against the EA complex are predominantly directed against the D component in IM and Oriental NPC while antibodies to the R component appear to be the principal response in BL and Caucasian NPC patients. The differential responses may be a reflection of the extent of lymphatic involvement. Thus, the absence or decline of anti-R antibodies in BL patients or anti-D antibody in Chinese NPC patients indicate a favorable prognosis. Similarly, anti-Ma antibody production declines 3 to 6 months prior to recognition of recurrent tumor during chemotherapeutically-induced remission of BL.

The search for virus particles and for capsid or early antigen producing cells in BL tumor ciopsies has not been successful. However, the majority of cells in most BL, but not NPC, biopsies exhibit EBV membrane antigen. Both BL and NPC biopsies are reactive for EBNA while an EBV-determined complement-fixing antigen, unrelated to VCA, MA or EA, has been measured in BL biopsy extracts. Similarities between this latter CF antigen and EBNA are currently under investigation. EBV DNA has been detected in virtually every BL and NPC biopsy and all tumor cell lines that have been examined. One to more than one hundred copies of EBV DNA were found per cell. The viral DNA appears to be present in both the epithelial and the lymphoid elements of NPC. It appears that every BL and NPC cell carries the EBV genome but only a small proportion of the cells contain selected EBV-related antigens. These results indicate a cellular association of EBV in which the viral genome is largely repressed.

EBV has a distinct growth stimulating effect on lymphoid cells. Exposure of peripheral lymphocytes from normal donors to EBV results in the establishment of continuous lymphoblastoid cell lines. Adult peripheral white cells may convert into such lines without the addition of extraneous virus, but the derived cell lines usually carry EBV. These cells acquire a chromosomal abnormality, a constriction of the long arm of human chromosome 10, which also occurs in many virus-producing cells in cultures of Burkitt's lymphoma. Several lymphoblastoid cell lines have been established by direct culturing of the BL or NPC itself. In contrast, cultures initiated with lymphoid cells

from donors without EBV-directed antibodies or from cord blood or fetal organs failed to grow. The available data indicate that EBV is the major, if not the sole, factor required for establishment of lymphoblast cell lines. Although the antigenic profile and virus productivity patterns vary among these cell lines, EBV-homologous nucleic acid sequences are, with rare exceptions, always detectable. Expression of the viral genome may be significantly increased by cultivation of the cells in arginine-deficient media, or by exposure of the cells to x-irradiation, mitomycin C, bromodeoxyuridine, or iododeoxyuridine. Thus, the virus genome persists in these cells *in vitro*, is expressed in varying degrees, and is transmitted during cell division.

Recently, EBV propagated in human or simian cells was shown to infect and induce lymphomatous reticuloproliferative disease or lymphoma in sub-human primates. This represents the first demonstration of the oncogenic potential of this virus *in vivo*. Although these results suggest that EBV is indeed involved in the oncogenic process, its role could be that of an accessory factor or passenger virus in the tumor. No EBV DNA has yet been found in American BL tissues even though this tumor is histopathologically similar to African BL. RNA sequences homologous to the RNA of Rauscher murine leukemia virus have recently been described in human lymphomas (including BL) suggesting the possible interaction of type C and herpesviruses in the genesis of this disease. The viral-related RNA is complexed with RNA $\rightarrow$ DNA polymerase in a particle possessing a density characteristic of the RNA tumor viruses. It is interesting that in non-neoplastic cells which contain EBV information, such as infectious mononucleosis, no RNA particles are found. This may explain the involvement of EBV infection with more than one type of disease. Should this virus prove to be a required co-factor in the induction of disease, control of infection would be of paramount importance.

Herpes Simplex Virus. An expanding body of evidence has strengthened the causal relationship of herpes simplex virus type 2 (HSV-2) with carcinoma of the human uterine cervix, the second most common malignancy in women in the United States. Genital HSV-2 infection is venereally transmitted, second in frequency only to gonorrhea. HSV-2 infection may be more common in males than females since this virus can be isolated from urogenital specimens taken from a high percentage of asymptomatic males. These observations indicate that the incidence of genital herpes infections could account for every reported case of cervical anaplasia; however, the epidemiological data are too limited to determine the number of infected women at risk to the development of cancer.

Women with cervical neoplasia tend to have higher titers of antibody to HSV-2 which are acquired earlier in life than normal controls. These differences between patients with cervical carcinoma and control groups are more impressive in Negro than in Caucasian women and in the U.S. than in several foreign countries, including Israel, Columbia, and New Zealand. Additionally, exfoliative cytology studies have shown more evidence of herpetic cytopathology in patients with cervical anaplasia than in matched controls.

Two aspects of the seroepidemiological studies of HSV-2 and cervical cancer

impose limitations upon the interpretation of the data. First, the degree of accuracy with which the present antibody systems detect past infections with HSV-2 is not known. Herpesvirus types 1 and 2 share common antigens and have one or more type-specific antigens. Infection with either virus results in the production of antibodies that will cross-react with the heterotypic virus. The immune responses to the two viruses are not independent: a prior infection with HSV-1 modifies the production of antibodies to HSV-2. Further, an infection with HSV-2 in an individual previously infected with type 1 virus may stimulate an anamnestic response to the shared antigens of the two types of virus. It is apparent that the isolation, purification, and characterization of HSV type specific antigens is crucial to definitive seroepidemiological studies.

HSV non-virion antigen(s) have been described in cells infected in vitro with these viruses. Antibody to this antigen(s) has not been measured in patients with active recurrent herpetic lesions, but was present in patients with tumors of the mouth, lip oropharynx, nasopharynx, kidney, urinary bladder, prostate, vulva, larynx, and cervix. These results indicate a possible involvement of this virus in the etiology of human tumors other than cervical carcinoma. It is of interest that an HSV-2 specific soluble antigen, AG-4, has been prepared from human cells harvested 4 hours after virus infection. Although it is not clear whether this antigen is virion, nonvirion or even a modified cellular antigen, it appears to be specifically associated with squamous cancer of the human cervix. Antibody directed against AG-4 is present in women with evidence of previous HSV-2 infection, correlates well with the progression to cervical cancer, and disappears following tumor removal.

Neither virus or virus-specific antigens nor herpes-induced cytoplasmic changes have been detected in biopsied neoplastic cells. However, tumor cells on the surface of neoplastic lesions, as well as exfoliated tumor cells, do possess HSV-2 antigens and all of the virus-associated cytological changes but no virus particles. Cultured cells, established from a biopsy of carcinoma in situ, failed to show evidence of viral antigens or virions unless the cells were exposed to media of high pH. This in vitro data suggests that cervical cancer cells harbor the complete HSV-2 genome in a latent or repressed state, and virus expression occurs following exposure of the tumor cells to conditions of stress.

The oncogenic potential of herpes simplex virus has been demonstrated in vitro. Cultured hamster embryo fibroblasts transformed with ultraviolet irradiated or dye-light inactivated HSV-2 are tumorigenic in newborn hamsters. HSV-2 antigens were detected in the cytoplasm of the cells transformed in vitro and virus-neutralizing and membrane-reactive antibodies appeared in the sera of the tumor-bearing animals, suggesting the presence of genetic information derived from HSV in the tumor. Weanling hamsters that were preimmunized by injection of HSV-1 or HSV-2 did not develop transplantation immunity to the HSV-2 transformed cells. The transformed cells metastasized after injection into hamsters and the number of metastases was enhanced by immunization with HSV-1 or HSV-2. Two additional human herpesviruses, HSV-1 and cytomegalovirus, have also been shown to transform cultured hamster embryo fibroblasts. It was noted recently that Cebus monkeys develop cervical infection and

herpetic lesions similar to that of humans following intravaginal inoculation of HSV-2. A large scale investigation is now in progress to determine whether the repeated infection of Cebus monkeys with HSV-2 will produce malignant changes of the cervix. This system would provide the first animal model for study of cervical cancer.

These findings strengthen the association of HSV-2 with cervical carcinoma. Studies to determine if this virus is an etiological agent in the induction of this disease will be continued. If genital herpes infection can be shown to be a factor in the development of cervical neoplasia, appropriate control measures may be developed to reduce the incidence of this human cancer.

Papovaviruses. Recently, several papovaviruses of the polyoma-SV40-virus group have been isolated from humans. These human viruses are classified into three antigenically distinct groups which cross-react to some extent with SV40 but not polyoma virus. Strains of virus belonging to two of the three groups have been isolated from brains of people dying from a rare neurological disease, progressive multifocal leukoencephalopathy, and strains from the third group were found in the urine of a renal transplant patient. Broad patterns of prevalence of antibodies to these viruses have been found in human populations; thus, human infection by certain small papovaviruses is rather common. Since papovaviruses are known to cause natural or experimental tumors and to transform cells in culture, human viruses of this group are now being studied as possible etiological agents of cancer in man.

Treatment and Control.

The most impressive evidence for the viral etiology and perhaps the best hope for prevention and control of cancer would be the demonstration that anti-viral agents or anti-viral immune responses will prevent or modify the disease. Rational approaches to tumor therapy may be possible even without the isolation of a bonafide human tumor virus. There is considerable evidence that, in animal systems, RNA tumor virus genomes are responsible for spontaneous and induced cancers even when there is little or no expression of infectious virus. As information on the biochemistry of tumor virus replication and on tumor virus structural and induced antigens has increased, new methods for treatment and control of oncogenesis have become possible.

Biochemical treatment. Many compounds have been tested for their capacity to inhibit replication of RNA tumor viruses. The studies are being approached as follows:

(1) Enhancement of the process of reversion to normal state. Recently two agents, colcemid and fluorodeoxyuridine were found to increase the reversion frequency of transformed S+L- cells to the normal state by a factor of 50. Any procedure or drug which would accelerate the rate of reversion from the transformed state has obvious potential curative value. An understanding of cellular controls could be exploited to yield a high frequency of reversion and be applied to tumors *in vivo*.

(2) Inhibition of RDDP. The systematic study of the mechanism of action of different inhibitors of RDDP is underway. Such agents as

streptonigrin, narcissus alkaloids, pyran copolymer, tilorone, rifamycins, and poly A have potential usefulness as inhibitors of reverse transcriptase. Although the mechanism of inhibition is not known for all of the inhibitors, some combine physically with the template without affecting the binding of template and enzyme. A few of the most active compounds have been tested in mice and proved effective in increasing survival of animals in induced remission.

(3) Interferon treatment. The graft versus host reaction was shown to be highly effective in activating murine leukemia virus (MuLV) with subsequent development of leukemias in mice. MuLV is activated from recipient lymphoid cells responding to foreign skin graft antigens in sites where lymphoid blastogenesis is maximum. Interferon treatment prevents this activation of virus and current studies suggest that interferon may also prevent GVH-virus-induced leukemogenesis.

Immunological treatment. Attempts to prevent or reduce the incidence of virus-induced neoplasms by immunological means fall into three categories:

(1) Non-specific stimulation of host immune mechanisms. Among the agents that stimulate the reticuloendothelial system nonspecifically, imidazolethiazole, pyran copolymers, tilorone, BCG and *Str. hemolyticus* have proven effective in increasing the survival times of leukemic mice. Some compounds exhibit dual effects: (a) direct, on tumor cells similar to that of conventional anti-tumor agents, and (b) indirect, through enhancement of host immune response to virus or virus-induced, tumor specific cell antigens.

(2) Vaccination with RNA tumor viruses. It now appears possible to use virus vaccine-induced antibodies to modify natural type C virus expressions and either delay or prevent tumors associated with these viruses. Early experiments established the feasibility of using formalin-killed virus vaccine in the prevention of murine leukemias induced by the Rauscher strain of MuLV. Inactivated murine type C virus vaccines also produce significant reduction in tumor incidence in mice treated with chemical carcinogens. Only vaccines prepared from the natural (endogenous) virus of the mouse strain were effective. Recently virus-specific antibodies which develop spontaneously in several strains of mice have been shown to have virus-neutralizing capacity. The development and use of viral vaccines are plausible because: (a) specific antigens can be packaged in viral particles that can be replicated in virtually unlimited amounts; (b) high concentrations of purified particles are available; (c) viral protein separation techniques provide additional opportunities for isolating and concentrating those tumor antigens specifically responsible for protection; and (d) growth of virus in various types of tumor and normal cells provide opportunities for selecting a variety of viral-coded, cellular membrane antigens as well as viral proteins.

Virus neutralization titers vary according to vaccine potency and immunological competence of the mouse strain. Although the humoral antibodies to type C viruses may not represent the immunological factors most responsible for protection, they do serve as indicators of vaccine potency. Cell mediated response stimulated by vaccination may actually provide more effective

immunity to tumors.

(3) Virus-assisted immunological stimulation. Homogenates of tumor cells infected with certain non-oncogenic viruses are more immunogenic than extracts of non-infected cells. This phenomenon has been studied thoroughly with viral-induced tumors infected with influenza virus. Homogenates of non-infected, SV-40 transformed cells did not induce immunity in BALB/c mice, whereas homogenates of SV-40 transformed cells infected with influenza virus or VSV caused suppression or complete regression of tumor transplants. The results stress the necessity of working with fully adapted viruses. Viruses which seem suitable for the purpose of "virus-assisted immunotherapy" in man have been adapted to human malignant tissues and a new research strategy towards human application now seems feasible.

2. Progress Highlights (not covered in Narrative)

Type C viruses.

The major glycoprotein which appears on lymphocytes transformed by type C viruses has been purified. This protein is found not only on the surface of cells but also on virion surfaces, a fact which is important for an understanding of the relationship between the host immune response and oncogenesis.

Specific antibodies to the RNA → DNA polymerase of murine type C viruses have been isolated from the renal glomeruli of both leukemic and non-leukemic AKR mice. This finding provides further evidence for the lack of immunologic tolerance in the mouse to its own endogenous viruses.

A highly significant and predictable association between endogenous type C viral expression in early life and leukemias and reticulum cell sarcomas with advancing age has been found in mice, suggesting that these viruses are a major determinant of these diseases.

Morphologic revertants of sarcoma virus transformed non-producer cells have been isolated. These show normal cell morphology but still contain the sarcoma viral gene. Both cellular and sarcoma viral mutants obtained by this approach are being characterized to define the viral and cellular functions involved in expression of sarcoma virus transformation.

Genetic studies have revealed that mouse genomes contain several biologically distinguishable endogenous RNA viruses which can be activated independently. Genetic factors affecting inducibility and persistence of different endogenous viruses have been detected and are currently being studied.

A few and highly potent class of chemical inducers of type C virus, protein synthesis inhibitors, was discovered. These inducers are able to activate endogenous viruses selectively from the same cell, indicating that there exist cellular regulatory mechanisms specific for each virus.

The Kirsten sarcoma virus was found to be a recombinant between Kirsten

murine type C virus and sequences in rat cells. At least, part of the rat sequences are homologous to rat type C virus produced from NRK cells. The results suggest that transduction of oncogenic information occurred.

A new host range class of endogenous type C viruses has been identified which are unable to replicate in any mouse cell line tested. The term "S-tropic", derived from the virus sensitive rabbit cell line SIRC, is used to identify these agents. By host range properties and by nucleic acid hybridization studies, this class of viruses is distinguishable from both "N" and "B" tropic murine type C viruses.

DNA sequences complementary to 70S AMV-RNA can be separated from the bulk of cellular DNA because of their high GrC content. This finding permitted studies which showed that the cellular content of viral DNA increases as early as one hour after infection of cells with AMV or RSV and that within 24 hours the viral DNA becomes covalently linked to host cell DNA.

H^3-labeled 35S AMV RNA can be exhaustively hybridized with excess normal chicken DNA leaving residual RNA which hybridizes only to leukemic chicken DNA but not to normal chicken DNA. If confirmed, these results indicate that DNA from leukemic cells contains viral specific sequences which are absent from DNA in normal cells.

Tumors induced in hamsters by bovine papilloma, polyoma viruses or DMBA contain hamster lymphoma virus. Although present in amounts too low to be detectable by EM, reverse transcriptase or gs antigen tests, the virus was detectable by its ability to produce visceral lymphomas after injection into newborn hamsters. Similarly, rat cells transformed by chemicals or polyoma virus, while free of demonstrable overt RNA virus, reverse transcriptase or gs antigen, were shown to contain significant amounts of virus-specific RNA. These findings suggest that RNA viruses may be involved as co-agents in all forms of cancer.

An intriguing new finding is the discovery of RNA → DNA polymerase in certain normal cells, specifically chick embryo cells and amphibian oocytes. It is suggested that this enzyme (and perhaps cancer viruses themselves) plays a part in normal embryonic development.

Type C viruses have been isolated from baboon placentas, spleens, lungs and testes. The DNA product of an endogenous reaction using these viruses as templates hybridizes to the normal baboon cell DNA and to the cellular DNA of other primates. Eight to twelve DNA copies are found per diploid genome in all normal baboons tested. This is the first demonstration of endogenous type C virus in primates.

To date type C particles have been found in 17/20 normal human placentas examined by electron microscopy. Placentas from patients with lupus, an auto-immune disease, have been found to be particularly rich in type C virus particles.

Virus-neutralizing envelope antibodies against one or more envelope antigenic types were found in the sera of 22-24% of cats with or without neoplasia but

not in the sera of 36 veterinarians or in 33 laboratory personnel working in two laboratories engaged in feline leukemia research.

Terminal transferase activity has been found in 6 out of 8 cases of childhood acute lymphoblastic leukemia but not in cases of chronic lymphatic leukemia, lymphosarcoma, cell leukemia and acute myeloblastic leukemia. The enzyme has also been found in a class of normal human infant thymocytes which predominate during fetal life and may be T lymphocyte precursor cells. These findings have important implications for diagnosis and studies of the origin and mechanism of induction of acute forms of leukemia.

Reverse transcriptases have been purified from three cases of human acute myelogenous leukemia. The enzymes have antigenic properties closely related to those of the type C viruses found in woolly monkeys and gibbons. However, hybridization studies indicate that the gibbon virus is not an endogenous primate virus but appears to be derived from rodents.

DNA produced in an endogenous reaction with particles from human acute myeloid leukemia cells was shown to hybridize most readily with RNA from simian sarcoma virus-1.

DBA synthesized in an endogenous reaction with 70S RNA containing particles from Hodgkin's and Burkitt's lymphomas was shown to hybridize with DNA from the lymphoma cells but not with DNA from normal cells. The unique DNA sequences found in the lymphoma tissues are related to each other but not to the DNA of the Epstein-Barr virus. These findings suggest that tumor virus information may not be transmitted through the germ line and that RNA viruses, as well as DNA viruses, may be involved in the etiology of Hodgkin's and Burkitt's lymphomas.

Type B viruses.

MMTV viral polymerase was shown to differ from type C polymerase, immunologically and biochemically. MMTV and MP-MV reverse transcriptases prefer Mg++ when synthetic templates are employed, as opposed to the enzymes of type C viruses which prefer Mn++. This distinction has provided an important tool for the identification and measurement of enzymes in cells which contain both viruses.

Phospholipase C can be used to prepare cores from MMTV and from human milk particles. Cores, unlike complete virions, band in gradients in a region comparatively free of cellular contaminants. Using this method, assays for particles become more sensitive and reliable.

Studies with clones of high and low expressor cells from MMTV cell lines suggest that transcriptional regulation of the integrated MMTV genome is a critical determinant in MMTV expression. In spite of 1000-fold differences in viral expression in vivo and absolute differences in mammary tumor incidence in different strains of inbred mice, no differences in the number of multiple viral copies in the cellular DNA were found.

Epidemiologic surveys showed no increased risk of breast cancer in man

associated with breast feeding.

A DNA probe synthesized from the RNA of human milk cores hybridizes significantly with the polysomal RNA from human malignant breast tumors but not to the polysomal RNA of benign breast tumors, normal breast tissue, sarcoma tissue, leukemic cells or spleen.

Immunologic evidence for a viral involvement in human breast cancer was demonstrated by studies which showed that the migration of leukocytes in breast cancer patients was inhibited by mouse milk containing MTV but not by milk which does not contain MTV. Studies using cryostat sections of breast tumors as antigens showed that small tumors were antigenic and patients with small tumors were able to respond immunologically to MTV or human breast cancer antigens. However, patients with advanced disease were unable to respond to breast cancer related antigens. Similar results were obtained with studies in mice. Of interest is the restoration of the immunological response of patients occurring after surgery. This finding has obvious implications for treatment of human breast cancer.

DNA Viruses.

The earliest effect of SV-40 infection on the host cell is a twofold drop in cAMP concentration which occurs within three hours of infection of contact-inhibited cells. The cAMP level gradually rises to the original level over the following 8-10 hours. cAMP has been implicated in a number of cellular regulatory mechanisms.

The human papovavirus, BK virus, can transform BHk_{21} clone 13 cells which are then tumorigenic in adult hamsters. Similarly, BK virus will transform specific marmoset fibroblast cell lines.

Genital infection of cebus monkeys with HSV-2 has resulted in persistent mild to severe cervical dysplasia in 5 animals one to two and a half years after inoculation.

Antibody to a non-virion HSV-2 antigen has been detected in the sera of patients with advanced carcinoma of the lip, mouth, oropharynx, nasopharynx, kidney, urinary bladder, prostate, cervix uteris and vulva. No antibody is found in serum from normal individuals, donors with current or recurrent HSV-1 or HSV-2 infection or patients with other cancers.

Studies on the genetics of Burkitt's tumor demonstrated a high incidence of HLA types 1 and 8 in patients with American BL; the same HLA types have been found in an increased frequency in patients with Hodgkin's disease.

The EBV genome was found to be linearly integrated into some, but not all, chromosomes of infected lymphocytes. A nuclear antigen, EBNA, induced by the virus associates with the chromatin fibrils in interphase nuclei and with the chromosomes in metaphase. EBNA is present in most of the tumor cells examined. It appears that only lymphocytes derived from B cells can be infected by EBV.

E. Projections:

The Viral Oncology Area set forth a long range research plan for the identification and control of virus-induced cancers of man. Many studies within the following broad categories have already been implemented and will be expanded in the coming year:

1. Virus (or virus-expression)-tumor relationships

a. Model Studies. Studies on animal, RNA and DNA, tumor viruses, known to cause malignancies in several mammalian species, will be continued. The results of these studies have already provided important information about tumor viruses that is applicable to the isolation and identification of human agents. Special emphasis will be given to determine the characteristics of several newly-isolated primate viruses, since these agents may provide the best probes for detection of Type C virus information in human cells. This work will remain an integral part of the Program.

b. Human Studies. Efforts to identify viruses or detect virus expression in human tumors have been underway for some time. The Program will continue to increase its activities in the search for viruses which induce malignancies of man.

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

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

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

(4) To extend existing and develop new methods to induce tumor virus expression in "normal" cells.

(5) To develop suitable reagents and to improve existing immunological and biochemical methods for mass diagnostic screening for candidate viruses.

(6) To characterize, biologically and biochemically, presumptive human virus isolates.

(7) To increase emphasis on understanding the relationship of environmental agents (e.g. chemical carcinogens) as co-factors in viral carcinogenesis.

2. <u>Molecular Studies</u>

Major progress in the understanding of the molecular pathways of tumor virus replication has been made within the past year. Such advances have already provided the basis for new, extremely sensitive methods for the detection of oncogenic viruses or virus expression. It is now possible to characterize agents detected in specimens of human cancer patients in terms of their content of high molecular weight RNA and of RDDP. Specific hybridization procedures already provide a method for further investigation of host-cell-virus relationships which have been extended into the study of human cancers. Preliminary results offer strong supportive evidence that certain human tumor cells contain genetic information related to that found in known oncogenic viruses.

a. <u>Basic Studies</u>. The program will continue to broaden its activities for detecting, identifying, and characterizing the spectrum of enzymes and their products required by tumor viruses for replication and/or transformation.

b. <u>Applied Studies.</u> As knowledge of the fundamental molecular events in virus-cell interaction increases, the Program will continue to apply this information to the study of human cancer as follows:

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

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

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

Ultimately these approaches will require an intensified program to develop drugs, anti-enzymes, gene repressors, or inhibitors effective at the molecular level.

3. <u>Immunological Studies</u>

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

a. Basic Studies. Investigations of selected model systems, representing tumors induced by Type C, Type B and herpesviruses, will be extended to further identify, characterize, and determine the viruses, viral antigens, and membrane antigens of tumor cells. The studies include development and application of improved techniques to detect cellular alterations induced by tumor viruses alone or as the result of interaction with other environmental agents. Research on spontaneous or naturally occurring tumors in model systems relevant to human cancer will be continued as a basis for a rational approach to prevention and treatment:

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

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

b. Applied Studies. As basic research provides the framework for identification and characterization of viruses, viral antigens, and virus-induced cell membrane alterations in human cancers, immunological methods will be applied:

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

(2) To identify and characterize intra- and inter-species viral antigens which are present in known mammalian tumors, as probes for detecting human tumor viruses or viral antigens.

(3) To determine the presence of cross-reacting antigens implying viral causation in various human tumors.

(4) To launch large-scale seroepidemiological surveys which will define populations at high risk to virus-induced cancers.

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

(5) Development of vaccines (viral or sub-viral components) from identified and fully characterized human tumor viruses.

(6) Determination of the role of host immune responses in virus-induced tumor recognition and rejection.

(7) Application of a. and b. in the prevention and control of human cancer.

4. Test Systems and Resources

a. Test Systems. In vitro and in vivo (animal) test systems will be

carefully selected to evaluate the work outlined in the previous research areas:

(1) To determine the oncogenic potential of candidate human viruses;

(2) To develop bioassay systems for testing viral and viral/chemical carcinogens;

(3) To begin viral vaccine (conventional or other) testing and immunization programs;

(4) To begin viral therapy testing programs;

(5) To explore special animal tumor systems, especially in primate species particularly relevant to human cancer;

(6) To develop and maintain well-characterized cell culture lines and animal stocks (small mammalian and primate species).

Many of these systems are being developed at the National Cancer Institute's Frederick Cancer Research Center.

b. Resources. Since research efforts undergo continual change in emphasis and scope as new leads emerge, a variety of resources will have to be developed, maintained, and coordinated.

(1) Human Resources-collection and storage of serum and tissue specimens; integration of data on clinical records, storage and distribution; computerization of specimen collection.

(2) Animal Models-maintenance of various mammalian animal colonies for basic research and special studies;

(3) Reagent production-large scale production of animal tumor viruses for basic research; production of standardized lots of purified viruses; and production of high quality diagnostic reagents;

(4) Candidate Human Virus Production-intensive developmental research effort to isolate and produce human viruses;

(5) Biohazards Control and Containment-controlled environment facilities are required for research on known oncogenic viruses and candidate human tumor viruses as well as for maintaining animal colonies which are protected from extraneous infections.

F. Reports of Meetings Sponsored by the Virus Cancer Program:

Participation in US-USSR Agreement. Developed within the framework of the US-USSR Agreement on Health Cooperation, a Memorandum of Understanding for further

cooperation in the study of the microbiology, the immunology, and the molecular biology of cancer viruses was signed on November 18, 1972. The Memorandum established procedures for joint studies through the exchange of information, materials, and scientists. A detailed account of the first year's proceedings is given in last year's Annual Report. On November 12, 1973, a meeting of the Joint Subcommittee on Oncology was held in Bethesda, Maryland. At a Working Group Conference on Tumor Virology, the discussions centered on: viruses isolated from the six cell lines sent to the U.S. last year: cytogenetic studies on these cell lines; and progress on the study of plasma samples from leukemic subhuman primates sent to the U.S. by Dr. B. Lapin. The first studies have resulted in a joint US-USSR publication which confirms the presence of virus-like particles in five different human cell lines. The virus from one of the lines, a human amniotic cell, was selected for further study by immunological and biochemical techniques. It was found to be closely related to MP-MV, first described in 1970 in biopsy material from a rhesus monkey mammary adenocarcinoma.

In further discussions, the U.S.S.R. side agreed: to conduct collaborative viral seroepidemiologic studies on ethnic groups within the U.S.S.R.; to make available human tumor cell cultures not yet producing virus particles; to provide materials from baboon and monkey leukemias induced by inoculation of human leukemic materials. The U.S. side agreed to make available: cell cultures containing the recently isolated type C particles from baboon placentas and human placentas, and antiserum to reverse transcriptases from selected mammalian tumor viruses. Both sides agreed that two groups per year of one to three young Soviet scientists would come to the U.S. for work and study in the laboratories of the National Cancer Institute and in collaborating laboratories of the Virus Cancer Program. Scientists will visit selected laboratories in the U.S.S.R. for participation in seminars, lectures, etc. The members agreed to prepare lists of appropriate viral oncology meetings to which scientists of both countries could be invited to attend and/or participate.

A U.S. delegation attended a joint Soviet-American symposium on biology of oncogenic viruses of animals and man in Moscow in May, 1974. Visits were made to institutions actively engaged in the field of virology and immunology in Moscow, Kiev, Sukhumi, Riga, Leningrad, and Tblisi. An account of these meetings will appear in the next Annual Report.

During the last few years a number of cases of leukemia in primates have occurred at the Institute of Experimental Pathology and Therapy at Sukhumi, U.S.S.R. Although some animals were injected with material from human leukemic patients, the origin of the disease in the primates is not clear. This year two baboons (Papio hamadryas) were sent to the U.S. for further study. The first one arrived in January, 1974. After exhaustive physical examinations, the animal was sacrificed; the diagnosis at autopsy was malignant lymphoma. Organs and tissues have been sent to several collaborating laboratories in the Program, and attempts to transmit the disease to other primates and to isolate a virus from the cells have begun. No definitive results are available at this time. The second animal arrived in March in

apparent good condition. The animal is now being observed and examined for signs of malignancy at a local contract facility.

Under the agreement, the following U.S.S.R. virologists have made official visits to the National Cancer Institute and collaborating laboratories within the U.S.:

Dr. Viktor Zhdanov, Ivanovsky Institute of Virology, and Dr. Frederick Seitz, Petrov Research Institute of Oncology, visited with scientists at the National Cancer Institute and attended the annual meeting of the Virus Cancer Program at Hershey, Pennsylvania. Dr. Seitz also visited several laboratories in the San Francisco area.

Dr. Konstantin Ilyin, Gamaleya Institute of Epidemiology and Microbiology, visited scientists at the National Cancer Institute as well as the M. D. Anderson Hospital and Tumor Institute in Houston, Texas and Flow Laboratories in Rockville, Maryland.

Franco-American Exchange Program. This program permits the exchange of U.S. and French scientists to work and study in laboratories of the respective countries for periods up to three months. This year the following scientists participated:

U.S.: Dr. Dharam Ablashi - International Agency for Research on Cancer, Lyon
Dr. Barbara Marczynska - Hopital Saint Louis, Paris
Dr. Robert Bassin - Institut Pasteur
Dr. Victor H. Zeve - College de France, Paris and International Agency for Research on Cancer, Lyon
Dr. Paul Levine - International Agency for Research on Cancer, Lyon

France: Dr. J. C. Chermann - Viral Oncology Area, NCI
Dr. Jean-Claude Chuat - Viral Oncology Area, NCI; Flow Laboratories, Rockville, Md.; and University of Southern California, Los Angeles, California

VI International Symposium on Comparative Leukemia Research

The World Committee of the International Association for Comparative Research on Leukemia and Related Diseases selected Nagoya-Ise-Shima, Japan, as the site for the sixth meeting, September 16-21, 1973. Dr. Yohei Ito of the Aichi Cancer Center was Chairman of the Organization Committee. The meeting brought together leading scientists from all over the world who work on the problem of leukemia and related diseases. Discussions and original scientific papers were presented on comparative animal model systems; neoplastic conversion of cells and tissues by leukemogenic agents; growth regulation and differentiation of hematopoietic cells; *in vitro* viral and chemical carcinogenesis; mechanisms of growth regulation and differentiation of leukemic cells; immunological approach; non-human primates in the study of human leukemia; detection and identification systems; viruses, genes and nucleic acids; the state and function of viral genome in transformed cells; the role of immunological, virological and chemical determinants; genetic, biochemical and immunological control; new therapeutic approaches; advances in genetic,

biochemical and immunological control; current trends in the management of leukemia and lymphoma; problems and progress in the control of bio-hazards in experimental cancer research.

National Conference on Virology and Immunology in Human Cancer

This American Cancer Society-National Cancer Institute co-sponsored meeting was held in New York City, November 29-December 1, 1973. Papers presented at this conference covered the current developments in research and clinical investigation in virology and immunology and the assessment and implications of this work in the prevention and treatment of human cancer.

The 1974 Abraham Flexner Symposium. This two-day conference on Viral Transformation and Endogenous Viruses was held in Nashville, Tennessee, April 1-2, 1974.

Conference on Papovaviruses. An informal meeting at NIH on April 8, 1974 brought together a number of investigators in the field of papovaviruses to discuss the possible role of this group of viruses in human cancer and to develop a system of nomenclature for these viruses.

Eighth Annual Joint Working Conference-Virus Cancer Program. The annual meeting of the Virus Cancer Program was again held at Hershey, Pennsylvania on November 4-7, 1973. The following topics were discussed: molecular basis of tumor virus expression; breast cancer; basic studies in the understanding of viral oncogenesis; the biology of virus-induced cancer; cancers associated with herpesviruses; endogenous RNA viruses; and the control and prevention of virus-induced neoplasia.

Atlantic Coast Tumor Virus Group (ACTVIGR)

The Atlantic Coast Tumor Virus Group was formed to provide an opportunity for investigators located in the eastern region of the country to discuss research activities of mutual interest. Its objectives are to promote exchange of information on current concepts and progress and to maintain integration of the research effort within the Program. The informal atmosphere of smaller discussion groups is conducive to active exchange among participants with special research interests.

Meetings are held under the chairmanship of Dr. Fred Rapp, who contributes his time to make these gatherings possible at facilities offered by different contractors. Attendance has been limited to one day, and each participant arranges his own travel. The constraints imposed by time and space require some limitation on attendance. Accordingly, selection of specific areas of research involving several laboratories is required in order to best achieve the objectives of these meetings. The various ramifications of an area are presented by different investigators drawing on their own and supporting data, thereby providing the basis for amplification through the ensuing discussion. Participation has not been restricted to scientists within the SVCP. Contributions from others have been invited to provide more comprehensive coverage of current research.

Arrangements were made by Dr. Fred Rapp to hold the first ACTVIGR meeting at Pennsylvania State University, Hershey, Pa., on March 22, 1971. The topics discussed were of general nature reflecting the broader aspects of Program. Dr. David Yohn arranged facilities for a meeting on June 7, 1971, at Ohio State University, where those attending considered the current knowledge on relationships between herpesvirus type 2 infections and carcinoma of the uterine cervix.

The first ACTVIGR meeting in 1972 was held on January 11, at the Institute for Cancer Research, College of Physicians and Surgeons, Columbia University, New York City. Dr. Sol Spiegelman was host for this workshop meeting to discuss molecular approaches to viral oncogenesis.

Dr. Joseph Pagano served as host for a meeting held at the University of North Carolina at Chapel Hill on June 6, 1972. Participants involved in the study of herpesviruses were invited to discuss several possible mechanisms whereby infection by these agents might effect neoplastic transformation.

The fifth ACTVIGR meeting was held in Houston, Texas, on January 16, 1973. Dr. Leon Dmochowski was host for this one day workshop to discuss cellular immune mechanisms in human neoplasia. Topics discussed included: cell mediated immune response to acute leukemia cells and soluble leukemia antigen; quantitative measurement of cell and antibody mediated immune reactions of patients to cultured sarcoma cells; cellular hypersensitivity to autologous breast cancer; immune responses of tumor-bearing marmosets; immunologic activation of oncogenic viruses; and, function of the host immune system in the regulation of endogenous RNA tumor virus expression.

Dr. Jeno Szakacs served as host for the sixth meeting held at St. Joseph's Hospital in Tampa, Florida, on May 14, 1973. Participants were invited to discuss their recent findings in molecular studies in viral oncology. Discussions centered about the following topics: techniques for detection and isolation of reverse transcriptase; partial transcription by type C particles in BALB/c mice; proteins and low molecular weight polypeptides of RNA tumor viruses; detection of EBV DNA in malignant tissue and cells; and relevance of RNA tumor viruses to human lymphomas.

The seventh meeting was held on April 26, 1974, at Emory University in Atlanta. Dr. Andre Nahmias was host for this workshop meeting to discuss viral vaccines, their potential usefulness and associated problems for prevention and therapy of neoplastic diseases in man and animals.

Pacific Coast Tumor Virus Group (PACTVIGR)

Only two PACTVIGR meetings were held during fiscal year 1974. Each presented preliminary findings (since published) of enormous significance in the search for the human type C RNA tumor virus, and possible approaches to cancer prevention and control through viral immunoprevention (vaccines) and better understanding of the mechanisms of switch-on and suppression of oncogenesis.

New discoveries and techniques included the visualization of type C RNA particles in human and other primate placentas; the presence of endogenous type C RNA (xenotropic) viruses which could not grow in their respective host cells but which were prime candidates as causes of naturally occurring cancer; methods of induction for uncovering the xenotropic viruses; and biochemical probes which provide an extremely sensitive method for establishing the probable species of origin of the numerous viral isolates being recovered, and which will eventually provide the proof of the human origin of "rescued" viral isolates.

These meetings provided a forum for establishing collaboration between laboratories and programs working in related areas to accelerate progress and make possible a pooling of resources.

The 15th PACTVIGR meeting was held on August 29, 1973 at the University of Southern California. Dr. Murray Gardner (USC) described a long-term study of a number of wild mouse colonies, in which a colony switched-on for type C RNA tumor virus demonstrated early cancer incidence and paralysis. Experimental follow-up confirmed the etiologic association between the type C virus, tumor and paralysis. The form of paralysis seen was clinically and pathologically almost identical to amyelotrophic lateral sclerosis in humans, and studies are now underway to search for an equivalent agent in humans, and to determine the host range of the murine viral isolate.

Dr. Brian Henderson, who is in charge of the epidemiology program at the University of Southern California, described what appeared to be an enhanced incidence of lung tumors in three areas of Los Angeles, all of which were in high petroleum refining districts. The increased incidence appeared to be associated with residence, but the data are preliminary. Studies are now underway to study occupational exposures, etc.

Dr. Frank Dixon, of the Scripps Clinic and Research Institute, discussed his work on the immunopathology and biological behavior of the type C virus derived from the NZB mouse, which has a high incidence of autoimmune disease associated with the type C virus. This disease strongly resembles lupus erythematosus in humans. The disease in both mice and humans is strongly genetic, and is associated with familial cancer in humans; the mice do not usually live long enough to develop cancer, but do develop leukemia if treated with immunosuppressants at an early age.

Dr. Dixon's group described a new oncornavirus surface antigen, and was now setting up a radioimmune assay for this.

Dr. Karl Hellstrom of the University of Washington presented a progress report on cellular immunity to tumor antigens in which he described his most recent findings on the blocking and unblocking factors originally described by Dr. Hellstrom; the presence of the former associated with loss of regulation and the cancerous state in humans and in animals. Animals with the blocking factor would accept tumor transplant cells, whereas those with the unblocking factor would not.

Dr. Hellstrom described some very promising preliminary studies in which lymphocytes cytotoxic to target cancer cells could be induced in animals lacking the blocking factor by treatment with sera from animals with the factor.

Dr. J. Taylor, of Dr. Michael Bishop's laboratory at the University of California in San Francisco, described their work on characterization of the 3' end in the 70s RNA complex, which had been found to be a 4s RNA They are currently doing some work with the Visna viruses which lack the 4s RNA, among other differences from the type C RNA tumor viruses. They hoped to determine whether the 3' 4s RNA precursor of the RNA virus template was the RNA on which oncogenic behavior was coded.

Dr. Eckhart of the Salk Institute described his most recent findings on the polyoma temperature sensitive mutants in efforts to determine which gene products were important in transformation. The ts A mutant was required for initiation of transformation, but not for maintenance of the transformed state; ts 3 was required for maintenance of lytic characteristics of transformed cells. The ts A's retained their characteristics; the ts 3 did not. Current emphasis was on whether translation of a given gene was involved in transformation of a normal cell. They were also looking at alterations in the mechanism of uptake of hexoses (Deoxyglucose) and possible correlations with transformation at permissive temperatures.

Dr. Henry Kaplan, Stanford University, described his efforts to culture malignant cells, with emphasis on Hodgkins disease and other lymphomas. Acknowledging the difficulty in growing Reed-Sternberg cells, he noted that his group had found enough malignant cells in the spleen to establish lines. The established lines would be studied intensively in efforts to detect and rescue a virus, particularly from the Hodgkins lines, since there was some epidemiological and other evidence of a possible viral etiology in Hodgkins disease.

Dr. Robert Huebner, National Cancer Institute, focused on the importance of the host immune response in the development and control of tumors. His group was attempting enhancement of the immune system by production of antibodies to the viruses involved, which would require antibody to the precise antigens involved in tumor induction. He described his vaccine trials and results leading to his conclusions.

Dr. Huebner also described tumor induction in hamsters by the bovine papilloma virus which apparently had derepressed the hamster's endogenous type C viral genome, resulting in a series of tumors which continued to come up and regress throughout the lifetime of the hamster. At the time of the experiments, the hamster leukemia virus had not been isolated. Sera from the afflicted animals, tested recently, revealed high levels of HaLV antigen. In addition, independently, the endogenous HaLV had been proved oncogenic after a period of about 18 months on test. He pointed up the necessity of now going back to tumors produced by other DNA viruses to ascertain whether they too operated through derepression of their endogenous RNA viruses.

Dr. John Stephenson, NCI, described his and Dr. Stuart Aaronson's work on genetic controls of virus expression using temperature sensitive murine virus mutants. It appeared that viral inducibility (IdU and BrdU) and persistence were dominant in some genetic crosses. Work was in progress to try to determine whether the genetic controls involved structural genes or regulatory genes.

Dr. Hans Meier of The Jackson Laboratory discussed his findings which revealed the type C virus or viral genome to be present in every mouse cell. Viral expression varied with respect to a number of inducible loci; and pathogenic expression was related to early virus expression.

Utilizing F_1 and F_2 backcrosses of high and low viral expression strains, Dr. Meier determined that the development of tumors was associated with gs+ mice, providing an important tool for identifying high risk individuals and groups.

Additional experiments indicated the presence of a repressor gene which regulated gs antigen expression in low tumor-incidence mice. Thus tumor formation may be repressed by repression of antigen expression.

Dr. Meier's group was also working on the use of carcinogens transplacentally to induce tumors in the offspring of five different strains which were chosen with respect to their inherent antigen or virus expression. The effect of the carcinogens was found to be dependent on the genetic strains and crosses used, with the greater hereditary influences reflected on the maternal side. Infectious virus was not necessary for pathogenesis or transmissibility of carcinogenesis; offspring derived from gs and virus-positive (high tumor) strains developed the same tumors normally seen in the strain at a much earlier age. These strains would be used to test the effects of vaccines used prophylactically. Dr. Meier summarized by stating that through genetic selection one could prevent cancer, identify genes associated with cancer, and possibly identify and make use of repressor genes.

Dr. Gilden (Flow Laboratories) described his studies on the evolution and relationships of the type C RNA viral genome in a number of species. He demonstrated a mammalian viral "family tree" in which he demonstrated the gibbon and woolly monkey viruses to be relatively closely related, the RD114 to be in a category by itself, and the hamster, rat and murine leukemia viruses to be relatively close at the lower spectrum.

At the 16th meeting, which was held in Berkeley, California, Dr. Jay Levy described the discovery of a "xenotropic" type C RNA mouse virus, present in virtually all strains, which was characterized by its inability to grow in its host cell. He described the technology used to uncover the presence of this virus and viral genome, which could be applied to other species. This finding was of enormous potential significance, since it provided insights into the natural history of the type C viral genome, its role in natural cancer, and the technology for rescuing an analogous virus/genome in humans.

A major finding had been the discovery in several laboratories of type C viral particles in human and primate placentas. This work was summarized by Dr. Kawakami of the University of California at Davis.

In view of the foregoing, a large segment of the meeting was devoted to a number of approaches in several laboratories in efforts to induce and/or rescue the human type C virus or viral genome. Participants included Drs. Walter Nelson-Rees and Adeline Hackett (Naval Biological Research Laboratory, Oakland), Drs. Robert Huebner and Paul Arnstein (NCI), Drs. John Riggs and Linden O'Shiro (California State Department of Public Health), Dr. Jay Levy (University of California Medical Center, San Francisco). and Dr. Thomas Kawakami.

Dr. Huebner followed with a brief summary of the evidence to date of the genetic transmission of oncogenic viral information, providing further confirmation of the universal presence of an oncogene normally suppressed under immunological controls until old age or other carcinogenic environmental influences. This pointed up the necessity to better understand the mechanism of immuno-control.

The meeting concluded with a discussion by Dr. Michael Bishop (University of California Medical Center, San Francisco) on the use of biochemical probes to demonstrate the presence of endogenous virus and to prove homology with given species. This was a highly sensitive method of demonstrating homology between viral isolates and their presumed tissues of origin and to rule out contaminants or agents which infected the host but were not part of its genetic heritage.

II. SUMMARY REPORTS OF OFFICES OF THE ASSOCIATE DIRECTOR

A. Frederick Cancer Research Center

The Viral Oncology Program (VOP) at the Frederick Cancer Research Center is implemented through the Scientific Coordinator for Viral Oncology who is physically located at Fort Detrick, Frederick, Maryland. Program direction for the VOP is derived from the Associate Director for Viral Oncology with the assistance of the FCRC Viral Oncology Advisory Group. The latter currently consists of seven NCI staff members plus at least three outside consultants. The major function of the Viral Oncology Advisory Group is to review all VO projects at FCRC on a continuing basis for the purpose of attaining and maintaining program excellence and to insure rapid response of the FCRC project operations to needs related to the overall NCI program objectives. The attainment of research objectives is further served through close associations between the FCRC Scientific Coordinator for Viral Oncology, and the NCI Project Monitors, the Contractor's Project Managers, the Office of Program Resources and Logistics, the Office of Biohazards and Environmental Control, and other NCI program elements.

During the second year of operations, Viral Oncology completely funded five projects and partially supported three others. New this year was Project 13, Basic Research in Viral Oncology, which consisted of a number of NCI intramural sub-projects that were initiated with contractor personnel on a collaborative basis. Also new this year was a research program in Project 6 to support the work of Dr. T. O'Connor on the evaluation of a possible human oncogenic virus candidate. A summary of these efforts and other VO projects at FCRC are presented below.

Those projects that are completely funded by Viral Oncology are the following:

Project 1, Virus Production. The objectives of this project were to attain a production level of Rauscher murine leukemia virus (RLV) of 150 liters per week, to concentrate and purify this and other oncogenic or suspected oncogenic material, to phase in the large-scale production of a second virus (woolly monkey fibrosarcoma virus), to produce and concentrate limited amounts of a variety of specially requested viruses on a one-time basis, and to distribute virus products as requested by the Office of Program Resources and Logistics, NCI.

Project 2, Developmental Research. The objectives of this project were to develop techniques applicable to scaling up cell culture production of Epstein-Barr virus and mouse mammary tumor virus, involving continued studies directed at optimizing parameters for growth, including concentrations of amino acids, oxidation reduction potential, pH and dissolved O_2 and CO_2, the induction of viral yields with chemicals, (e.g. IdU, hormones, DMSO, cyclic AMP), host range screening of cell cultures for infectivity and replication, development and/or improvement of testing and assay procedures *in vitro*, and the development of a mouse colony to supply tumors, milk and animals for MMTV testing.

Project 3, Viral Diagnostic and Test Reagents. The major objectives of this program were to purify group specific antigens (p30) and viral reverse transcriptase (RDDP) enzyme from Rauscher leukemia virus and to prepare specific antisera in various laboratory animals using the purified proteins as antigens; this project also had the responsibility for the quality control of FCRC products until the formation of a new separate Quality Evaluation Section in November.

Project 5, NCI Office of Biohazards and Environmental Control. Project 5 provides and maintains laboratories, animal holding and administrative space for use by the NCI Office of Biohazards and Environmental Control in Building 550. The Project will evaluate potential hazards associated with research activities in the viral oncology laboratory. Methods will be developed for identifying and evaluating viral hazards. Nonhazardous model systems for evaluating standard laboratory procedures and equipment will be developed. Safer techniques, procedures, equipment and devices will be developed and made available to all NCI contractors.

Project 13a, Studies on Herpesvirus. The major objective of Project 13a during this report period has been the implementation and support of the Herpesvirus program of Dr. Albert B. Sabin. Specific objectives included the following: (1) preparation of nonvirion HSV antigens in guinea pig kidney (GPK) and HEp2 cultures, (2) preparation of virion antigen in HEp2 cultures, (3) preparation of antisera in guinea pigs to the HSV nonvirion antigen(s) (NVA), (4) absorption and preparation of human sera for complement fixation (CF) testing, (5) CF testing of large numbers of human sera from patients with a variety of cancer types.

Project 13b, Virus Disease Modification. The goal of this project is to conduct a multidisciplinary approach to the detection, prevention, and/or control of viral-induced and spontaneous neoplasias (specifically, leukemias, sarcomas, carcinomas, and melanomas). The research efforts are concentrated in three general areas of investigation: (1) antitumor therapy; (2) antiviral and immunostimulation effects of drugs *in vitro*; and (3) biochemical assessment of active compounds on the basis of inhibitory effects on viral polymerases and the synthesis of protein and nucleic acids by both virus and host cells.

Project 13c, Purification and Characterization of Virus-Associated Tumor Antigens. The major contract objective of Project 13c is to isolate and characterize virus-augmented tumor transplantation antigens using the following virus-mouse-tumor systems: (1) SV40-transformed BALB 3T3-induced fibrosarcoma in BALB/c mice, (2) Moloney virus-induced ascites lymphoma in A/He mice, and (3) mammary tumor virus-induced Adenocarcinoma in C3H mice. Other objectives are to develop protocols for the use of concentrated antigens in immunotherapy and to support the human clinical program of the Cell Biology Section, Viral Biology Branch, NCI.

Project 13d, Primate Virus Section. The objective of this unit is to develop and staff facilities for the study of the virology, biochemistry, immunology, and pathology of human and nonhuman primate oncogenic viruses. The Primate Virus Section is oriented to conduct and devote its efforts towards the role of human and nonhuman primate viruses in the induction of neoplasia and all its studies have either direct or indirect relevance to the understanding and prevention of cancer in humans. The Primate Virus Section will also provide laboratory facilities and training for visiting scientists from the U.S.A. and abroad for research in the above areas.

Those projects that are partially funded by Viral Oncology are the following:

Project 4, Environmental Control. Control of the total work environment to minimize risk of human exposure to biological agents and chemical carcinogens used in experiments, to safeguard against accidents involving radioactive materials, toxic chemicals or physical apparatus are the objectives of the Environmental Control Project. The control measures that protect the experimenter also protect the validity of the experiment, which could be compromised by accidental contamination or cross-contamination of cultures, cell lines and animals. Environmental Control personnel are attempting to assess risks, define problems and develop solutions for the effective control of environmental hazards. Viral Oncology supported approximately 35 percent of this effort.

Project 6, Developmental Electronmicroscopy. The objectives of this project are to: interface the TEM and light microscope with the automated image analysis system to increase the capabilities of these instruments for quantitative and qualitative measurements; perform collaborative research within the FCRC utilizing the image analysis system to improve efficiency and accuracy of quantitative and qualitative measurements for quality control and specimen evaluation; and perform collaborative research within the FCRC utilizing the combined resources of the TEM and SEM.

SA-4 Project. Objectives are to develop adequate expertise for the purpose of examining and evaluating human oncogenic candidate viruses. The first of such candidates, introduced by Dr. Timothy O'Connor in January, 1973 is associated with studies conducted on cultured cells (SA-4) derived from a human liposarcoma. A major effort was made to produce large volumes of cells and to process supernatant fluids necessary for evaluation of 60-70S complexes in the simultaneous detection procedure. Plans were also implemented to prepare adequate amounts of virus concentrate for nucleic acid hybridization studies for species determination at FCRC and other laboratories collaborating in this endeavor.

Viral Oncology supported approximately 40 percent of the total Project 6 effort which includes all of the above mentioned work.

Project 12, Animal Farm. Project 12 provides for the production of inbred, hybrid and outbred laboratory animals of various species and strains, free of spontaneous diseases and of known genetic background; these animals are to be produced in sufficient quantities to meet the needs of the FCRC and other NCI operations as directed by the Project Officer. Viral Oncology supported approximately 25 percent of this project.

The FCRC Viral Oncology Program will undergo some alteration next year. The general scope of effort within which the Contractor will be required to perform consists of four main segments of work: (1) Virus and Viral Reagent Production, designated Project 1; (ii) Viral Oncology Research, consisting of Project 13, Basic Research in Viral Oncology and Project 2, Developmental Research on Viruses and Viral Components; (iii) Product Characterization and Standardization, designated as Project 3; and (iv) Biohazards and Environmental Control, designated as Project 5.

It should be emphasized that the FCRC Viral Oncology effort is to be performed as a total integrated activity which will allow for a high degree of flexibility in response to overall program needs and for broader latitude in creating and pursuing critical fundamental research objectives both in-house and on a collaborative basis with outside acknowledged experts. As work in the given areas is performed, particular research objectives may receive changes in emphasis; new objectives may be introduced and/or previously established ones may be postponed or eliminated.

B. Office of Biohazards and Environmental Control

The office is responsible for developing safety standards with regard to oncogenic viruses and chemical carcinogens. It assists contractor and, where applicable, intramural and grantee programs in carrying out cancer research safety policies. Performs research to develop effective control practices and establishes risk assessment criteria. It conducts basic research pertaining to the physiological and environmental factors that alter the host's susceptibility and response to oncogenic viruses and carcinogens.

The Biohazard Section conducts research to elucidate the mechanisms involved in host immunocompetence and the consequences of endocrine imbalance on such competence and oncogenesis. These factors are being investigated by *in vivo* and *in vitro* as are the genetic factors associated with hormonal activation of protein synthesis associated with tumor virus expression.

In collaboration with the Southwest Foundation for Research and Education (a collaborative contract laboratory) we recently observed the presence of type C viruses in normal baboon and human placental tissue. The significance and function of type C viruses in reproductive tissue of primates as well as rodents is being further evaluated. Conditions normally necessary for the expression of such particles are also under study. We are further exploring the nature and significance of the M-7 type C virus presumably isolated from baboon placental tissue.

The Environmental Control Section develops and recommends equipment, facilities, procedures and standards for the proper handling of potentially biohazardous materials and disseminates this information to the scientific community through publications, training programs, site visits and consultation activities. Procedures, equipment and facilities are evaluated to identify inherent safety deficiencies and to provide basic information for developing corrective measures. The Section operates the NCI virus containment facility and prototype containment laboratories.

The Section conducts applied research to evaluate potential hazards associated with research activities in viral and other areas of oncology and to develop adequate protective measures. This program is performed at the Frederick Cancer Research Center.

Minimum Standards for Biological Safety and Environmental Control have been issued to all contractors within the VCP. Consultation and training activities have been expanded to assist contractors in implementing these standards.

Minimum Safety Guidelines for Research Involving Chemical Carcinogens have been developed by the Section in collaboration with the NCI Cancer Research Safety Committee. The Section has provided technical assistance to the Carcinogenesis Program in areas of laboratory safety and environmental control. Studies have been undertaken to determine the impact of the Department of Labor Carcinogen Standard on cancer research and to select practical methods for the implementation of this Standard.

C. Office of the Coordinator for Ultrastructural Studies

This office and the Virus Studies Section continue to be involved in a number of projects concerned with viral replication and oncogenic virus-host cell interactions. These projects are as follows:

1. A search for viruses in biopsies and tissue culture isolates of human solid tumors and other human tissues.

2. The study of ultrastructural variations in different oncogenic and related viruses.

3. Electron microscopic and biochemical analysis of particles found in guinea pig leukemia and normal guinea pig cells.

4. Ultrastructural and biochemical studies on viral RNAs.

5. Investigation of viral nucleic acid metabolism during the eclipse phase after infection using radioautography and pulse labeling of Actinomycin D, uridine and thymidine.

6. Ultrastructural changes during transformation following RSV infection.

Search for Viruses

A total of 10 cell lines have developed from sterile biopsies of human solid tumors. Six of these are monolayers, 4 are suspension cultures. In addition 4 tissue cultures from placentas have been obtained, one from an ectopic pregnancy, 2 from caesarian section and one from a term placenta. Suspension cultures as would be expected from previous work have the Epstein-Barr viral genome but to date no virus has been identified in monolayer cultures. Eighteen of the cultures presently being carried in this laboratory have been sent to Jon Fogh at Sloan-Kettering partly for safekeeping, partly so that Dr. Fogh can carry out studies not contemplated for this laboratory. The placenta cultures are being stimulated with BUdR to determine whether a type C viral genome can be induced to replicate type C virus.

Ultrastructural Variations in Virions

Recently a comparison was made between the type C viruses of the mouse, cat, gibbon ape and baboon with the placental virus of the rhesus monkey, the baboon and human. All typical type C viruses exhibit an electron lucent space between the envelope and the nucleoid during the bud stage. The large majority of placental virions in all 3 species have no such space. This

suggests the possibility that two type C viruses are present in placentas and that in tissue culture of placentas, a typical type C virus has been selected while the placental virus has not been isolated.

Viruses vs. Host Cells

Studies on the particle associated with guinea pig leukemia have indicated that the particle is not a type C particle but an intracisternal A particle. This particle in practically 100% of the cases is attached to another particle or to the membrane of the endoplasmic reticulum. It would appear that this particle escapes from the cell only at the time of cell death and is probably not responsible for the induction of leukemia.

Normal guinea pig cells (guinea pig embryo cells) do not contain intracisternal A particles but on stimulation with BUdR produce a particle which has most of the ultrastructural features of a mouse mammary tumor virion. Typical intracytoplasmic A particles develop which are incorporated into buds which in turn separate from the cell. Many of the particles exhibit eccentric nucleoids and possess surface spikes.

These results indicate that if a type C genome exists in guinea pigs, its phenotype has yet to be demonstrated.

Viral RNAs

After many months of painstaking effort and variable results and numerous failures, the structure of the RNA of avian myeloblastosis virus and of MC29 has been determined. These RNAs have lengths of 0.7, 1.1 and 1.9μ. Under comparable conditions the RNAs of vesicular stomatitus and Newcastle's disease viruses exhibit lengths of 8-10μ which correspond to their molecular weights. Thus previously described molecule lengths of some viral RNAs are incorrect and probably represent contaminating DNA.

Radioautography Studies

Quantitative autoradiographic studies by light and electron microscopy indicate that following Rous sarcoma virus infection, a virus induced amplification of ribosomal cistrons in nucleolar chromatin occurs as a result of integration of the viral genome and derepression of cellular (oncogenetic) DNA. These events occur within 80 minutes of infection.

Structural Changes During Transformation

Cells transformed by Bryan (high titer) Rous sarcoma virus develop numerous vacuoles which apparently contain mostly water. Other Rous sarcoma virus strain do not induce vacuole formation. The membranes bounding these vacuoles have features different from other cellular membranes. A temperature sensitive mutant derived from BHK_{21} cells deficient in cytoplasmic 28S RNA was used to determine whether cells of this type could be infected with either a hamster leukemia virus containing a murine sarcoma virus genome or with SR RSV. Neither virus would infect.

Preparation of sections for electron microscopy included 867 embeddings of which 724 were sectioned.

A total of 28 tissue culture lines are presently being carried in the tissue culture unit. Twelve of these were developed during the past fiscal year.

The photographic unit produced 5,634 prints. In addition to the work for the staff in the Office of the Coordinator for Ultrastructural Studies, work was done for Dr. Moloney, Dr. Ablashi, Dr. Perk, Dr. Boone, Dr. Orme, Dr. Chirigos, Dr. Albert, Dr. Gruber, and Dr. Pearson. These included 150 light micrographs and prints, 25 large lantern slides, 500 2x2 slides, 600 4x5 copy negatives and 20 16x20 exhibit prints.

Lecturers in the Virus Studies Section in the seminar series: Dr. C. Weichan, Dr. K. Perk, Dr. E. de Harven, G. Schidlovsky, Dr. J. Dahlberg and Dr. G. Weber.

Dr. Kalman Perk has been associated with the Office of the Coordinator Ultrastructural Studies as a visiting scientist.

D. Office of Program Resources and Logistics

The Office of Program Resources and Logistics within the Office of the Associate Director for Viral Oncology is responsible for the review and scientific management of collaborative research contracts providing research resources and logistical support to intramural investigators within Viral Oncology and collaborating laboratories participating in the NCI Virus Cancer Program, and, when adequate resources are available, to the general scientific community. The Office is also responsible for the day-to-day general management and direction of resources distribution. This Office was established in 1972 to centralize the scientific administration and management of research resources and logistical functions and to unify these activities within the Office of the Associate Division Director. In this way individuals responsible for coordinating these support activities are able to provide to the entire Program with an awareness of the overall scope of activities. This has avoided any special consideration to a particular area, unnecessary duplication of effort, and the appearance of undesirable competition within the Program.

Many of the research investigations carried out in the Viral Oncology Area depend on the availability of clinical and laboratory materials of optimal purity, viability, and potency. Comparable studies in an integrated program of international scope, as encompassed in the VCP, make more meaningful and rapid progress when adequate quantities of standardized reagents, cell cultures, and test animals are available. The Office of Program Resources and Logistics provides these supportive activities through contract operations representing four general areas of activities. These include:

1. Activities directed toward production and characterization of purified viruses and viral reagents.

2. Activities concerned with acquisition, collection, storage, inventory and distribution of normal and malignant human specimen material.

3. Activities concerned with animal resources, including production of pathogen-free and germ-free species of animals, breeding of primates, maintenance of animal colonies, and containment-type holding facilities.

4. Activities directed toward the provision of specialized testing services for the examination of experimental materials.

The overall requirement for research resources and the number and extent of requests received frequently exceed the availability of Program resources. This is due, to some extent, to the high cost of producing or preparing certain scarce reagents, to fiscal considerations which limit the ability to prepare every potentially necessary item, and to a valid reluctance to

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prepare highly specialized and expensive materials which can be utilized by only a few laboratories. Because requests do exceed availability, the distribution of resources is influenced by the relative value of specific contract research in relation to overall Program goals. These relative values, or priorities, are determined by the joint Program Segment Chairmen, the individual Segment Working Groups, the OPR&L Advisory Group, and the VO Branch and Associate Branch Chiefs. The distribution of resources is patterned on the evaluations and recommendations of these groups.

During the past year, this Office has coordinated the distribution to NCI intramural investigators and VCP participants of a wide variety of biological, chemical, and materiel resources. The amount of material processed has been enormous. To illustrate the scope of activity, during this year the purified and concentrated products from over 25,000 liters of tissue culture-grown viruses, propagated in over 30 different cell lines, were distributed to over 100 participating laboratories throughout the world. Additionally, approximately 750 grams of a single agent, not propagated in tissue culture, was sent to over 80 different investigators in over 130 shipments. Furthermore, the Program repositories handled or shipped out over 50,000 items. In addition to these activities, the Office has coordinated the distribution to U.S.S.R. scientists of a variety of resources in keeping with the Memorandum of Understanding signed in Moscow in 1972 covering the mutual exchange of cancer research materials. The materiel supplied included purified and concentrated viruses of murine, feline, avian, and primate origin; specialized viral proteins and antigens; normal and infected tissue culture cell lines; various tumor-derived cells; a wide range of standard reagents and chemicals for tissue culture utilization, including dried powder media, radioisotope-labelled chemicals and nucleotides, and enzymes and hormones; a variety of antisera prepared in a wide range of host animals; and various other substances.

An analysis covering a twelve-month time period of the distribution of Program resources by type of recipient indicated the extent to which cancer investigators have been supplied with research resources. NIH intramural investigators received 18%, collaborating VCP laboratories 71%, and non-Program participants 11%, respectively, of the total resources effort. For both the collaborating laboratories and the non-participating investigators, approximately one-half of the total resources were distributed to scientists affiliated with academic institutions.

The Viral Oncology Program at the NCI-Frederick Cancer Research Center includes aspects involving production and purification of viruses and viral components, and preparation of selected antisera. Monitoring and evaluation of these special efforts is conducted by OPR&L personnel, and the Office is responsible for general management of the distribution of such materials to FCRC intramural investigators and to VCP collaborating laboratories. During the period February 1973 to February 1974, over 8,000 liters of virus materials were produced and purified, and over 7,600 ml. of virus concentrates were released to investigators as directed by this Office. All Program requests for viruses available from FCRC have been met and approximately

3,000 ml. of virus concentrates remain available at the FCRC virus repository to meet any additional needs.

The OPR&L is also concerned with the preparation of an annual catalog listing and describing the research resources available to collaborating laboratories within the Program. Usually the information provided for each item includes designation, origin, and processing procedure. This year, as in last year, a revised new edition of the catalog was prepared and distributed to Program participants. Loose-leaf binding, adapted last year, was retained to permit efficient future updating of information, and the format and contents were revised to expedite the recovery of items or services and reflect current research interests. Additionally, an element of the OPR&L is concerned with the development of a computerized central inventory for the sera, tumor tissue, cell out-growths, and other human specimen materials continuously being acquired by the Program. The central inventory facilitates matching investigators requests for human materials with specimens available, regardless of the geographic location of the repository or laboratory at which it is stored.

Because of the dynamic nature of the Viral Oncology Program and the increasing and expanding responsibilities of the Office of Program Resources & Logistics in support of that Program, administration within the Office was streamlined and expanded during this fiscal year. An element from the former Office of Program Analysis & Communications, responsible for providing data and information management in support of the Office of Program Resources & Logistics, was transferred to OPR&L and will assist OPR&L in the general areas of systems analysis, analytical statistics, computerized data and information storage, and retrieval and distribution of resource material. Because of its increasing responsibilities, it is anticipated that sections concerned with the management of specific areas of resources will ultimately be established. These newly designated sections would involve viral resources activities, and the human and animal resource activities. Additionally, a third section comprised of the elements responsible for data and information management would be appropriate. Currently the specific activities of the Program Data and Analysis Unit within OPR&L continue to involve the following:

<u>Automated Inventories of VCP Resources</u>

1. VCP Serum Collection: Maintains a computerized inventory of 150,000 sera specimens held in seven local and regional repositories; PDAU manages the continuous input, updating, and distribution data for these specimens stored for utilization by program scientists.

2. VCP Tissue Collection: Planned, installed, and maintains a computerized inventory of human tissues from 1500 donors.

3. Environmental Control Specimens: The NCI Office of Biohazard and Environmental Control collects sera from research and related activity employees of Viral Oncology supported contracts.

Specimens are collected annually from individuals, and also at the time of employment, termination, injury, and during convalescence. PDAU maintains storage, donor, and distribution data for this collection of specimens.

4. Local Systems: The PDAU provides continued support for previously developed and installed inventories in three laboratories participating as resource repositories for the VCP: Naval Biomedical Research Laboratories, Huntingdon Research Center, and Simpson Memorial Institute-University of Michigan.

5. Central PDAU Inventory: The PDAU has promoted compatible automated systems and codes for specimens in all institutions participating in the VCP. This includes the capture and automation of complete clinical, demographic, and laboratory information on specimen donors. The goal has been a central specimen inventory at NIH making available for all of VCP the many large specimen repositories throughout the United States. PDAU receives a tape copy semiannually of the independent satellite repository inventories which is merged with the PDAU system to prepare a central inventory file.

Data Services

The PDAU prepares monthly an inventory listing for each of the satellite repositories maintained in the VCP Serum Bank and Tissue Bank system. Ad hoc reports are prepared when required by OPR&L.

Statistical and Systems Services

All laboratories in the VCP are offered a comprehensive statistical service (consultation on problems of research design, data procurement, data management, and statistical analysis of survey or experimental findings). A completely automated data processing routine directed by experienced technicians maintains direct access to the main NIH computer. Consultation and systems planning service is also rendered to VCP contractors in developing, planning and installing local automation systems for managing and processing experimental data.

Progress Reports

During the major part of the fiscal year, a system of managing semiannual progress reports was maintained. PDAU alerted contractors when progress reports were due, and received and disbursed copies to appropriate personnel (project officers, segment chairmen, contract officers, and Viral Oncology management). PDAU also compiled and distributed to program scientists condensed summaries of the actual progress reports. This function has now been assumed by the VO Information Unit.

Within the Office of Program Resources & Logistics, principal support is provided by three staff scientists, a computer programmer, and a secretary who assist in responsibilities for the management of the collaborative contract operation and the coordination of resources distribution.

E. Scientific Information Management

The Information Unit continued to focus attention on scientific information retrieval in the area of viral oncology, and its dissemination to program scientists. Sources of information are scientific journals, books, and other notifications and summaries of current research in the field. Major contributions of the Information Unit in FY 1974 were as follows:

(1) Bibliographic Service: A semi-automated system is maintained for storage, rapid search, identification, and reference print-outs covering almost any desired topic in the published literature on viral oncology.

(2) Cooperative Literature Searching Service: Cooperative arrangements from this office have been established with the Information offices of six other federal agencies whereby their literature collections and other user services are made available to Viral Oncology program staff members on request.

(3) Viral Oncology Contractor Directory: This quarterly publication contains the names, addresses, and telephone numbers of contractors, principal investigators, project officers, and contract specialists within the Viral Oncology Program. Its purpose is to facilitate and expedite communications between staff members and contractors.

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

Other responsibilities of the Information Unit are: Administration of the Viral Oncology library facility containing subscriptions to seventy scientific journals and also a collection of 350 reference books; continuous compilation of the VCP bibliography containing citations to all papers published by Viral Oncology staff members and research contractors; and the preparation of special bibliographies both manually and by computer on request.

III. BRANCH REPORTS

A. VIRAL BIOLOGY BRANCH
July 1, 1973 - June 30, 1974

The Viral Biology Branch conducts research on virus and host factors related to carcinogenesis and the development and evaluation of measures for cancer control in experimental systems. Investigations are conducted to detect and identify the nature of virus activity in tumor tissue, study the effect on tumorigenesis of interaction between viruses co-infecting the host, determine the biological behavior of neoplastic cells, characterize virus and tumor specific antigens, and examine biochemical events related to viral infection and cell transformation. Ultrastructural studies permit detection and morphological characterization of viruses associated with disease processes and their effect upon the internal organization of the cell. Selected experimental animals and cell cultures are used to evaluate viral relationships to the carcinogenic process and the effectiveness of combined chemotherapeutic and immunotherapeutic measures in the control of virus activation and tumor growth.

The Section of Cell Biology investigates malignant cell behavior and the cell surface neoantigens associated with malignant transformation. Methods are developed to study the cell surface changes which accompany cell transformation. Increase in host immune responsiveness to tumor cell antigens through induced modifications of cell surface is sought as an approach to improved immunotherapeutic control of neoplasia. The Section of Microbiology has emphasized investigation of herpesvirus-induced malignancies in non-human primates in conjunction with current interest in association between members of this group of viruses and human cancers. Host immune responses to herpesvirus-induced antigens are correlated with the progression of disease. Similar studies related to immunological control of RNA virus-induced neoplasia are conducted. The Section of Human Tumor Studies combines viral, immunochemical and biochemical approaches to detect, isolate and characterize viruses associated with human and some animal tumors. Protein products of virus gene expression, *in vitro* viral protein synthesis, and the relationship of these proteins to cellular transformation are under investigation. The Section of Virus and Disease Modification is concerned with the development of effective measures for the control of virally-induced neoplasms. Combined chemotherapeutic and immunotherapeutic measures are evaluated in terms of prolonged life and suppression or elimination of tumor growth in animals. The Section of Electron Microscopy had devoted efforts to the detection of viruses in biological materials, viral morphology, and the virally induced alterations in cellular ultrastructure. The Section will be integrated into the Office of the Coordinator for Ultrastructural Studies. The Office of the Chief coordinates the research conducted in the Sections with due recognition to the scientific freedom of the individual investigators. The Office is responsible for establishing collaborative efforts between investigators in the Branch and other laboratories at NIH or elsewhere. The Chief serves as Chairman of the Developmental Research Segment of the

Virus Cancer Program and is assisted in this capacity by the Vice Chairman and Executive Secretary for the Segment. As such, the Chief is responsible for the extramural research contracts contributing to the Segment's mission.

Studies on the biological behavior of cultured cells indicate that decrease in cell-substrate adhesivity is related to the malignant properties of the cells. Non-neoplastic cells do not grow and divide on substrates to which the cells cannot adhere, but will undergo mitosis under these cultural conditions in the presence of low concentrations of N^6, $0^{2'}$-dibutyryl cyclic AMP. Since CAMP aids in assembly of cellular microtubules and microfilaments, it is postulated that these structures promote adherence of cells to substrate. Cyclic AMP also appeared to restore anchorage dependence for growth of a spontaneously transformed malignant cell line.

A type C RNA virus has been rescued from an apparently virus-free human mammary tumor cell line, HBT-3, by treatment with testosterone followed by iododeoxyuridine. The virus is 85 to 100 nm in diameter with a 40 to 58 nm core and had a density of 1.15 in a sucrose gradient. The particulates contain 28S and 70S RNA. The antigenic properties of the agent are under investigation. Preliminary observations indicate the presence of gs-3 interspecies antigen. Further, there is no co-electrophoretic identity between the protein components of the viruses from HBT-3 cells and with the major structural protein, p30, of simian feline or murine viruses.

An agent isolated from a human cell line produces a cytopathogenic effect on transformed or type C virus-shedding simian, bovine and murine cells, but is without effect on normal cells or primary fibroblast cultures on which tests have been made. A plaque assay has been developed using Vero and monkey embryo spontaneously transformed (MEST) cells. Purified preparations of the agent titering 10^9 PFU/ml are obtained using a combination of polyethylene glycol precipitation and Renografin equilibrium sedimentation. The agent has a density of 1.16 in Renografin and contains DNA. The extracted DNA has a molecular weight of 10^8 daltons as estimated by co-sedimentation with HSV·DNA and a density of 1.70 in cesium chloride. While these biochemical characteristics are similar to herpesvirus, thin sections of infected cells or negative stains have failed to reveal any particulate forms resembling herpesvirus. Normal human sera neutralize the agent and the reaction appears to be complement dependent.

Plaque purified herpes simplex virus type 1 (HSV-1) repeatedly passaged through JLSV9 cells retained its characteristic plaque morphology. Simultaneously passaged HSV-1 in Rauscher murine leukemia virus-infected JLSV-9 cells altered the phenotypic expression of the virus. Plaque morphology changed from the round cell form to a giant cell syncytial form. Syncytia were also produced on chicken embryo fibroblasts (CEF) in contrast to the parental virus which produced no cytopathogenic effect on these cells. The plaque morphology was conserved on subsequent passage in Vero and CEF. The virions produced in the doubly-infected

cells were examined by electron microscopy. Some particles possessed two distinct membranes surrounding the nucleocapsid and the outer membranes contained surface projections 85 angstroms in length. To determine whether phenotypic co-encapsidation is responsible, neutralization assays, immunofluorescence microscopy and ferritin tagging experiments are in progress.

Purified, biologically active herpesvirus saimiri (HVS) is prepared by polyethylene glycol precipitation followed by equilibrium sedimentation or flotation in Renografin. Up to 70 percent of the biological activity is recovered in a single, virtually pure 1.16 density band. Following infection with HVS, owl monkeys develop antibodies to virus-induced cell membrane antigens (MA) and late virus antigens (LA) regardless of the presence or absence of the development of neoplasia. However, antibodies to early virus-induced antigens (EA) are only detected in those animals that developed leukemia and/or lymphoma following infection. The antibodies usually appear prior to the onset of disease, indicating the anti-EA response is diagnostic for lymphoproliferation. There is no evidence that malarial infection activates HVS in the squirrel monkey, the natural carrier of this virus.

The P3J-HRIK strain of Epstein Barr Virus (EBV) is infectious for marmosets. Inoculated animals develop antibodies reactive with virus capsid antigens and splenic enlargement which eventually subsides. A transient response to EA accompanied by cervical node enlargement occurred in one of five animals. A cell line originated from bone marrow of an infected animal is positive for EB virus-induced nuclear antigen (EBNA). These cells lack T-cell markers and can be superinfected by EBV.

A sarcoma virus-specific polypeptide of 57,000 daltons has been identified by co-electrophoresis of purified feline leukemia virus (FeLV) and murine leukemia virus (MLV) pseudotypes of the Moloney murine sarcoma virus (MSV) with their respective helper viruses. These studies also confirm biological and immunological data on the 4:1 ratio of MSV focus-forming units to FeLV replicating units. Preliminary experiments have shown significant differences between the leukemia viruses and the sarcoma pseudotypes in the region of the membrane glycoproteins (70,000 daltons).

The co-electrophoretic method that has been developed is in itself a powerful tool in that the degree of relatedness between different RNA tumor viruses is reflected in the number of the viral polypeptides that have electrophoretic identity.

Translation products that co-electrophorese with avian myeloblastosis virus (AMV) capsid proteins have been synthesized in vitro using AMV messenger RNA in a protein synthesizing system derived from fetal bovine kidneys. Autoradiographic immunodiffusion analysis of the in vitro translation product with carrier AMV virus polypeptides, using an antiserum prepared agianst Tween-ether disrupted AMV, gave one major radioactive band tentatively identified as p30.

The RNA directed DNA polymerase of a reptilian type C virus resembles the polymerase of AMV in its elution pattern from phosphocellulose but has a molecular weight of 109,000 daltons in contrast to that of the avian enzyme (180,000 daltons).

The mechanism of action of different inhibitors of AMV·RNA directed DNA polymerase was studied. Whereas streptonigrin binds to the RNA template, an alkaloid extract of Narcussus tazetta L combines physically with the template without affecting the binding of template and enzyme. Pyran copolymer interacts with the polymerase at a region other than the template site and inhibition is overcome only by excess enzyme. Tilorone is an exceptionally potent inhibitor of purified viral DNA polymerases. The degree of inhibition was template specific for the templates tested. Chemically modified polyA is unable to base pair with corresponding bases and, therefore, cannot be primed to act as a synthetic substrate for polymerase. Since the modified polyA cannot effectively base pair with nucleic acids normally involved in cellular processes, it has potential usefulness as an inhibitor of oncogenic viral polymerases.

Immunity to a transplantable Moloney virus-induced lymphoma can be transferred to mice by sera possessing high antibody titers against mouse sarcoma tumor cell membrane antigens. Such sera are obtained only from donor mice bearing regressing allogeneic sarcomas. Protection is particularly effective when the serum treatment is used in combination with drug therapy. Thus, titer of serum against tumor cell membrane antigens provides a measure of effectiveness for the control of tumor growth. Such sera also enhance the cytotoxic activity of normal lymphoid cells for target cells carrying these membrane antigens. The enhancing factor is contained in the IgG serum fraction.

The immunogenicity of tumor cell membrane antigens is increased considerably if the tumor cells are infected by influenza or vesicular stomatitis virus. Such virus-augmented tumor antigens also are more effective than the noninfected tumor antigens in elicidation of a delayed hypersensitivity reaction in tumor-immune animals. A factor present in necrotic tumors specifically depresses cellular immunity in mice bearing the tumor.

Evaluation of nonspecific stimulation of immunity for the control of the growth of virus-induced neoplasms in mice was continued. A number of chemical and biological materials which were ineffective alone did produce substantially increased periods of remission following chemotherapeutic reduction of the tumor burden. The antibiotic, streptonigrin, is quite effective at low levels in inhibiting both virus replication and MSV-induced tumor growth. The most active inhibitors in tests in vitro are alkaloids of the narcissus bulb, adriamycin, tilorone and its derivatives, and methylene blue.

Other Activities of the Branch:

During this reporting period, investigators in the Branch were senior

authors on 23 published manuscripts, on 9 papers in press, and were collaborating authors on 33 published reports and 19 reports in press.

Members of the Branch presented lectures by invitation to research groups in this country and abroad and discussed research observations at different scientific meetings. The Branch also hosted visiting scientists desiring training in experimental procedures. Senior members of the Staff served as Project Officers on research contracts, and participated in site visits to different laboratories in the research contract program of the Institute.

The Electron Microscopy Section assisted intramural investigators in the Viral Oncology Area by providing ultrastructural studies on specimens submitted. The Photographic Unit assisted investigators requiring photographic records of specimens and by processing electron photomicrographs for inclusion in manuscripts.

B. VIRAL CARCINOGENESIS BRANCH

July 1, 1973 - June 30, 1974

Introduction

The mission of the Viral Carcinogenesis Branch is concerned with the natural histories of tumor viruses in animals and man and their established and suspected roles as causes of cancer; the ultimate objectives are the isolation and characterization of postulated human cancer-inducing viruses and the prevention of such cancers by antiviral vaccines or inhibitors.

As can be seen in the bibliographies of this and previous reports of the Viral Carcinogenesis Branch (VCB), the research program is part of an extensive collaborative research effort involving large numbers of scientists working on research projects and programs located in other Branches and Laboratories of VCP-NCI (Todaro, Scolnick, Benveniste), in the National Institute of Allergy and Infectious Diseases (Rowe, Hartley), and a number of VCP-STS research contracts (see list). Much of this collaboration had been established by Dr. Huebner prior to his appointment as Chief of the VCB in 1968; VCB had been and continues to develop and furnish RNA and DNA tumor virus diagnostic tissue culture systems and sero-epidemiological services to the other programs. The work of these collaborating laboratories are so interlocking, supplementary and complementary to each other that separate descriptions of their joint accomplishments would be quite incoherent. Thus where indicated they will be discussed together.

Background

Since 1966 the VCB and collaborative programs have been primarily concerned with the RNA tumor (oncorna) viruses and their subunits found in normal as well as tumorous tissues of a number of classes of animals and cells; the chief question has continuously been, "What are they doing there?" Since the known well-established type C and B RNA tumor viruses are clearly tumorigenic, it was logical to assume that their partially expressed naturally occurring counterparts in cells also carried oncogenic genes.

In order to establish this it was necessary to develop sensitive *in vitro*, as well as *in vivo*, techniques for isolating, assaying and characterizing the indigenous RNA tumor viruses and the viral specific immune responses they produced. Working in close collaboration with a number of other programs in NCI and NIAID, VCB scientists succeeded in developing a number of critical techniques for doing precisely this. The application of these new procedures (complement-fixation tests for viral antigens and antibodies, COFAL, COMUL, XC test, reverse transcriptase assay for virus in tissue cultures, production of virus specific antisera to gs (p30) and viral envelope antigens using CF, IFA, radioimmune assay, gel diffusion, neutralization tests, etc.), to RNA tumor virus research have been described in previous annual reports and numerous publications.

The Viral Oncogene Hypothesis

Studies beginning in 1962 of RNA tumor virus expressions and behavior in a number of natural mammalian species led to the Viral Oncogene Hypothesis in 1969 which postulated that oncorna viruses have their origins in cellular genes of most if not all vertebrates. Proof of this hypothesis would provide a useful unitary theory concerning a basic cellular cause of cancer in vertebrates. During the 4 years since 1969 the validity of this hypothesis has gathered considerable support and although not proven in its entirety it is now apparent that in natural species certain endogenous type C RNA tumor viruses indeed are transmitted and regulated primarily through genetic mechanisms although other RNA tumor virus representatives are also in some circumstances transmitted epigenetically and even horizontally as will also be described below. During FY 1974 it was finally shown (vide supra) that a number of apparently universally prevalent endogenous viral genomes (mice, rats, cats, hamsters, chickens, and pheasants) have oncogenic potential as demonstrated by (1) injection and long term observation of susceptible experimental animals, and (2) by Mendelian cross-breeding experiments.

In FY 1974 immunization with RNA virus vaccines led to virus specific neutralizing antibodies in mice and rats to their own viruses; it is anticipated that this new development should lead to some degree of control of virus expression and hopefully also of cancer in these and other species, and finally hopefully in man.

The major research effects of VCB and collaborating programs have been and still are directed towards achieving evidence concerning the following major questions or tasks:

Task I: Are endogenous RNA tumor virus genomes transmitted as part of the natural inheritance of host cell DNA? What experimental and natural evidence supports this concept and how universally is it true?

Task II: What is the evidence in those vertebrate species available for testing, where the technology is adequate, that spontaneous or induced cancers and transformed cells are caused by oncogenes of RNA tumor viruses?

Task III: Do endogenous and exogenous factors, including defective genes, hormones, chemicals, radiation, and DNA tumor viruses, known to induce various cancers also activate or derepress endogenous RNA tumor virus genomes as endogenous basic co-carcinogens?

Task IV: Can virus specific immune responses occurring naturally or produced by virus specific vaccines prevent or otherwise modify cancer occurring spontaneously and/or induced in well-studied experimental animal systems and when human RNA tumor virus is available in man?

Major Accomplishments Relating to Endogenous (Genetic) Origins of RNA Tumor Viruses.

Delineation of X-tropic type C RNA tumor viruses (Task I): In 1970 Dr. Jay Levy of VCB succeeded in isolating the first representative of a new class of type C virus from New Zealand Black (NZB) mice; this virus was unique in that it would not infect any mouse cells, but would replicate in rat cells. In early 1973, Todaro, Arnstein and Huebner reported the isolation of the AT124-type viruses from human cells (RD line) derived by culture of these cells in the brains of antithymocyte-treated NIH Swiss mice and C57L/J mice. These viruses could not be grown in mouse cells but did grow regularly in human and rat cells. At about the same time Aaronson isolated a rat cell tropic virus from BALB/c cells which were immunologically and by genetic segregation distinct from the N- and B-tropic viruses.

Subsequently, in late 1973, Levy (now working at the University of California in Berkeley) found that similar viruses could be isolated in his rat cell system from NIH Swiss and other "virus-free" mouse strains; since these viruses do not infect and replicate in mouse cells but do infect and replicate in rat and other non-mouse cells, for these viruses Levy coined the term "xenotropic" viruses (Science 182:1151, 1973). These viruses were entirely similar not only to those described above but to the BALB/2 virus described in 1973 by Aaronson of VCB. Subsequently, but before Levy's paper appeared, Drs. Todaro and Benveniste of the Viral Tumor Detection Branch described isolations of similar viruses from numerous strains of mice using rabbit cells (SIRC) and a variety of other cells. These were independently reported as X-tropic viruses; they also could not infect mouse cells. Shortly after this, Aaronson and Stephenson of VCB reported that sera from 14 of 15 mouse strains (sera taken at 2 months of age were tested) had high level neutralizing antibodies to the X-tropic viruses. In addition to adding another technique for detecting endogenous virus expression, Aaronson's group found that all X-tropic mouse viruses were of the same sero-phenotype, thus suggesting that these viruses are rigorously controlled by murine cell regulating genes, making it very likely that they are wholly genetic both in their origins and transmission.

It should be emphasized that X-tropic type RNA tumor viruses are not peculiar to mice but were demonstrated in 1972 in cats (RD114 virus reported by McAllister et al.), hamsters (HaLV), rats (RaLV) and chickens (ALV strain E). Presumptive evidence for similar X-tropic viruses have been reported by VO and DCCP scientists for pigs, cows and baboons. It is clear that the X-tropic viruses represent a major new class of oncornaviruses, which deserve intensive study.

Genetic (Mendelian) studies which reveal independent segregations of several endogenous RNA tumor virus genomes in inbred mice and the existence of virus regulating genes have provided impressive additional evidence for genetic origin and transmission of both virogenes and oncogenes (Tasks I and II):

Segregation of two endogenous viruses:

Genetic studies have revealed multiple genetic loci for RNA tumor virus induction in mouse cells of different strains. These biologically distinguishable viruses have been activated at distinct loci thus establishing that different structural genes for 2 different viruses segregate independently in backcross and F_2 progeny (Aaronson, Stephenson and associates).

Viral genomes in DNA of normal cells:

These same investigators demonstrated with Gelb and Martin that virus specific DNA is present in the high molecular weight DNA of mouse cells. Cooperative studies with Hatanaka and Gilden (Flow Laboratories) have revealed similar virus specific DNA in rat, hamster and cat cells, using rat (RaLV), hamster (HaLV), and cat (FeLV and RD114) virus specific probes.

Use of dominant anti-viral genes to switch off virus and cancer:

In collaboration with Meier and Taylor (The Jackson Laboratory) and with Gardner, Officer and Henderson (USC), overt RNA tumor virus infections occurring in 100% of female mice in a large wild mouse deme, Lake Casitas (LC), have been virtually eliminated in F_1 progeny of a cross with C57BL/10/Sn males. Studies of virus expression in F_2's and backcrosses confirmed earlier findings by Meier and Taylor (with AKR mouse crosses) that the C57BL/10/Sn mouse carries homozygous dominant genes for switching off the murine RNA tumor virus expressions (this includes not only the major viral protein, gs antigen, but the infectious virus as well).

Since the wild type RNA viruses in the LC wild mice have been shown to be responsible for high frequencies of motor neuron paralysis and lymphoma in these mice, the elimination of virus expression by the dominant genes of the C57BL/10/Sn in most of the progeny predicts that they will continue to be relatively free of spontaneous lymphomas and expressions of paralysis (both diseases have been prevented by virus-specific neutralizing antibody in already completed transmission experiments) (Henderson, Gardner, Officer and Huebner).

Multiple gene loci in regulation of endogenous virus:

Rowe, Hartley and their associates followed up their 1973 findings confirming that endogenous mouse tropic RNA tumor viruses could be predictably induced *in vitro* in virus-free mouse cells by mutagenic agents (IdU, BrdU). The viruses were found to be regulated by at least two independent loci. More recent studies revealed three independently segregating virus-inducing loci-determined virus expressions on the high virus mouse strain C3H/Fg. Another high virus mouse strain, C58/J, revealed 3 or possibly 4 virus-inducing loci; the evidence suggests that these virus-inducing loci are not allelic sites in the cell genome, but more likely represent random insertions of MuLV genetic information into the genome of the mouse.

One or several endogenous viruses?

Virus induction and genetic studies have revealed a very complex situation in various strains of inbred mice. Some strains which are relatively resistant to virus infection and expression reveal only one endogenous virus--the X-tropic virus (which to date has been found to be universally present in all laboratory strains): the NIH Swiss and C57L/J fall into this category. Other strains like AKR and C57BL/6 contain two inducible viruses, one N or B-tropic and the other X-tropic. BALB/c mouse cells, on the other hand, have at least 3 endogenous viruses, N-tropic, B-tropic and X-tropic. Thus in the inbred mouse geneticists now have the opportunity to study two different bags of genes, including up to 3 phenotypically different oncorna virus genotypes and at least 3 or 4 different virus inducing loci. Since cats have 3 host cell tropic viruses (FeLV types 1, 2 and 3), and RD114 (an X-tropic virus), the problems aren't any simpler in this species.

However, in hamsters, rats, pigs and baboons, only one class of type C RNA tumor virus has so far been demonstrated (Klement, Huebner, Gilden, Sarma, Todaro). In each instance the wild type virus isolates do not infect their host cells and therefore appear to be X-tropic.

Significance of X-tropic oncornaviruses:

The discovery of highly prevalent type C RNA tumor viruses (X-tropic) which cannot infect cells of natural hosts (mice, cats, rats, hamsters, chickens and others) provided strong new evidence supporting the concept of naturally inherited oncogenic viral information. These new virus genomes apparently can be spontaneously derepressed *in vivo* and *in vitro* where they are then continuously generated in certain cells. The viruses produced are able to penetrate other host cells and carry "defective" information (as when rat virus is linked with the defective sarcoma genome) and presumably could integrate and produce oncogenic effects.

Quite apart from their great potential significance as cancer-inducing viruses, the endogenous oncornaviruses of both mouse-tropic and X-tropic types can be regarded a time bomb in normal cells that are constantly susceptible to being triggered by a variety of agents and factors. These include, besides mutagens, radiation, carcinogens, DNA tumor viruses, graft versus host reactions (GVHR) and simple aging in *in vivo* and *in vitro* systems (long-term cultures) (Freeman). The presence of known potential oncogenic viruses as endogenous genomes in all cells of many mammalian species therefore presents a potential problem of considerable urgency and dimensions.

New evidence for induction of RNA tumor viruses in vitro (Task I):

In FY 1973, Rowe, Aaronson and Todaro, and a number of VCP-supported scientists (Klement, Freeman, Price, Gilden) reported induction of endogenous mouse cell tropic RNA tumor viruses not only in virus resistant inbred mouse, rat and chicken cells, but also in hamster, pig and baboon cells. Using RT probes these induced viruses were shown to have homologous DNA sequences with the DNA of the cells of homologous species (Gilden, Scolnick, Todaro).

Evidence for induction of RNA tumor viruses in conjunction with spontaneous and induced tumors of hamsters (Task I):

Huebner, Lane and Hill (manuscript in preparation) found that tumors induced in hamsters by bovine papilloma, polyoma viruses, and DMBA each contained hamster lymphoma virus (HaLV). Although present in amounts too low to be detectable by EM, reverse transcriptase or gs antigen tests, it was possible to detect infectious virus in cell-free (Moloney procedure) preparations by injecting newborn hamsters. Visceral lymphomas were invariably induced within 100 to 150 days; further passages of the lymphomas when transmitted by cell-free viral preparations yielded tumors much earlier and, in some, detectable gs antigen and virus particles. These particles apparently do not infect hamster cell cultures in vitro and therefore are not hamster tropic; however one strain of HaLV derived by endpoint dilution from a MSV(HaLV) pseudotype (Huebner, Gilden and Kelloff) produced lymphomas by 21 months in 60% of hamsters infected as newborns. Controls were essentially negative.

Evidence that wild type endogenous RNA tumor viruses are oncogenic (Task II):

It is often stated in the literature (with no good evidence one way or another) that wild type isolates of type C RNA tumor viruses are not oncogenic; this claim is unsubstantiated.

(1) Direct inoculation tests of newborns: Peters, Kelloff and Huebner produced lymphomas in 39% of BALB/c mice; controls had 10% (published FY 1973). Cell-free extracts of spontaneous BALB/c tumors were injected into newborn BALB/c mice. Only those tissues which contained mouse B-cell tropic virus produced tumors; N-tropic virus-containing preparations did not. Since several hundreds of mice were used, the differences were quite significant.

(2) As described above, wild type hamster viruses were also shown to be oncogenic (Huebner, Lane and Gilden, unpublished).

(3) Meier and Taylor demonstrated that a wild type N-tropic virus isolated from SWR/J mice produced lymphomas and other tumors in newborn SWR/J mice (unpublished data.)

(4) Greenberger and Aaronson, in tests of the biological activity of chemically activated type C virus, produced lymphatic leukemias in newborn mice of a low leukemia incidence strain. Gardner, Theilen, Rickard and Sarma produced leukemias and sarcomas by injecting newborns with wild type cat viruses.

Finally, several investigators have reported that viruses activated in lymphomas induced by graft versus host reactions readily induced lymphomas when injected into newborns of the parental line.

Evidence that natural high expression of endogenous viruses are associated with high levels of early spontaneous cancer and the evidence concerning the influence of host genes (H2) and immune responses. (Tasks II, III, IV):

It is nearly axiomatic that mouse strains having extremely high rates of leukemia early in life have adequately high titers of infectious RNA tumor viruses in their tissues; they also have a relatively short life span. Examples of the extreme situation are AKR, C58, DBA/2 strains, and the hr/hr hairless congenic variant of the HRS/J strain. The latter is most interesting and has been studied by Meier, Taylor and Heiniger, and Huebner. The hr/hr homozygote develops 50-60% lymphomas within 12 months, but the congenic hr/+ heterozygote develops only 2-7%; both categories of mice which appear in the same litter seem to have approximately the same amount of wild type infectious virus in their tissues. Further studies of the possible effect of immune responses on the discordant tumor incidences revealed the following:

(1) Treatment of hr/+ and +/+ tumor-resistant mice for several months with azothioprine, a powerful immunosuppressant, resulted in 50-60% incidences of lymphoma in both strains; treatment of the hr/hr mice did not increase the tumor incidence appreciably. It was concluded that the hr/hr mice lacked immune responses present in hr/+.

(2) Quantitative studies of immune responses of the hr/+ and hr/hr mice revealed significantly lower humoral responses to tetanus toxoid in the hr/hr mice; suggesting that the defectiveness of immune surveillance mechanisms in hr/hr may be responsible for the high tumor incidence (Heiniger and Meier).

Correlations of magnitude of virus expressions with incidences of lymphoma:

In our FY 1973 report we described a collaborative study with Meier and Taylor of the Jackson Laboratories wherein a predictable correlation was found between RNA virus gs antigen expressions detected shortly after birth and the incidence of tumors which occurred much later in life. It was possible to predict that 80-90% of the gs-positive mice would develop cancer.

A recent report by Lilly (Einstein College of Medicine) provided highly significant data on F_2's and backcross progeny from BALB/c-AKR matings which showed a high degree of correlation between infectious RNA virus

titers and subsequent leukemias and other cancers during the lifetime of the mice. The virus titers were determined in quantitative XC tests of tail extracts (Klement, Rowe and Hartley technique):

LEUKEMIA INCIDENCE AMONG (BALB/c x AKR) x AKR BACKCROSS MICE, ACCORDING TO MuLV TITER AT 6 WEEKS OF AGE

Virus Titer	$H\text{-}2^k/H\text{-}2^k$	$H\text{-}2^k/H\text{-}2^d$	Total
3.3 - 3.0	11/15 (73%)	7/9 (78%)	18/24 (75%)
2.9 - 2.0	23/27 (60%)	17/38 (45%)	39/75 (52%)
1.9 - 1.0	9/22 (41%)	1/14 (7%)	10/36 (28%)
0.9 - 0.3	1/14 (7%)	3/14 (21%)	4/28 (14%)
None	6/64 (9%)	1/86 (1%)	7/150 (5%)

These findings plus the RNA virus-suppressing power of the homozygous gs- gs- C57BL/10/Sn mice when used in crosses with the nearly 100% virus-positive LC wild mice (described above) which normally develop high incidences of virus-induced lymphoma and paralysis provide a strong case for the presence of specific oncogenes in endogenous wild type RNA tumor viruses.

As is the case for non-oncogenic viruses of mammals, the characteristic generic properties of the RNA tumor viruses, which in all cases that have been properly tested are shown to be present, should not be assumed to be absent in other situations merely because a specific new isolate or strain hasn't yet been tested properly.

Although most natural viruses of natural hosts rarely produce serious disease, most often producing inapparent or subclinical infections, no one doubts that poliovirus causes poliomyelitis, or that adenoviruses, paramyxoviruses or rhinoviruses cause a variety of respiratory illnesses. The evidence for oncogenic capacity on the part of bonafide type C and B RNA tumor viruses is just as unequivocal. While it will be necessary to establish without question the oncogenic capability of new wild type isolates, particularly the new xenotropic viruses, the logical assumption must be that they do possess the properties of the generic groups to which they belong: greater responsibility for providing proof resides with those who would assume that oncornaviruses are not oncogenic. Prevalent viruses natural to a species rarely show anything like a 1:1 relationship of virus with disease; it is likely to be closer to 1:1000. Many factors, including dosage, virulence, genetic factors, age, immune responses, etc., must conspire together to produce overt disease. A congregation of these same factors are required in the case of RNA tumor viruses.

RNA tumor viruses in in vitro transformation of cells:

(1) Rat cells treated with appropriate doses of IdU followed by appropriate treatment of 3MC presented evidence of activation of endogenous rat type C virus (RaLV gs antigen) simultaneously with accelerated neoplastic transformation (Freeman, Price, Gilden, Huebner; published).

(2) Ten BALB/c mouse cell lines, each infected with a wild type B-tropic BALB/c virus, became transformed within 10 subcultures and when inoculated produced tumors in syngeneic newborn mice (Freeman, Zimmerman, Huebner).

(3) Rat cells transformed by chemicals or polyoma virus, while free of demonstrable overt RNA virus, reverse transcriptase or gs antigen, were shown by Hatanaka, Gilden and Rhim to contain significant amounts of virus-specific RNA.

These findings tend to confirm studies reported earlier that rat, hamster and mouse cells infected with appropriate exogenous or with switched-on endogenous RNA tumor viruses undergo greatly accelerated transformation in some instances with, and in other cases without, carcinogen treatment.

Current studies designed to determine specificity of the viral influence on cell transformation include the use of both chemical and specific immunological inhibitors of RNA tumor virus replication applied to the cells at various periods prior to the expected transformation.

Attempts to prevent or reduce cancer incidence with RNA tumor virus vaccines (Task IV):

Studies of natural immunological surveillance mechanisms of various types show effective protection against early neoplastic disease in inbred mice and also in rats, cats and humans. Similarly, as described earlier in this report, the use of immune suppressant agents are known to accelerate the onset of neoplasia in both humans and mice. The latter is apparent in humans with organ transplants who are maintained on azothioprine and other immunosuppressants. Reports by several groups describe increased incidence of leukemia in NZB and hr/+ mice when given prolonged treatment with azothioprine starting in early life (Mellors, Meier and Heiniger).

Earlier in this report we described virus-specific neutralizing antibodies which develop spontaneously in most strains of mice versus the so-called X-tropic virus (Aaronson, Hartley, Huebner and Capps). Immune response genes are known to determine high and low incidences of neoplasia and of RNA tumor virus expressions. Since this information is comparatively well-defined in numerous inbred and hybrid mouse strains, VCB scientists (Huebner, Lane, Capps and Kelloff) in collaboration with scientists at The Jackson Laboratory (Meier, Taylor, Heiniger), Flow Laboratories (Gilden and associates), and Microbiological Associates (Peters, Nims) developed a systematic program to see if formalin-killed virus-specific RNA tumor virus

vaccines could be made which would produce neutralizing antibodies capable of preventing of modifying not only virus expression and replication but also the leukemias and other cancers of inbred mice.

Earlier experiments by Rauscher, Sibal and Fink established the feasibility of formalin-killed vaccines in prevention of the murine leukemias produced by Rauscher virus. Our preliminary results show that it is quite possible to produce virus-specific neutralizing antibodies to natural endogenous viruses in the sera of mice and rats of a number of strains. The virus neutralization titers produced varied according to vaccine potency and the immunological competence of the mouse strains used. Certain strains of mice were "tolerant" to vaccines made from their own virus. These mice, however, invariably produced large amounts of complete virus during embryonic and early post-natal life and do not represent the situation found in most natural species wherein infectious virus expression is low or nonexistent during early life.

Using strains of mice having resistance to type C RNA virus expression early in life and potent formalin-killed virus of the type known to be associated with spontaneous tumors, it has been possible to produce high titered neutralizing antibody responses during early life, i.e., by 2 months of age. Antisera from vaccinated mice at dilutions of 1:20 to 1:80 have neutralized as much as 100 focus-forming units of the live virus tested in tissue cultures.

The viral specificities of such antisera are critical in establishing that the antibodies neutralize virus in specific fashion and do not produce non-specific precipitation. Thus immunologically distinct viruses were used for vaccine production. When given to weanling mice (3 to 4 injections at 14-day intervals) of those strains known to give good immune responses, quite respectable amounts of neutralizing antibodies were produced which were specific. The results shown in the following table clearly indicate that high titered virus-specific neutralizing antibodies can be produced to endogenous mouse cell-tropic RNA tumor viruses and that these antibodies can be produced early in life (<70 days) at a time when virus expressions in resistant mice are still low or undetectable.

NEUTRALIZATION OF SPECIFIC VIRUSES

Vaccine	AKR			SLV			WM			RLV		
made from	10	20	40*	10	20	40	10	20	40	10	20	40
AKR (virus)	100**	98	75	6	0	0	0	0	0	0	0	0
SLV	0	0	0	100	99	92		NT		0	0	0
Wild Mouse	0	0	0	0	0	0	100	99	70		NT	
RLV	29	0	0	0	0	0	0	0	0	73	41	0

*Reciprocal of serum dilution.
**Percent foci neutralized.

Thus it appears quite feasible to utilize vaccine-induced antibodies to modify natural virus expressions and in the process delay or probably prevent tumors associated with demonstrable virus later in life.

In order to reduce the time factors in getting needed answers, careful selection of test systems and of appropriate vaccines become very critical. Also, the costs of vaccine production are very high.

Current vaccine projects are being done in collaboration with Meier and Taylor using AKR and Gross vaccines produced by Flow Laboratories and Electro-Nucleonics. The experiments will be performed in various mouse strains which will be treated by chemical carcinogens, and in SWR/J/AKR and BALB/c/AKR hybrids. The female parent will be immunized and the young progeny as well. The incidences of tumors in these hybrids is 100% at 4 months of age when ENU is given at an appropriate dose on the 16th day of pregnancy.

Important Specific Observations by VCB Scientists on a Section Basis.

Molecular Biology Section:

Two genetic loci for induction of RNA type C virus in BALB/c mouse embryo cells have been shown to segregate independently in backcross embryo cell lines. The viruses at these loci are shown to be biologically distinguishable. The first codes for activation of a virus that grows preferentially in NIH Swiss embryo cells. The second locus codes for activation of the virus that grows poorly, if at all, in NIH Swiss or BALB/c cells, but replicates well in a rat cell line. These findings suggest that the loci for virus-induction in BALB/c cells represent structural genetic information for type C viruses.

The immunologic response of different strains of mice to the natural expression of their endogenous type C viruses has been examined. High titered neutralizing antibodies have been detected to endogenous virus of the class that grows in rat, but not in mouse, cells. The findings indicate the widespread occurrence of endogenous viruses of this class and the ability of the mouse to immunologically respond to a type C virus whose mode of transmission is identical to that of cellular genes.

Under conditions by which viruses have been induced from tissue culture cell lines derived from several different strains of mice, NIH Swiss mouse embryo cells were consistently virus-negative. Antigens which immunologically cross-react with two mouse type C viral polypeptides, p30 and p12, were partially purified from NIH Swiss cell extracts. The type-specific antigenicities of these polypeptides were distinct from those of known strains of virus including the two endogenous type C viruses of BALB/c mouse cells, but were indistinguishable from the corresponding polypeptides of a type C virus obtained by passage of human tumor cells in immunosuppressed NIH Swiss mice. These findings suggest that the block to expression of an endogenous virus of NIH Swiss cells is at a level beyond that of production of two of its structural polypeptides.

Genetic studies have revealed the presence of multiple genetic loci for virus induction in mouse cells of different strains. Biologically distinguishable viruses can be activated at distinct loci. These findings, along with findings that virus-specific DNA is present in the high molecular weight DNA of mouse cells, indicate that type C viruses are naturally integrated within the mouse cell genome. Genetic factors affecting inducibility and persistence of different endogenous viruses have been detected and are currently being studied.

Morphologic revertants of sarcoma virus transformed non-producer cells have been isolated. These show normal cell morphology but still contain the sarcoma viral genome. Both cellular and sarcoma viral mutants have been obtained by this approach. These are being characterized to define the viral and cellular functions involved in expression of sarcoma virus trnasformation.

MSV non-producer cells have been of particular interest because they provide a model system with many similarities to the human tumor cell. Non-producer cells have been found to lack any detectable transplantation antigens, in contrast to RNA virus-producing transformed cells or DNA transformed cells, which are highly antigenic. The non-producer cell has, however, recently been shown to contain an MSV-associated cell surface antigen, which is being studied to determine its involvement in the transformation process.

Ecology and Epizoology Section:

This section recently reported the induction and isolation of a virus identical with RD114 from virus-free sublines of an established cat kidney cell culture (Crandell). In more recent studies, evidence was obtained to suggest that the RD114 viral genome is widespread in apparently normal cats. Thus, virus-free cultures of diverse cat cells derived from normal or tumor tissues contained covert RD114-like viruses inducible with 5-iododeoxyuridine (IdU). Infectious virus was isolated from each of 16 cat cell cultures derived from 9 cats, which included 6 cultures of diverse, normal embryonic tissues from 6 cats, one subline, CRFK, of the Crandell cat cell line, and 7 single cell clones we prepared from subline C-C of the same Crandell cat cell line.

Continuous cell lines of cat origin were examined for covert endogenous type C viruses by induction and cell co-cultivation techniques. Nine cell lines examined yielded a virus similar to RD114 virus. In addition, four of the nine cultures also yielded FeLV-like isolates which replicate preferentially in human rhabdomyosarcoma cell line RD. Studies are in progress to characterize these suspected endogenous FeLV isolates. These preliminary studies suggest that cats have two completely different, genetically transmitted endogenous type C viruses, the RD114 virus and FeLV

Virus-neutralizing envelope antibodies against one or more envelope antigenic types were found in the sera of 13 of 59 (22%) cats without neoplasia and in 9 of 38 (23.7%) cats with neoplastic disease, but not in the sera of 36 veterinarians or in 33 laboratory personnel working in two laboratories engaged in feline leukemia research.

Viral Genetics Section

Primate type C viruses (woolly and gibbon) were found to be highly related to each other, immunologically, using radioimmunoassays and ^{125}I-labelled purified viral polypeptides. Viral antigen was detected in spontaneous primate tumors from woolly and gibbon tissue, but not in rhesus tumor tissue. The finding of antigen in the tumor tissue suggested that these primates might be higher mammalian analogues of mice and cats and that type C viral DNA transcript did not show that gibbons and woolly monkeys carry homologous type C viral genomes. Consequently, these "primate" type C viruses may represent an example of oncogenic epigenetic transmission and/or novel natural expression of type C viruses in primates.

Murine mammary tumor viral (MMTV) systems are being intensively studied. Clones of high and low expressor cells from MMTV cell lines suggest that transcriptional regulation of the integrated MMTV genome (as DNA) is a critical determinant in MMTV expression. All mouse strains examined contained multiple copies of MMTV information as DNA and differences between strains were not noted in spite of 1,000-fold differences in viral expression in vivo and absolute differences in mammary tumor incidence in different strains of inbred mice.

MMTV viral polymerase in the milk of mice was shown to differ from type C polymerase in size, immunologically and biochemically. Both MMTV and MP-MV RNA-dependent DNA polymerases prefer Mg^{++} as a divalent cation when synthetic templates are employed and type C viruses prefer Mn^{++}. This distinction in type C and B viral enzymes has provided an important means of quantifying MMTV in tissue culture.

Levels of MMTV-RNA and antigen in murine cell lines can be stimulated 10-50-fold using dexamethasone. For the first time it is possible to detect significant levels of MMTV specific DNA polymerase in murine cell cultures. This method of increasing in vitro MMTV production may provide a source of large quantities of MMTV for biological, immunological and biochemical characterization.

Solid Tumor Virus Section

The association of herpesviruses with some human and animal tumors has been amply documented in the literature. Essentially all of the studies carried out to date have attempted to directly implicate herpesviruses as oncogenic agents based on "guilt by association". Although studies within the VCB have taken note of this association between herpesviruses and tumors, analysis of the data suggests that while herpesviruses may play an ancillary role in oncogenic transformation, the herpesvirus genome, in itself, has not been shown to be oncogenic. In order to acquire more direct evidence concerning herpesviruses and tumors, studies have been directed towards elucidating the mechanism of herpesvirus latency, with particular emphasis on the Epstein-Barr (EB) virus in human lymphoblastoid cells.

Evidence that the EB virus genome resides in human cells in a repressed state was obtained using a producer cell line (P3HR-1) made resistant to BudR. The P3HR-1(BU) cells carrying a repressed virus genome showed no expression of thymidine kinase (dTK) enzyme, while cells where spontaneous virus activation had occurred did show a positive dTK pathway of DNA synthesis. Subsequent studies showed that the virus genome in non-producer cells could be activated by incorporation into DNA of thymidine analogues (IdU or BudR). These findings suggested a means for detailed studies concerning the association between the EB virus genome and the cell genome.

When producer and non-producer cells synchronized by the double thymidine blocking technique were exposed to IdU for 60 min. intervals, it could be shown that virus activation required incorporation of the analogue into DNA during a critical period localized in the cells' early S phase (S-1 period). This suggests that the DNA made during the S-1 period contains unique sequences which control activation and repression of the EB virus genome. When the DNA from synchronized non-producer cells was isolated at various times during the S phase and hybridized with virus specific cRNA, it was shown that replication of the resident repressed EB virus genome occurs during the S-1 period, which coincides with the critical period for virus activation by IdU. These findings suggest that EB virus activation is initiated at or near the site of association of the resident virus genome with cell DNA and that the resident virus genome is physically associated with early replicating cell DNA.

The findings, thus far, have particular relevance to the ultimate objectives of the VCB and the VCP. First, this is the first direct evidence that a naturally occurring latent human herpesvirus genome may be associated with specific cellular DNA. Second, the procedures developed for EB virus should have direct application in studies relating to latency by RNA tumor viruses. Finally, the findings to date in conjunction with ongoing studies may furnish direct evidence concerning the role of herpesviruses in oncogenic transformation, a role which undoubtedly is of importance although it probably does not include a direct oncogenic potential of the herpesvirus genome in itself.

C. VIRAL LEUKEMIA AND LYMPHOMA BRANCH
July 1, 1973 - June 30, 1974

The Viral Leukemia and Lymphoma Branch conducts research designed to elucidate the role of viruses in the etiology of human neoplasms, particularly leukemias, lymphomas and sarcomas. A variety of scientific approaches are used which provide a broad base of knowledge applicable to the identification and isolation of human oncogenic agents and the prevention or control of the disease as it occurs in man. More specifically, the Branch encompasses a range of scientific disciplines including molecular biology, genetics, immunology, biochemistry, pathology, and cell culture techniques. In the past year, the emphasis has been away from model systems to the more direct study of human materials.

The Section of Viral Pathology exerts a multidisciplinary approach towards the *in vivo* and *in vitro* study of viral oncogenesis. The areas of study include virology, immunology, pathogenesis and the interferon system, and are pursued emphasizing several viral induced and spontaneous leukemias and sarcomas. The Section of Genetics 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 Primate Viruses conducts studies to determine the pathologic factors of both tumor viruses and the hosts which they infect that are involved in the oncogenic process. Particular emphasis is placed on herpes virus oncogenesis and cellular "susceptibility" genes, particularly those genes of man that predispose individuals to the development of cancer. The Section of Viral Biochemistry plans and conducts studies to detect viruses and their constituents in human tumor cells by biological and biochemical methods. 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 Clinical Studies utilizes information and laboratory techniques derived from investigations in viral oncology and applies these techniques to clinical studies on the etiology and control of cancer. The Office of the Chief coordinates the research of the various sections while recognizing the scientific freedom of the individual investigators. The office is responsible for establishing collaborative efforts with investigators in other areas of NIH and elsewhere such that information derived from studies within the Branch is constantly being applied in investigations leading to a better understanding of the etiology of human neoplasia.

Potential RNA-containing tumor viruses have been recognized by a number of methods based on biological, biochemical, and immunological properties. The reverse transcriptase has provided another potentially extremely sensitive method for virus detection.

The discovery that certain RNA tumor viruses have an enzyme capable of transcribing the viral RNA back into DNA has led to the possibility of using extremely sensitive biochemical probes to search for evidence of viral etiology of cancers, and especially, cancers in man. Some of the potential applications to the etiology and control of human cancers are:

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

2. The use of highly effective synthetic templates and optimal enzymatic conditions to search for viral reverse transcriptase in human tumor cells.

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

Each of the above approaches are being actively followed by members of the Viral Leukemia and Lymphoma Branch.

Type C viruses have been isolated from baboon tissues. The first isolate was obtained from a baboon placenta and the virus was found to grow well in a variety of heterologous cells, but especially in a dog thymus line (FCf2Th). Several additional viruses were isolated from baboon spleen, lung, and testes. The DNA product of an endogenous reaction hybridizes to normal baboon cell DNA and also to the cellular DNA of other primates. Viral expression (RNA, gs antigen) can be detected in normal baboon spleen, testes, and placenta. Eight to 12 DNA copies are found per diploid genome in all normal baboons tested. This is the first demonstration of a group of endogenous type C viruses in primates. The finding of related genetic information in other primates (rhesus, green monkey) indicates that they, too, have endogenous type C virus information.

Following the initial isolation of a cat type C virus (CCC) with properties very much like the RD114 virus, several additional viruses have been isolated from normal cat tissues which can be shown to constitute a group of viruses closely related to one another. There is also partial sequence homology between the cat (RD114/CCC) virus group and the primate virus group; the gs antigens and reverse transcriptases of viruses of these two groups share antigenic determinants with one another and their

pseudotypes interfere with one another. The results indicate a closer evolutionary relationship between these two groups of viruses that would not have been expected based on the extensive genetic divergence between the species.

A reverse transcriptase has been purified from three cases of human acute myelogenous leukemia in collaboration with Dr. Robert Gallo's group. The enzymes have antigenic properties closely related to those of the type C viruses found in woolly monkeys and gibbon apes. The gibbon ape viruses, which are highly infectious for primates, do not seem to be endogenous primate viruses and may have at one time been derived from rodents. However, the enzyme in the particles in the human leukemic cells has the properties of a type C viral enzyme, and its reverse transcriptase is particularly strongly inhibited by antisera to the gibbon type C reverse transcriptase group. If further cases of leukemia show the same characteristic enzyme, it would strongly implicate infectious, as opposed to endogenous, type C viruses in human acute leukemia.

In 1969, it was proposed that the cells of most or all vertebrate species contain type C RNA virus genomes that are vertically transmitted from parent to offspring. Depending on the host genotype and various modifying environmental factors, either virus production or tumor formation, or both, may develop at some time during the life of these animals or in their cells when grown in culture. The evidence for this concept was derived both from cell culture experiments and from a variety of seroepidemiologic studies and was presented as a unifying concept that would be consistent with the facts as they were known at the time.

In the time that has followed, a great deal more evidence has accumulated that provides strong support for the general theory. One particular prediction that was made was that the genetic information for making an RNA tumor virus, being present in a repressed form in all cells, would be potentially inducible by carcinogenic and/or mutagenic agents. Recent evidence from single cell clones of mouse embryo cells of both the high susceptible strain, AKR, and the low susceptible strain, Balb/c, indicate that every cell clone in culture does contain the information for producing a type C virus. Infectious virus can be induced from clonal lines of mouse, rat, and Chinese hamster cells, normal as well as transformed, which provides dramatic support for the original hypothesis.

One of the major advances in the past year has been the characterization of the endogenous viruses contained within most, and very likely all, vertebrate cells. These viruses differ in certain of their properties from the horizontally transmitted viruses isolated from the same species. The differences are most extreme in the case of the cat where there are two essentially unrelated type C viruses. The first (FeLV) is a virus which can be horizontally transmitted and can produce tumors. The second, an endogenous virus, can be isolated from cell lines and primary cultures from a variety of cat tissues such as, cat embryo cells, kidney cells, and lung cells. The endogenous type C virus is unable to grow in most cat

cells, but grows readily in primate cells. It will appear spontaneously from continuous lines of cat cells and the probability of appearance can be increased by treating the cells with thymidine analogs. Six independent cat cell cultures have yielded, either spontaneously or upon induction, a virus with properties indistinguishable from the prototype endogenous cat virus, RD-114. The cat, then, offers a unique species in which to test the relative contribution of endogenous and exogenously added viruses in the development of naturally occurring tumors in that species.

As a general rule, endogenous viruses of several species are unable to reinfect the cells from which the virus is produced. The cat viruses (RD114/CCC group) do not grow in most cat cells, but grow readily in primate cells. The baboon viruses do not infect most normal baboon cells, but grow readily in dog cells. A group of mouse viruses grow readily in rabbit cells, but not in mouse cells. In addition, endogenous viruses have been obtained from several other species; Chinese hamster, Syrian hamster, rat and pig. In all of these cases, the virus does not infect and grow well in clones of the species that produce the virus. Virus production in several species begins spontaneously in long-term cell cultures; the probability of virus release, however, can be increased by treating the cells with thymidine analogs, such as iododeoxyuridine. The findings in the past year in mouse cell lines suggest the possibility that spontaneously transformed cells more readily release their endogenous virus than do untransformed cells. The following table shows those species in which it has now been shown that complete virogene information is present in normal somatic cells of the species.

Species Where a Complete Virogene is Known to be Present in Normal Cells
Chicken
Chinese hamster*
Syrian hamster
Mouse*
Rat*
Cat*
Pig
Baboon

* Single cell clones spontaneously produce virus in long term culture

The isolation of type C viruses from reptiles, birds, as well as mammals would suggest that they have evolved as the organism has evolved for many millions of years and that the species specific proteins will have evolved in much the same way that serum albumins, globulins, and other proteins have evolved. The genetic relatedness of the group-specific antigens and the reverse transcriptases may well, then, be used as an index of the genetic relatedness of the species from which the type C

virus was derived. Obviously, those viruses derived from higher mammals, and especially primates, will be the most related to the viruses that come to be obtained from man. The VLLB is concentrating on the endogenous primate type C viruses, because they should have enough genetic relatedness so that an antiserum produced to the purified polymerase or the purified group specific antigen should show some ability to recognize type C viruses isolated from human tissues, unless, of course, a situation exists in primates similar to that in cats where the endogenous type C virogene is very different from a horizontally transmitted virus also in that species.

The system involving the activation of the type C virus has obvious superficial similarities to the lysogenic system in bacteria. However, in many ways virogene induction might be considered more analogous to the switching on of a differentiated function by vertebrate cells. Either spontaneously or after addition of a small molecule, the cell begins producing proteins whose genes are normally repressed and assemble a rather complex package for export from the cell. BrdU has been known to greatly affect the differentiated state in culture and to both increase and decrease the rate of only partial expression of viral genetic information. However, upon induction, new viral RNA rapidly appears. Clearly, then, one major control in this system is at the level of transcription. Whether there will be additional controls at the level of translation remains to be resolved. The induction of a lysogenic prophage involves the excision of that genetic information from the bacterial chromosome and also the lysis of the cell. In the induction of type C virus, the cells produce virus but do not die; whether this, too, involves an excision mechanism is not clear. It is possible that the system works entirely by reading off cellular genes. So in a sense, the cell lines that produce endogenous virus would not be replicating the virus, but would, rather, be transcribing and translating information that is part of their natural genetic makeup.

Other Research Developments in the Branch

The Kirsten sarcoma virus was found to be a recombinant between Kirsten murine type C virus and sequences in rat cells. At least part of the rat sequences are homologous to rat type C virus produced from NRK cells. The results suggest that transduction of oncogenic information has occurred.

Employing an ^{3}H-DNA copy of mouse mammary tumor virus RNA or the Kirsten strain of murine leukemia virus RNA, it was shown that the DNA from a variety of mouse cells, even those not producing virus, gave readily detectable hybridization reactions. With the type C probes, the DNA from NIH 3T3 and feral mouse cultures had lower levels of hybridization than seen with DNA from RIII, Balb/c or C57 Bl/6 mice. These differences might represent partial homology between the viral genomes present in NIH Swiss and feral mouse cells and the Kirsten murine leukemia probes, and/or

of cells which survive the MuLV superinfection. These effects have been quantitated and have been compared to MuLV infection of normal and non-producer mouse cells where no obvious changes of this type are seen.

S+L- 3T3FL cells are equally sensitive to N- and B-tropic viruses unlike almost all other cell lines reported to date. The presence of the S+L- MSV genome in these cells is not responsible for the enhanced sensitivity; S+L- Balb/3T3 and S+L- NIH/3T3 cells still retain their original host range restrictions. Surprisingly, untransformed 3T3FL cells also are equally susceptible to N- and B-tropic leukemia viruses even though the original 3T3 line from which they were derived is clearly type B. Cloning experiments showed that the 3T3FL line is not a mixture of B- and N-type cells, and thus they appear to represent a cell line in which the Fv-1 gene is either missing or is not expressed.

Mouse x human hybrid cells, uniquely segregating mouse chromosomes, have been used to determine the influence of the human and mouse genomes on the replication of mouse tropic and xenotropic strains of MuLV. The complete human genome does not inhibit the replication of mouse N-, B-, or NB-tropic viruses. The Fv-1 locus exerted its viral restrictive functions in the hybrid cells. The replication of a xenotropic MuLV strain, AT-124, was completely inhibited in hybrids having a complete or nearly complete mouse chromosome content.

Certain avian nontransformed fibrosarcoma cells, which are gs+ and cellular helper factor positive, absorbed the activity of the chicken anti-Bryan strain Rous sarcoma virus (B-RSV) serum to the envelope antigen of B-RSV, indicating the presence of CSA common to B-RSV-envelope antigen. This suggests that possible human oncogenic viruses can be searched for by examining VEA on the cell surface with anti-VEA serum.

The H-2^b antigen on EL4 cells and the G surface antigens of E♂G2 cells, treated once with 0.1% formalin and stored at 4°C in EBSS for 3 months were well preserved; formalin-treated tumor cells may thus provide a stable antigen source, for immunotherapy and prophylaxis, without biohazard problems.

Specific antibodies to the RNA-dependent DNA polymerase (RDDP) of murine type C viruses have been isolated from the renal glomeruli of both leukemic and nonleukemic AKR mice. The antibodies were shown to have sedimentation coefficients of 26S to 28S and 5S to 7S and to be both IgM and IgG classes. This further proves the lack of immunologic tolerance.

The American Burkitt Lymphoma Registry has been continued and expanded. An additional twelve patients have been added in the past six months and a cell line has been established from one of these patients. Although sensitive biochemical assays have demonstrated that four patients with American BL had no EBV genome in any of their tissues, specimens taken from a more recent case of American BL contained EBV, and virus-associated antigens were readily induced by IdU. Serological studies demonstrated

might be related to the fact that neither of these cells are inducible to form a complete type C virus, and may lack an essential viral component necessary for complete viral expression.

The mouse cell line Balb/c 3T3 and its derivatives transformed either spontaneously or by treatment with a variety of agents including methylcholanthrene and X-irradiation were analyzed for cytoplasmic RNA complementary to DNA products from the Kirsten strain of murine sarcoma-leukemia virus and from an endogenous type C virus of Balb/c 3T3. While none of these clonal lines spontaneously release virus they all contained RNA which was partially homologous to a portion of the 35S RNA isolated from these viruses. The parental cell line, Balb/c 3T3, contained a low level of viral-related RNA. There was an increased amount of this RNA in some of the transformed cells.

A new host range class of endogenous murine type C viruses has been identified which are unable to replicate in any mouse cell line tested so far, but replicate well in a variety of other mammalian cells, including rhesus monkey, dog, mink, and rat cells. Because of the sensitivity of the rabbit cell line, SIRC, for detecting this group of viruses, they have been called "S-tropic" viruses. By host range properties and by nucleic acid hybridization studies, this new class of viruses can be distinguished from both "N-" and "B-" tropic murine type C viruses. The S-tropic virus is preferentially induced by halogenated pyrimidines from the Balb/3T3 line, although low levels of N-tropic type C virus are also produced. S-tropic virus has also been shown to be spontaneously released from certain Balb/3T3 derived cell lines. Balb/c splenocytes preferentially release S-tropic type C virus after induction and also after graft-versus-host or mixed splenocyte reactions. Spleens from older Balb/c animals generally contain S-tropic virus, whereas young animals do not. Normal weanling mice of C57Bl, CBA, DBA, NZB, C58, and AKR strains have S-tropic virus. The S-tropic viruses from Balb/c cells and the S-tropic viruses from NIH Swiss cells are related, but different.

A tissue culture system using an SV40-transformed human cell line has been developed to study the transforming effect of various sarcoma virus pseudotypes. This continuous line offers several advantages compared to diploid cells; transformed foci are recognized within five days, and the system lends itself to easy quantitation of transformation. Another transformation system using a mink cell line has proven to be useful for transformation assays since the S-tropic mouse viruses as well as both groups of cat viruses and both the infectious and endogenous primate viruses grow readily in these cells. Pseudotypes of mouse sarcoma, feline sarcoma, and woolly monkey sarcoma virus have been produced and the same mink cell has been transformed by sarcoma viruses from these three different species. Using these cells, it has been possible to generate feline sarcoma transformed non-producer cells as well as mouse sarcoma virus transformed non-producer cells. Each of these can be

rescued by a variety of type C viruses enabling the study of pseudotypes with sarcoma and leukemia virus information derived from different species.

The single hit interaction of mouse cells with the murine sarcoma virus results in sarcoma positive-leukemia negative (S+L-) cells which release a noninfectious particle. The RNA of these fragile particles or nucleoids from one S+L- line consists of several sizes. The 28S virion RNA is interesting in that this size of RNA does contain sarcoma-leukemia virus specific sequences, although this is smaller than the murine leukemia viral subunit size. Additional demonstration of its viral nature is the presence of covalently linked polyadenylate sequences at the 3'-terminal. In another S+L- mouse cell subline apparent multimeric aggregates of 28S RNA exist, seen as 56, 68, and 80S structures.

Human S+L- cells have been investigated in detail. Sarcoma virus was rescued from the human amnion S+L- cells with RD114 virus and was used to generate new human S+L- cells in L-132 human lung cells. These new human lung S+L- cells were analogous to the human amnion S+L- cells. Both were transformed, expressed the stable gs-1 marker of murine leukemia virus and contained cryptic murine sarcoma virus rescuable by various helper viruses. Neither human S+L- cell line had type C particles. After induction by several chemical agents, human S+L- cells failed to yield infectious sarcoma or helper viruses.

The understanding of the process of reversion of S+L- cells to apparently normal cells was approached in several ways. Initially, it was asked whether there are chemical agents which can significantly enhance reversion frequency. Two such agents, colcemid and fluorodeoxyuridine each could raise the reversion frequency by a factor of fifty. These revertants were compared to spontaneous revertants in terms of sarcoma virus rescue, agglutinability, chemical induction, saturation density, growth in soft agar, virus susceptibility, spontaneous retransformation, and chromosomal makeup. All the features of chemically derived revertants were essentially identical to properties of spontaneous revertants except that fluorodeoxyuridine-derived revertants had a lower chromosome number than the parental S+L- cells. An important finding was that in rare instances a back-transformed cell demonstrated a rescuable sarcoma virus genome, implying that reversion from the transformed state in S+L- cells was phenotypic. To determine which chemical or physical agents increase the reversion frequency, a rapid screening assay was developed for the detection of revertants if these occurred at very low frequencies. This was based on the fact that S+L- mouse cells but not revertants infected with leukemia virus underwent a secondary morphological change accompanied by reduced adhesion to the surface. On simple washing of the leukemia virus infected monolayers, only revertants were seen as colonies.

The S+L- type of MSV-transformed mouse cell responds to MuLV superinfection by a characteristic morphological change and a markedly decreased plating efficiency, followed by the eventual selection of a subpopulation

the need for measuring all EBV-associated antibodies since a previously "EBV-negative" American BL serum (no VCA, EA, or MA) was shown to have antibody to the EBV-associated nuclear antigen (EBNA). Retesting of all the American BL patients demonstrated that only one case is entirely negative for antibody to all of the EBV-associated antigens. Antibody to the leukemia associated nuclear antigen (LANA), which has been described in the two EBV-negative cases of African Burkitt's lymphoma, was found in 30% of sera from American BL cases and generally correlated with a poor prognosis. Studies on the genetics of Burkitt's tumor demonstrated a high incidence of HLA type 1 and 8 in patients with American BL. It is of interest that these HLA types have also been found in an increased frequency in patients with Hodgkin's disease. Skin test studies performed in lymphoma patients using antigens prepared from EBV-containing cell lines and control cell lines indicate that lymphoma patients show an increased reactivity against antigens on the surface of lymphoid lines derived from cancer patients, but not from cell lines derived from normal individuals. In vitro studies evaluating cell-mediated immunity to EBV also indicate that there is a parallel relationship between humoral and cell-mediated immunity to EBV-associated antigens in lymphoma patients, although the correlation is not a complete one.

Two-hundred sera from five stages of nasopharyngeal carcinoma (NPC), African Burkitt lymphoma (AfBL) patients, controls and patients having tumors of the cervix, head and neck and liver when tested to four EBV antigens (early antigen (EA), virus capsid antigen (VCA), EB nuclear antigen (EBNA), and soluble antigen (S)) showed that antibodies to two of these EBV antigens were quite significant. The EA antibody in NPC sera gradually increased (from clinical stage 1 - 5) and even though EA titers were higher in AfBL, these were not as high as in NPC. All other groups have practically no antibody titers to EA. Therefore, EA antibody titers suggest a direct relationship to the progression of NPC. Similarly, the titers to S antigen in NPC sera was significantly higher in clinical stage 1 of NPC, whereas titers to EA, VCA and EBNA were much lower. This finding also suggests that S antigen may be of prognostic value in NPC.

Sera from owl and marmoset monkeys developing lymphoma and leukemia contained Herpes virus saimiri (HVS) EA antibody which reacted with nuclear antigen in two distinct patterns similar to those reported for EBV. No EA antibody was detectable in owl monkeys which did not develop neoplasia; however, other antibodies to HVS antigens (CF and late antigen, neutralizing) were present. This finding suggests that antibody response to EA may be a very useful marker to indicate lymphoproliferation or cell transformation. The squirrel monkey which is the natural host of HVS rarely contains EA antibody.

Other Activities within the Branch

In addition to their intramural research activities, several of the senior investigators within the Branch spent a substantial portion of their

time in support of the Virus Cancer Program. The members serve as Chairmen, Vice-Chairmen, Work Group members and Executive Secretaries of segments of the Program. They provide scientific guidance as Project and Assistant Project Officers on research contracts supported by the Virus Cancer Program.

The activities of the VCP 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 VCP to date. The broad scientific perspective developed by these investigators in their VCP 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.

IV. CONTRACT PROGRAM

A. Research Logic for the VCP (See Chart.)

B. Major Program Modifications of Viral Oncology Contracts

Flexibility within a program is necessary if effective program management is to provide responsiveness to changing program needs. This requires constant monitoring and evaluation of each scientific project in terms of need, priority and relevance to specific program objectives within the framework of available funds. This task is time consuming and requires constant attention because of the number of contracts to be monitored, the complexity of many of the contracts, and the necessity for reappraisal of their scientific objectives as priorities, degrees of relevance, and need, change in the light of accomplishments, failures, and newer information.

One early accomplishment of the planning team of the Special Virus Cancer Program was the recognition of this need for flexibility so that the program would be responsive to changing requirements. Indeed, it is program management of this type which in part separates the contract support mechanism from the grant support mechanism.

This report is a summary documentation of major modifications of contracts within the Viral Oncology Area for the period ending June 30, 1974. The actions include (1) termination, (2) modification of workscope, (3) change of emphasis within existing workscope, and (4) initiation of new contracts.

B. SUMMARY: MAJOR MODIFICATIONS OF VIRAL ONCOLOGY CONTRACTS

July 1, 1973 - June 30, 1974

ACTION	PREDOMINANT REASONS	NO. OF CONTRACT MODIFICATIONS	
Modification of existing workscope	Decrease activity of low priority	9	14
Modification of existing workscope	Expansion of activity of high priority	5	
Change of emphasis workscope	To exploit new information, to respond to changing program priorities, to provide maximum program flexibility	3	
Termination	Work successfully completed	3	18
Termination	No longer high priority	9	
Termination	Insufficient progress	4	
Termination	Transfer to other programs	2	
New contract	Satisfy program needs	27	30
New contract	Provide better management of large efforts	3	
TOTAL NUMBER OF CONTRACT MODIFICATIONS		65	

SPECIFIC MAJOR PROGRAM MODIFICATIONS OF VIRAL ONCOLOGY CONTRACTS

July 1, 1973 - June 30, 1974

BREAST CANCER VIRUS SEGMENT

PURPOSE	CONTRACTOR	DATE CONTRACT INITIATED	MODIFICATIONS	
			ACTION	REASON/PURPOSE
In vitro cultivation of human and mouse mammary tumor virus	University of California (33253)	1972	Modification of workscope	Increased effort to characterize Murine mammary tumor virus *in vitro*
Etiological studies of breast cancer in human milk and breast cancers	Institute for Medical Research (33339)	1968	Modification of workscope	Increased effort in biochemical and immunologic characterization of "virus-like" particles from human milk and tumors
Studies of primate and other mammary tumor viruses	Mason Research Institute (33358)	1970	Modification of workscope	Decreased emphasis on rate R-35 virus studies and hormonal monitoring of test monkeys

BREAST CANCER VIRUS SEGMENT CONT'D

PURPOSE	CONTRACTOR	DATE CONTRACT INITIATED	MODIFICATIONS	
			ACTION	REASON/PURPOSE
Studies on milk in high risk breast cancer families	Michigan Cancer Foundation (33347)	1971	Modification of workscope	Increased work on characterization of "virus-like" particles associated with human milk and breast tumor-derived cell lines
Virological studies of human and animal breast cancers	Pfizer, Inc. (33239)	1967	Modification of workscope	Decreased work on development of molecular hybridization techniques
Studies on viral etiology of human mammary adenocarcinoma	Georgetown University (50053)	1965	Termination	No longer high priority within the goals of the Virus Cancer Program

BREAST CANCER VIRUS SEGMENT CONT'D

PURPOSE	CONTRACTOR	DATE CONTRACT INITIATED	MODIFICATIONS	
			ACTION	REASON/PURPOSE
Hormone effects on *in vitro* viral propagation in breast cancer	Medical College of Wisconsin (81010)	1968	Termination	No longer high priority within the goals of the Virus Cancer Program
Collection of sera from breast cancer and control population groups	Memorial Hospital for Cancer and Allied Disease (43208)	1971	Termination	Work successfully completed
Correlation of molecular virology studies to diagnosis of breast cancer	Howard University (43287)	1974	New	To examine diagnostic non-pregnancy excretions for detectable markers of "oncorna-virus-like" activity
Molecular studies of human and animal cancer with emphasis on breast cancer	Meloy Laboratories Inc. (43223)	1974	New	To develop molecular virology techniques for studies on relationship between viruses and breast cancer

BREAST CANCER VIRUS SEGMENT CONT'D

PURPOSE	CONTRACTOR	DATE CONTRACT INITIATED	MODIFICATIONS	
			ACTION	REASON/PURPOSE
Study of the release of RNA tumor viruses and their genetic control	Radiobiological Institute (43328)	1974	New	To characterize the effect of aging and environmental factors on the genetic control of virus expression

DEVELOPMENTAL RESEARCH SEGMENT

PURPOSE	CONTRACTOR	DATE CONTRACT INITIATED	MODIFICATIONS	
			ACTION	REASON/PURPOSE
Studies on viruses related to cancer	Baylor College of Medicine (33257)	1963	Modification of workscope	Termination of peripheral studies relative to human leukemia, gene linkage mapping and transcription
Expression of the RNA tumor virus genome in animal and human malignant cells	Duke University (33308)	1973	Modification of workscope	Expansion of effort to characterize proteins of type B viruses
Support for National Conference on Virology and Immunology in Human Cancer	American Cancer Society, Inc. N-CP7-4152A	1973	Termination	Meeting successfully completed
Application of feline virus systems in the study of viral relationships	Cornell University (33346)	1965	Termination	No longer high priority within the goals of the VCP

DEVELOPMENTAL RESEARCH SEGMENT CONT'D

PURPOSE	CONTRACTOR	DATE CONTRACT INITIATED	MODIFICATIONS	
			ACTION	REASON/PURPOSE
Aerosal transmission of oncogenic viruses	Ohio State University Research Foundation (43217)	1965	Termination	No longer high priority within the goals of the VCP
Studies of genetic acquisition of oncogenic potential by non-oncogenic RNA viruses	Rutgers University (12077)	1971	Termination	No longer high priority within the goals of the VCP
Studies on the relationships of viruses to human neoplasia	University of Texas (33304)	1965	Termination	No longer high priority with the goals of the VCP

DEVELOPMENTAL RESEARCH SEGMENT CONT'D

PURPOSE	CONTRACTOR	DATE CONTRACT INITIATED	MODIFICATIONS	
			ACTION	REASON/PURPOSE
Sero-epidemiologic study of Herpes type 2 in cervical cancer	Boston Hospital for Women (43379)	1974	New	To provide information of herpes type 2 as the etiological agent of cervical cancer
Studies on viruses in relation to cancer using non-human primates	Litton Bionetics, Inc. (12025)	1974	New	To provide information on host-virus relationships established by potentially oncogenic viruses

IMMUNOLOGY-EPIDEMIOLOGY SEGMENT

PURPOSE	CONTRACTOR	DATE CONTRACT INITIATED	MODIFICATIONS	
			ACTION	REASON/PURPOSE
Develop coordinated Program on genetics of cancer	University of California (43211)	1971	Modification of workscope	Deemphasis of large scale cell mediated immunity studies
Evaluation of unusual epidemiological situation relevant to the viral etiology of cancer	Center for Disease Control (40202)	1967	Modification of workscope	Termination of nasopharyngeal carcinoma studies
Study of immunological reactivity of cancer patients to tumor and viral associated antigens	George Washington University (23251)	1972	Modification of workscope	Deletion of laboratory studies

IMMUNOLOGY-EPIDEMIOLOGY SEGMENT CONT'D

PURPOSE	CONTRACTOR	DATE CONTRACT INITIATED	MODIFICATIONS	
			ACTION	REASON/PURPOSE
Studies of anti-tumor reactivity in cancer patients	Johns Hopkins University (3337)	1971	Modification of workscope	Deemphasis of serological studies
Stimulation of Immunity against tumors in animal and human systems	Mt. Sinai Hospital (43225)	1972	Modification of workscope	Expansion of developmental research in viral oncology and human breast cancer
Determine relationships between animal and human leukemias	University of Texas (33301)	1972	Modification of workscope	Deemphasis of in vivo human studies
Immunological and Epidemiological studies on Japanese cancer patients	Aichi Cancer Center (33290)	1969	Termination	No longer high priority

IMMUNOLOGY-EPIDEMIOLOGY SEGMENT CONT'D

PURPOSE	CONTRACTOR	DATE CONTRACT INITIATED	MODIFICATION	
			ACTION	REASON/PURPOSE
Studies on relationship of fetal antigens to the etiology of cancer	Atomic Energy Commission (30210)	1965	Termination	Readvertisement of effort
Epidemiologic investigation of Burkitt's Lymphoma in Uganda	Makerere University (70047)	1967	Termination	Work successfully completed
Studies of immune responses to breast cancer	University of Miami (33218)	1973	Termination	Readvertisement of effort; relocation of principal investigator
Study of immunological reactivity of cancer patients to tumor specific antigens	University of Minnesota (92061)	1969	Termination	Transfer to Tumor Immunology

IMMUNOLOGY-EPIDEMIOLOGY SEGMENT CONT'D

PURPOSE	CONTRACTOR	DATE CONTRACT INITIATED	MODIFICATIONS	
			ACTION	REASON/PURPOSE
Development of vaccines for prevention and cure of cancer	Research Foundation of State University of N.Y. (33373)	1971	Termination	Transfer to Tumor Immunology Program
Characterization of tumor specific antigens	Robert B. Brigham Hospital (12172)	1967	Termination	Insufficient progress
Purification and characterization of viral antigens and antibodies	TRW Systems Group (33252)	1970	Termination	Readvertisement of effort; insufficient progress
Studies on the genetics of cancer	Biotech Research Laboratories (43365)	1974	New	To study human genetic variation in susceptibility to transformation and to characterize high risk cancer groups

IMMUNOLOGY-EPIDEMIOLOGY SEGMENT CONT'D

PURPOSE	CONTRACTOR	DATE CONTRACT INITIATED	MODIFICATIONS	
			ACTION	REASON/PURPOSE
International investigation on the epidemiology of lymphomas	International Union Against Cancer (43292)	1974	New	To define the role of viruses in the etiology of human lymphomas
Study of cellular and humoral immune response of cancer	Johns Hopkins University (43330)	1974	New	To study a possible etiological association between herpes simplex virus and cervical cancer
	Emory University (43393)	1974	New	(two RFP awards)
Serological and virological studies on human cancers	Litton Bionetics, Inc. (43252)	1974	New	To apply serological techniques to studies on the viral etiology of human cancer

IMMUNOLOGY-EPIDEMIOLOGY SEGMENT CONT'D

PURPOSE	CONTRACTOR	DATE CONTRACT INITIATED	MODIFICATIONS	
			ACTION	REASON/PURPOSE
Immunological assays for DNA and RNA viruses	Litton Bionetics, Inc. (43333)	1974	New	To apply immunological techniques to studies on the viral etiology of human cancer
Studies of immune responses to breast cancer	University of Miami (43358)	1974	New	To determine the possible role of host immune reactivity in viral carcinogenes
	University of Texas (43370)	1974	New	(two RFP awards)
Characterization of viral antigens	Medical College of Pennsylvania (43319)	1974	New	To isolate and purify EBV associated antigens and study relationship to human cancer

IMMUNOLOGY-EPIDEMIOLOGY SEGMENT CONT'D

PURPOSE	CONTRACTOR	DATE CONTRACT INITIATED	MODIFICATIONS MODIFICATIONS	
			ACTION	REASON/PURPOSE
Immunological studies on the relationship of embryonic antigens to virus-induced tumor antigens	University of Tennessee (43325)	1974	New	To provide information on the mechanism of viral oncogenesis and on the use of fetal antigens in the immunological control of virus-induced tumors
	Duke University (43395)	1974	New	
	University of Alabama (43394)	1974	New	(three RFP awards)

SOLID TUMOR VIRUS SEGMENT

PURPOSE	CONTRACTOR	DATE CONTRACT INITIATED	MODIFICATIONS	
			ACTION	REASON/PURPOSE
Studies on type C viruses in relation to oncogenic potential	Flow Laboratories, Inc. (43388) (formerly part of 33247)	1971	Administrative	Contract 33247 was divided into 3 separate contracts (43387, 43388) 43389) to provide better management
Virus and reagent production and purification	Flow Laboratories, Inc. (43387) (formerly part of 33247)	1971	Administrative	To provide better management
Herpesvirus studies	Flow Laboratories, Inc. (43389) (formerly part of 33247)	1971	Administrative	To provide better management
Studies of type C RNA tumor viruses	Microbiological Assoc.,Inc. (43240) (formerly part of 70-2068)	1970	Administrative	Contract 70-2068 was divided into 2 separate contracts to provide better management

SOLID TUMOR VIRUS SEGMENT CONT'D

PURPOSE	CONTRACTOR	DATE CONTRACT INITIATED	MODIFICATIONS	
			ACTION	REASON/PURPOSE
Molecular virology studies	University of California (33293)	1971	Change of emphasis of workscope	Expansion of efforts on molecular hybridization and renovation of facilities
Immunologic study of RNA (type C) viruses	Scripps Clinic and Research Foundation (43375)	1972	Change of emphasis of workscope	Expansion of efforts on the isolation of human tumor virus
Search for genetic material in human cancers and studies on the mechanism of oncogenesis by RNA and DNA tumor viruses	St. Louis University (43359)	1967	Change of emphasis of workscope	Decrease of activities of lower priority

SOLID TUMOR VIRUS SEGMENT CONT'D

PURPOSE	CONTRACTOR	DATE CONTRACT INITIATED	MODIFICATIONS	
			ACTION	REASON/PURPOSE
Studies on surface alterations in RNA tumor virus transformed cells	Princeton University (71-2372)	1971	Termination	No longer high priority within the goals of the VCP
Growth regulation of normal and transformed cells and immunological approaches to tumor rejection and prevention	Salk Institute for Biological Studies (72-3207)	1972	Termination	No longer high priority within the goals of the VCP
Electron microscope studies of tumor virus nucleic acids	California Institute of Technology (43306)	1974	New	A new approach to cancer etiology at the cellular level

SOLID TUMOR VIRUS SEGMENT CONT'D

PURPOSE	CONTRACTOR	DATE CONTRACT INITIATED	MODIFICATIONS	
			ACTION	REASON/PURPOSE
Isolation of human xenotropic viruses	University of California (43381)	1974	New	A new approach to - the isolation of the putative human tumor virus
Genetic studies on endogenous RNA tumor viruses	Department of Agriculture (Beltsville) (40214)	1974	New	An additional model system to further study the genetic control of RNA tumor virus expression
Virologic, immuno-logic and biologic characterization of Hodgkin's disease	Stanford University (43228)	1974	New	Intensive search for the etiologic agent of Hodgkin's disease

TUMOR VIRUS DETECTION SEGMENT

PURPOSE	CONTRACTOR	DATE CONTRACT INITIATED	MODIFICATIONS	
			ACTION	REASON/PURPOSE
Integrated program on host factors of significance in carcinogenesis	Atomic Energy Commission (20208)	1972	Modification of workscope	Phase-out research of low priority
Study host restriction of Friend Leukemia Virus	Albert Einstein College of Medicine (43380)	1974	New	To elucidate the molecular mechanism of restriction induced by the FV-1 gene
Study regulatory proteins in polyoma and SV40	University of California (43298)	1974	New	To find out if the regulatory proteins are involved in transformation

TUMOR VIRUS DETECTION SEGMENT CONT'D

PURPOSE	CONTRACTOR	DATE CONTRACT INITIATED	MODIFICATIONS	
			ACTION	REASON/PURPOSE
Attempt to isolate type C virus from cultured human leukocyte cells	Children's Cancer Research Foundation (43269)	1974	New	Once a human type C virus is isolated, its role in the etiology of human cancer can be studied
Molecular and genetic studies of Rous Sarcoma Virus	Foundation Curie Institut du Radium (43219)	1973	New	To learn more of this important model system
Study of relationship between cells transformed by DNA and RNA tumor viruses	Harvard University (43299)	1974	New	To identify common cellular functions following transformation by either DNA or RNA tumor viruses
Study new papovaviruses isolated from man	Johns Hopkins University (43266)	1974	New	To learn the relationship of human papovaviruses to human cancer

C. TABLE I - ANALYSIS OF CONTRACTS BY SEGMENTS*
(By Type of Institution)
Viral Oncology Area, DCCP, NCI

Segment	Profit		Educational		Non-Profit		Other***		Total	
	No.	Amount**	No.	Amount	No.	Amount	No.	Amount	No.	Amount
Program Management	1	2,951,282	1	574,726	0	0	2	972,917	4	4,498,925
Developmental Research	5	2,301,567	18	4,392,704	3	397,226	0	0	26	7,091,497
Solid Tumor Virus	6	4,339,524	14	6,263,135	4	1,370,968	2	217,500	26	12,193,127
Immunology	3	554,322	22	2,224,398	3	257,154	4	986,111	32	3,976,325
Resources and Logistics	20	9,152,494	18	1,070,502	8	323,170	3	238,731	49	10,784,897
Breast Cancer Virus	3	793,764	8	953,160	4	461,464	0	0	15	2,208,388
Tumor Virus Detection	4	2,753,155	13	1,591,379	1	174,900	2	648,000	20	4,584,234
TOTAL	42	22,846,108	94	17,070,004	23	2,984,882	13	3,063,259	172	45,337,393

*Chart based on FY 74 operating level
**Dollars in thousands
***Included interagency agreements

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

Contractor	Cont. No.	Description of Work
Biotech Res. Labs	43365	Genetic studies on high risk populations
Bost. Hosp. Women	43379	Retrospective cohort study of HSV-2 in cervical cancer
Calif., Univ. of	43211	Relationship of HL-A type to cancer incidence
CDC	40202	Epidemiological studies of cancer clusters
Child. Hosp. (Phila)	33272	Immunological studies of EBV-associated cancers
Georgetown Univ.	50053	Studies on populations at high risk to breast cancer
Hebrew Univ.	33342	Epidemiologic studies of Hodgkin's Disease and other lymphomas
Howard Univ.	43287	Studies on populations at high risk to breast cancer
IARC	43296	Seroepidemiological study of BL and NPC in SE Asia and Africa
Inst. for Med. Res.	33339	Studies on populations at high risk to breast cancer
Johns Hopkins Univ.	33345	Seroepidemiology of cervical carcinoma
Johns Hopkins Univ.	33337	Immunological studies of leukemia/lymphoma patients and families
Johns Hopkins Univ.	43266	Seroepidemiological study of papovaviruses in human cancer
Karolinska Inst.	33316	Immunological studies of EBV-associated cancers
Litton-Bionetics	43249	Study of human milk for viral markers
Mich. Canc. Fdn.	33347	Studies on populations at high risk to breast cancer
Minn., Univ. of	33357	Studies of high risk groups with immunodeficiency diseases
S. Calif., Univ. of	53500	Cancer surveillance and epidemiologic studies in LA County
Texas, Univ. of	33304	Serological studies of certain human cancers
UICC	43292	Epidemiological studies of lymphoma

Step 2: SOURCES OF VIRUS OR SUBVIRAL MATERIAL

Contractor	Cont. No.	Description of Work
Baylor Univ.	33257	Acquire clinical data and specimens of human neoplastic tissues
Bost. Hosp. Women	43379	Acquire specimens from cervical cancer patients and controls
Calif., Univ. of	43211	Acquire clinical data and specimens of normal and neoplastic tissue
CDC	40202	Collection of clinical data and specimens from human leukemias
Child. Canc. Res. Fdn.	43269	Clinical data and specimens from human leukemias
Georgetown Univ.	50053	Acquire clinical data and human milk and breast cancer specimens
G. Washington Univ.	23251	Acquire clinical data and specimens-breast cancer and lymphoma
Harvard Univ.	33390	Virus detection in non-human primates

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
Hebrew Univ.	33342	Acquire clinical data and specimens-Hodgkin's disease and lymphomas
Inst. for Med. Res.	33339	Acquire clinical data and specimens from human breast cancer patients
IARC	43296	Acquire clinical data and specimens from BL and NPC
Johns Hopkins Univ.	33245	Acquire clinical data and specimens from pediatric cancer patients
Johns Hopkins Univ.	33337	Acquire clinical data and specimens from leukemia patients
Johns Hopkins Univ.	43266	Acquire clinical data and specimens from brain tumor patients
Johns Hopkins Univ.	33345	Acquire clinical data and specimens from patients w/cervical carcinoma
Karolinska Inst.	33316	Acquire clinical data and specimens from BL, NPC, and leukemias
Memorial Hosp. (NY)	43208	Supply data and specimens for human breast cancer studies
Mich. Canc. Fdn.	33347	Acquire data and specimens for human breast cancer studies
Minnesota, Univ. of	33357	Data and specimens from patients with immunological deficiency diseases
Mt. Sinai Hosp.	43225	Clinical data and specimens from leukemia and breast cancer patients
N. Y. Med. Coll.	33398	Clinical data and specimens from breast cancer patients
S. Calif., Univ. of	53500	Acquire clinical data and specimens of human neoplastic tissues
Texas, Univ. of	33301	Acquire clinical data and specimens from leukemia patients
Texas, Univ. of	33304	Acquire clinical data and tumor specimens

Step 3: DETECTION OF VIRUS OR VIRUS EXPRESSION

Contractor	Cont. No.	Description of Work
Aichi Cancer Center	33290	Immunologic and epidemiologic studies on Japanese cancer patients
Alabama, Univ. of	43394	Immunological detection of viral and fetal antigens
Atomic Energy Comm.	20208	Detection using immunological, biochemical, tissue culture techniques
Baylor Univ.	33257	Detection using immunological and cell culture techniques
Bost. Hosp. Women	43379	Detection by immunological techniques
Calif. SDPH	43209	Studies on the role of oncogenic viruses in cancer of man
Calif. Inst. Tech.	43306	EM studies of tumor virus nucleic acids
Calif., Univ. of	33242	Detection techniques in comparative leukemia/sarcoma virus studies
Calif., Univ. of	43211	Detection using cellular immunology techniques
Calif., Univ. of	43381	Isolation of human xenotropic viruses
Calif., Univ. of	33283	Detection using biochemical techniques
Child. Hosp. (Phila)	33272	Immunological detection of EBV-associated antigens in human cancer
Columbia Univ.	33258	Screening human cancer specimens with biochemical 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
Atomic Energy Comm.	43210	Relationship of fetal antigens to tumor and virus-associated antigens
Cornell Univ.	33346	Isolation, characterization ov viruses from human cancer specimens
Duke Univ.	43395	Immunological detection of viral and fetal antigens
Emory Univ.	43393	Herpes antigens in cervical cancer patients
Flow Laboratories	43388	Immunological studies of mammalian Type C viruses
Flow Laboratories	43389	Immunological studies of herpesviruses
Georgetown Univ.	50053	Biochemical techniques for human breast cancer detection
Harvard Univ.	33390	Virus detection in non-human primates
Hazleton Labs	33212	Immunological/biochemical detection of virus in animal/human tumors
Hebrew Univ.	33342	Immunological detection of virus in Hodgkin's disease/other lymphomas
Hebrew Univ.	33310	Detection using biochemical techniques
Howard Univ.	43287	Biochemical detection of virus in human breast secretions
Illinois, Univ. of	43318	Detection using biochemical and tissue culture techniques
IARC	43296	Immunological studies of BL, NPC
Inst. for Med. Res.	33339	Immunological/biochemical studies on murine and human breast cancer
Jackson Labs	33255	Genetics of susceptibility to cancer in mice
Johns Hopkins Univ.	33345	Immunological studies on herpesvirus antigens in cervical carcinoma
Johns Hopkins Univ.	33337	Immunological studies of human leukemia and lymphoma
Johns Hopkins Univ.	43330	Immunological studies on HSV antigens in cervical cancer
Karolinska Inst.	33316	Immunological studies of EBV-associated human neoplasia
Litton Bionetics	43224	Screening of human/primate neoplastic tissue for virus activity
Litton Bionetics	43333	Detection using immunological techniques
Litton Bionetics	43252	Detection using virological and serological techniques
Litton Bionetics	43249	Detection using molecular techniques
Mass. Inst. Tech.	33348	Detection using biochemical techniques
Med. Coll. Wisc.	81010	Hormonal effects on virus expression
Meloy Labs	43236	Immunological and biochemical detection of virus
Meloy Labs	43207	Detection using EM, immunological, biochemical, tissue culture tech.
Meloy Labs	43223	Biochemical detection of virus in human/mammalian breast cancer

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
Mich. Canc. Fdn.	33347	Virus detection in human breast cancer by biochemical techniques
Micro. Assoc.	43240	Detection using immunological and cell culture techniques
Micro. Assoc.	33248	Bioassay of murine leukemia/sarcoma viruses
Minn., Univ. of	33357	Immunological and virological studies of immunodeficiency diseases
Nether. Canc. Inst.	33368	Immunological detection of natural MTV expression
Ohio State Univ.	43217	Immunological detection of feline viruses in cats
Penn. State Univ.	02024	Biological, biochemical studies of human herpesviruses
Pfizer, Inc.	33239	Immunological/morphological detection of viruses in mammalian tissue
Radiobiol. Inst.	43328	Genetic and environmental control of RNA tumor virus expression
Rockefeller Univ.	33306	Development of methods for isolation of virus from human neoplasia
Rush-Presbyterian	33219	Immun., biol., tissue culture studies of primate tumor viruses
Scripps Clinic	43375	Development of new immunological techniques for detection of virus
Scripps Clinic	33204	Immunological studies of virus antigen-antibody complexes
S. Calif., Univ. of	53500	Immunological studies of human fetal and tumor tissues
Southwest Foundation	43214	Selection of virus by EM and tissue culture techniques
Stanford Univ.	43244	Development of cell culture methods for human tissue
St. Louis Univ.	43359	Detection using tissue culture and biochemical techniques
Tel Aviv Univ.	23237	Biochemical detection of tumor viruses in human breast cancer
Tennessee, Univ. of	43325	Immunological detection of viral and fetal antigens
Texas, Univ. of	33304	EM/tissue culture/immunological studies of human neoplastic tissues
Texas, Univ. of	33292	Immunological methods for detection of human tumor antigens, antibodies
Washington, Univ. of	33372	Development of immunological tests for activation of viruses
Washington, Univ. of	33236	Immunological studies on canine tumors
Wisconsin, Univ. of	22022	Immunological, biochemical, tissue culture techniques
Wistar Inst.	33250	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 Univ.	33257	Human leukemia transmission studies in non-human primates
Biolabs, Inc.	22068	Development and improvement of *in vitro* production of HSV
Calif. SDPH	43209	Isolation and characterization of human tumor viruses
Calif., Univ. of	33237	Evaluation of cell cultures for viral oncology research
Calif., Univ. of	33332	Effect of hormones on virus replication
Calif., Univ. of	33242	*In vitro* and *in vivo* studies of simian virus infectivity & replication
Calif., Univ. of	33253	Development of methods for *in vitro* propagation of MTV
Cornell Univ.	33346	Isolation and characterization of human tumor viruses
Flow Labs, Inc.	43387	Large-scale production of RNA tumor virus diagnostic reagents
Harvard Univ.	33390	Replication of primate HSV
Hazleton Labs	33212	Isolation and production of ts-mutants of RNA tumor viruses
IARC	43296	Replication of HSV in NPC and Burkitt's lymphoma specimens
Illinois, Univ. of	43318	Replication of papovaviruses
Johns Hopkins Univ.	43266	Replication of papovaviruses
Karolinska Inst.	33316	Growth and replication of herpes-type viruses
Life Sciences	33291	Production and characterization of avian leukosis viruses
Litton-Bionetics	43224	Inoculation of primates with various virus materials
Litton-Bionetics	23294	Development and improvement of techniques for virus and reagent produc.
Med. Coll. Wisc.	81010	Stimulation of Type C virus production in human cells by hormones
Meloy Labs	43236	Replication of murine mammary tumor virus
Meloy Labs	43207	Growth of mammalian Type C viruses for tissue culture/biochem. studies
Mich. Canc. Fdn.	33347	Replication of virus in cultures from human breast tumors
Micro. Assoc.	43254	Characterization, transmission studies of mammalian, avian tumor virus
Naval Biol. Res. Lab	40200	Studies of environmental factors influencing virus-host interactions
Ohio State Univ.	43217	Studies on factors affecting horizontal transmission of viruses
Penn. State Univ.	02024	Herpes-type virus replication in human and animal cells
Pfizer, Inc.	33239	Test systems for growth of putative mammary tumor viruses
Rush-Presbyterian	33219	Mammalian tumor virus infectivity in nonhuman primates
S. Calif., Univ. of	53500	Production of mammalian RNA tumor virus and candidate human agents
Southwest Fdn.	43214	Replication of Type C viruses isolated from simian and human placentas

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
St. Louis Univ.	43359	*In vitro* cultivation of various mammalian Type C tumor viruses
Tel Aviv Univ.	23237	Replication and infectivity of viruses from mammary cancer
Texas, Univ. of	33304	Isolation and characterization of candidate human Type C viruses

Step 2: INITIAL CHARACTERIZATION

Contractor	Cont. No.	Description of Work
A. Einstein Med. Coll.	33311	Immunological characterization of RNA tumor viruses
Baylor Univ.	33257	Comparative characterization of human Herpes-type viruses
Calif., Univ. of	33293	Comparative studies on simian leukemia/sarcoma viruses
Calif., Univ. of	33253	Characterization of murine MTV
Child. Hosp. (Phila)	33272	Immunological, tissue culture characterization of EBV
Columbia Univ.	33258	Biochemical characterization of mammalian Type C viruses
Cornell Univ.	33346	Immunological characterization of feline tumor virus isolates
Duke Univ.	33308	Immunological characterization of RNA tumor viruses
Flow Labs	43388	Immunological, tissue culture studies of mammalian RNA viruses
Flow Labs	43389	Immunological, tissue culture studies of mammalian DNA viruses
Hazleton Labs	33212	Characterization of ts mutants of RNA tumor viruses
Inst. Med. Res.	33339	Characterization of MTV and human milk derived particles
IARC	43296	Isolation and characterization of HSV in cultures of BL and NPC
Johns Hopkins Univ.	43266	Biochemical and immunological studies of papovaviruses
Karolinska Inst.	33316	Immunological and biochemical characterization of EBV
Med. Coll. Penn.	43319	Isolation and characterization of EBV-associated antigens
Meloy Labs	43207	Biochemical characterization of mammalian Type C viruses
Meloy Labs	43236	Immunological characterization of murine MTV and mammalian Type C
Meloy Labs	43223	Molecular studies of animal and human breast carcinomas
Mich. Canc. Fdn.	33347	Characterization of particles from human milk and breast tumors
Microbiol. Assoc.	43254	Immunological characterization of mammalian Type C tumor viruses
Neth. Canc. Inst.	33368	Characterization of MuMTV types
Penn. State Univ.	02024	Biological, biochemical, immunological studies of HSV transformed cells
Pfizer, Inc.	33239	Characterization of suspected oncogenic viruses

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
Rush-Presbyterian	33219	Characterization of Herpesviruses of nonhuman primates
Scripps Clinic	43375	Development and improvement of specific viral diagnostic reagents
Scripps Clinic	33204	Isolation and immunological characterization of viral antigens
S. Calif., Univ. of	53500	Immunological characterization of mammalian tumor viruses
Southwest Fdn.	43214	Characterization of viruses isolated from primate placentas
St. Louis Univ.	43359	Biochemical characterization of oncogenic RNA and DNA viruses
Texas, Univ. of	33304	Characterization of candidate human Type C oncogenic virus
Wisconsin, Univ. of	22022	Isolation and characterization of subunits of RNA tumor viruses
Wistar Institute	33250	Isolation and characterization of virus-induced tumor antigens

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

Contractor	Cont. No.	Description of Work
A. Einstein Coll. Med.	43380	Host range restriction of FLV
Atomic Energy Comm.	20208	Induction virus expression by cell fusion techniques
Baylor University	43355	Development of "nonsense" suppressor mutant cell lines
Baylor University	43385	Regulation of gene expression in murine mammary cancer
Calif. SDPH	43209	Attempt to rescue defective viral genome from tumors
Calif., Univ. of	33237	Culture human neoplastic tissue for induction of virus replication
Calif., Univ. of	33332	Effect of hormones on virus expression
Child. Canc. Res. Fdn.	43269	Induction of Type C virus from leukemic cells
Flow Labs	43388	Cell hybridization techniques to rescue "defective" viruses
Harvard Univ.	43299	Study permissivity of cells to polyoma mutants
Hazleton Labs	33212	Transmission and induction of oncogenic virus expression
Hopital St. Louis	33365	Induction and transmission of oncogenic virus expression in human cells
Institut du Radium	43219	Viral replication in synchronized cells
Litton-Bionetics	43249	Studies of virus expression and transmission
Mass. Gen. Hosp.	43222	Studies of activation of Type C viruses by immunological techniques
Med. Coll. Wisc.	81010	Co-cultivation of human breast cancer and hormone-secreting cell lines
Meloy Labs	43207	Virus rescue from non-producer transformed cells
Meloy Labs	43236	Effect of hormones on virus expression

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 (continued)

Contractor	Cont. No.	Description of Work
Mich. Canc. Fdn.	33347	Virus replication in human breast cancer cell lines
Micro. Assoc.	43240	Chemical carcinogens and Type C viral genome expression
Nether. Canc. Inst.	33368	Immunogenetic studies of breast cancer and leukemia
Penn. State Univ.	02024	Induction and maintenance of human Herpes-type virus oncogenicity
Radiobiol. Inst.	43328	Genetic and environmental control of RNA tumor virus expression
Rockefeller Univ.	33306	Rescue of viruses from human tumors
Salk Institute	43243	Studies on the activation of Type C virus genome by polyoma virus
S. Calif., Univ. of	53500	Studies of virus expression in human fetal and tumor tissue
S. Calif., Univ. of	43242	Methods for recognition and/or rescue of tumor virus expression
Stanford Univ.	43244	Methods to induce virus replication in human tumor cells
Tel Aviv Univ.	23237	Infectivity of particles from human milk and breast tumors
Texas, Univ. of	33304	Attempts to induce viral replication in human cell lines
Wisconsin, Univ. of	22022	Effect of chemical carcinogens on virus expression

Step 2: INITIAL CHARACTERIZATION

Contractor	Cont. No.	Description of Work
A. Einstein Coll. Med.	33311	Characterization of viral-specific enzymes and other proteins
Atomic Energy Comm.	20208	Biochemical studies on regulation of gene expression
Biolabs	22068	Characterization of HVS DNA
Calif., Univ. of	33293	Molecular studies of avian tumor-virus-associated enzymes
Calif., Univ. of	43298	Characterize polyoma and SV-40 mutants
Calif., Univ. of	43212	Structure of oncogenic viruses and charact. of viral-specific enzymes
Calif., Univ. of	33332	Growth regulatory mechanism in normal and neoplastic cells
Calif., Univ. of	33253	Characterization of MMTV replication in cell cultures
Columbia Univ.	33258	Biochemical characterization of viral-specific enzymes
Duke Univ.	33308	Biochemical and immunological characterization of viral proteins
Flow Labs	43388	Characterization of tumor virus expression in mammalian systems
Harvard Univ.	43299	Characterization of cell membrane changes in transformation
Hazleton Labs	33212	Cellular and subcellular alterations in viral transformation
Hopital St. Louis	33365	Biochemical characterization of viral enzymes and NA
Illinois, Univ. of	43318	Characterization of papovavirus proteins
Inst. Med. Res.	33339	Characterization of particles from human milk and breast tumors

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
Johns Hopkins Univ.	43266	Characterization of papovaviruses
Litton-Bionetics	43249	Characterization of viral-specific enzymes
Litton-Bionetics	33211	Characterization of viral-specific enzymes and nucleic acids
Mass. Gen. Hosp.	33366	Characterization of nucleic acids of AMV
Mass. Inst. Tech.	33348	Biochemical characterization of viral-specific enzymes
Meloy Labs	43207	Characterization of virus-expression and mediators of replication
Meloy Labs	22020	Characterization of virus-enhanced tumor transplantation antigens
Meloy Labs	43236	Characterization of viral-specific enzymes
Meloy Labs	43223	Characterization of mammary tumor viruses
Miami, Univ. of	43358	Characterization of virus and tumor-specific breast cancer
Mich. Canc. Fdn.	33347	Characterization of particles from human milk and breast tumors
Micro. Assoc.	43240	Immunological identification of antigens related to known tumor viruses
Minn., Univ. of	33357	Cell membrane changes following viral transformation
Nether. Canc. Inst.	33368	Characterization of MuMTV types
N. Carolina, Univ. of	33336	Biochemical identification of Herpes DNA viral genome in human cells
Rockefeller Univ.	33306	Cellular and subcellular alterations in malignant transformation
Scripps Clinic	43375	Immunochemical methods for detection of cell membrane changes
S. Calif., Univ. of	53500	Immunological identification of antigens related to known tumor viruses
S. Calif., Univ. of	43242	Methods for identification of virus-induced transformation
St. Louis Univ.	43359	Characterization: oncogenic virus expression, mediators of replication
Tel Aviv Univ.	23237	Biochemical identification of subviral expression in breast cancer
Texas, Univ. of	43370	Characterization of virus and tumor-specific breast cancer antigens
Wisc., Univ. of	22022	Detection of virus expression in chemically-induced tumors

Phase III-A: COMPLETE CHARACTERIZATION AND DEFINITION OF PRESUMPTIVE DISEASE RELATIONSHIPS
Step 1: PRESUMPTIVE DISEASE RELATIONSHIPS

Contractor	Cont. No.	Description of Work
Alabama, Univ. of	43394	Relationship of fetal antigens to virus-induced tumors
A. Einstein Coll. Med.	33311	Relationship of Type C viruses to human cancer
A. Einstein Coll. Med.	33249	Genetic studies on tumor/virus susceptibility
Atomic Energy Comm.	20208	Interaction of RNA tumor viruses and host immune mechanism

TABLE II Analysis of Contracts by Activity

Phase III-A: COMPLETE CHARACTERIZATION AND DEFINITION OF PRESUMPTIVE DISEASE RELATIONSHIPS
Step 1: PRESUMPTIVE DISEASE RELATIONSHIPS (continued)

Contractor	Cont. No.	Description of Work
Baylor Univ.	33257	Studies on presumptive disease relationships of HTV
Child. Hosp. (Phila)	33272	Relationship of EBV to human lymphoma
Columbia Univ.	33258	Biochemical studies on relationship of Type C and Type B
Delta Reg. Pri. Ctr.	33396	Determination of response of natural host to Herpesvirus saimiri
Duke Univ.	33308	Relationship of Type C viruses to human cancer
Duke Univ.	43395	Relationship of fetal antigens to virus induced tumors
Emory Univ.	22301	Determination of host response to HSV in cervical cancer
Emory Univ.	43393	Immunological studies of HSV-2 in cervical cancer
Flow Labs	43388	Characterization of RNA viruses/viral antigens in mammalian tumors
Flow Labs	43389	Characterization of DNA viruses/viral antigens in mammalian tumors
Georgetown Univ.	50053	Studies on Type B particles associated with human breast cancer
George Wash. Univ.	23251	Cell-mediated immunity to human cancers
Harvard Univ.	33390	HSV and oncogenic disease in primates
Hebrew Univ.	33342	Epidemiological studies of Hodgkin's disease
Illinois, Univ. of	43318	Relationship of papovaviruses to human cancer
IARC	43296	Seroepidemiological studies of Burkitt's lymphoma, NPC
Inst. Med. Res.	33339	Studies on Type B particles associated with human breast cancer
Jackson Labs	33255	Host gene control of RNA tumor virus expression
Johns Hopkins Univ.	33345	Relationships of HSV-2 to cervical cancer
Johns Hopkins Univ.	43266	Relationship of papovaviruses to human cancer
Johns Hopkins Univ.	43330	Immunologic studies of HSV-2 in cervical cancer
Karolinska Inst.	33316	Immunologic studies on the etiology of EBV-associated diseases
Life Sciences	33205	Studies on Marek's disease herpesvirus
Litton-Bionetics	33211	Relationship of Type C viruses to human leukemia
Litton-Bionetics	43249	Relationship of Type C viruses to induction/maintenance of oncogenesis
Litton-Bionetics	43224	Relationship of herpes and other viruses to cancer in primates
Litton-Bionetics	23294	Immunological, biological studies of HSV-related oncogenic diseases
Mason Research Inst.	33358	Oncogenic potential of M-PMV
Meloy Labs	43207	Biochemical studies on relationship of Type C viruses to human cancer
Meloy Labs	43236	Relationship of Type B viruses to breast cancer in animals and man
Meloy Labs	43223	Studies on Type B particles associated with breast cancer

TABLE II Analysis of Contracts by Activity

Phase III-A: COMPLETE CHARACTERIZATION AND DEFINITION OF PRESUMPTIVE DISEASE RELATIONSHIPS
Step 1: PRESUMPTIVE DISEASE RELATIONSHIP (continued)

Contractor	Cont. No.	Description of Work
Mich. Canc. Fdn.	33347	Studies on "viruses" in human milk and breast tumors
Microbiol. Assoc.	43240	Evaluation of cocarcinogenic factors in viral oncogenesis
Microbiol. Assoc.	33248	Type C virus expression in embryogenesis and spontaneous cancers
Minnesota, Univ. of	33357	Immunologic evaluation of host response to human tumors
Naples, Univ. of	33314	Isolation and characterization of HSV-induced antigens
New York Med. Coll.	33398	Immunopathology of human breast cancer
Ohio State Univ.	43217	Immune response to viral antigens in model systems and man
Rush-Presby. Hosp.	33219	Oncogenic potential of selected Type C and herpesviruses
S. Calif., Univ. of	53500	Possible role of animal tumor viruses, environmental cocarcinogens
St. Louis Univ.	43359	Relationship of Type C viruses to human and animal cancer
Tennesse, Univ. of	43325	Immunological studies on fetal antigens
Texas, Univ. of	33292	Immunological studies on host reaction to tumor antigens
Texas, Univ. of	33301	Reaction to murine leukemia virus antigens in human cancer patients
UICC	43292	Epidemiological studies of human lymphomas
Washington, Univ. of	33372	Immunologic reactivity to tumor antigens in patients with cancer
Washington, Univ. of	33236	Immunologic reactivity to canine sarcomas
Wisconsin, Univ. of	22022	Detection and quantitation of immunity to oncogenic viruses/antigens

Step 2: COMPLETE CHARACTERIZATION

Contractor	Cont. No.	Description of Work
A. Einstein Med. Coll.	33311	Biochemical characterization of oncogenic viruses
Duke Univ.	33308	Biochemical characterization of oncogenic viruses
Flow Labs	43388	Characterization of oncogenic viruses
Harvard Univ.	33265	Biochemical characterization of Type C avian virus proteins
Hebrew Univ.	33310	Biochemical, biophysical characterization of EBV
Johns Hopkins Univ.	43266	Characterization of papovaviruses
Karolinska Inst.	33316	Immunological characterization of EBV
Life Sciences	33205	Biological characterization of MDHV
Mass. Gen. Hosp.	33366	Biochemical characterization of avian Type C virus nucleic acids
Meloy Labs	43207	Biochemical, biophysical, immunologic characterization-Type C viruses
Meloy Labs	43236	Biochemical, biophysical, immunologic characterization-Type B viruses

TABLE II Analysis of Contracts by Activity

Phase III-B: COMPLETE CHARACTERIZATION: DEMONSTRATION OF VIRUS-MEDIATED FUNCTIONS ESSENTIAL FOR INDUCTION AND MAINTENANCE OF NEOPLASIA

Contractor	Cont. No.	Description of Work
A. Einstein Coll. Med.	33311	Determine molecular pathways of oncogenic virus expression
Baylor Coll. Med.	43385	Regulation of gene expression in mouse mammary cancer
Calif., Univ. of	33293	Biochemical determination of viral gene expression
Calif., Univ. of	33283	Biochemical characterization of viral gene expression
Columbia Univ.	33258	Search for specific viral gene expressions in human cancer
Duke Univ.	33308	Biochemical characterization of viral gene expression
Hebrew Univ.	33310	Search for EBV gene expression in human cancer
Hopital St. Louis	33365	Search for viral gene expressions in human leukemia
Johns Hopkins Univ.	43266	Molecular hybridization using papovavirus probes
Life Sciences, Inc.	33205	Co-carcinogenic factors in the etiology of Marek's disease
Litton-Bionetics	33211	Biochemical markers of Type C virus gene expressions in human cancer
Mass. Inst. Tech.	33348	Determine the nature of oncogenic viral gene expression
Meloy Labs	43207	Molecular hybridization studies on human cancers
Meloy Labs	43236	Molecular hybridization studies on human cancer
Nether. Canc. Inst.	33368	Immunogenetic studies on breast cancer and leukemia
N. Carolina, Univ. of	33336	Molecular hybridization studies of human lymphoma, cervical carcinoma
Radiobiol. Inst.	43328	Genetic control of release of RNA tumor viruses
Salk Institute	43243	Viral gene expression in the induction and maintenance of oncogenesis
S. Calif., Univ. of	53500	Biological and biochemical determinations of viral gene expressions
St. Louis Univ.	43359	Search for specific viral gene expressions in human cancer

Phase IV-A: IMMUNOLOGICAL CONTROL
Step 1: DETERMINE SUITABLE IMMUNOLOGICAL CONTROL

Contractor	Cont. No.	Description of Work
Atomic Energy Comm.	20208	Effect of poly(Am) in boosting immune response
Flow Labs	43388	Vaccines against natural mouse Type C viruses
Inst. Med. Res.	33339	Immunological studies of murine mammary cancer
Meloy Labs	22020	Evaluation of various approaches to immunotherapy in model systems
Microbiol. Assoc.	43240	Viral vaccines/interferons in protection against chemical carcinogens
Microbiol. Assoc.	33248	Immunoprevention of spontaneous neoplasms
Merck and Co.	12059	Developmental research for virus vaccine production

TABLE II Analysis of Contracts by Activity

Phase IV-A: IMMUNOLOGICAL CONTROL
Step 1: DETERMINE SUITABLE IMMUNOLOGICAL CONTROL (continued)

Contractor	Cont. No.	Description of Work
Mt. Sinai Hosp.	43225	Enhancement of tumor cell immunogenicity by neuraminidase
Ohio State Univ.	43217	Development and evaluation of Type C feline viral vaccines
Radiobiol. Inst.	43328	Immunological studies of murine mammary cancer
S. Calif., Univ. of	53500	Wild mouse endogenous virus vaccines and antisera

Phase IV-B: BIOCHEMICAL CONTROL
Step 1: DETERMINE SUITABLE METHODS FOR BIOCHEMICAL CONTROL

Contractor	Cont. No.	Description of Work
Atomic Energy Comm.	20208	Poly A inhibition of viral polymerases
Litton-Bionetics	23294	Immuno-chemotherapy for treatment/prevention of viral-induced tumors
St. Louis Univ.	43359	Screening of various chemicals as inhibitors of viral polymerases

RESOURCES

Contractor	Number	Type	Species
California, Univ. of	33237	cell cultures	human, animal
California, Univ. of	72-2202	animal	feline
Chicago Park District	33271	animal	primate
Child Research Center	33333	service	cell cultures
Colorado, Univ. of	33400	tissues	human
Connecticut, Univ. of	33221	animal	avian
Cornell University	02224	service	feline
Dow Chemical Co.	33243	service	biohazards
Duke University	33308	virus	avian
Electronucleonics Labs., Inc.	23249	virus	human, animal
Electronucleonics Labs., Inc.	33335	virus, cells	human, animal
Electronucleonics Labs., Inc.	43334	virus	human, animal
Emory University	33343	animal	primate
Flow Laboratories, Inc.	33391	animal	rodent
Flow Laboratories, Inc.	33201	repository, service	sera, tissues, reagents, virus

RESOURCES (Cont'd)

Contractor	Number	Type	Species
Georgetown University	33404	tissues	human
Goodwin Institute	23261	animal	avian, rodent
Health Research, Inc.	23247	tissues	human
Hektoen Inst. Med. Res.	43344	tissues	human
Hospital for Sick Children	23266	tissues	human
Huntingdon Research Center	33223	antisera	animal
Illinois, University of	43345	tissues	human
Jewish Hospital & Med. Ctr.	43251	tissues	human
Johns Hopkins Hospital	33245	tissues	human
Life Sciences, Inc.	33210	animal	avian, murine
Life Sciences, Inc.	33291	virus	avian
Litton Bionetics, Inc.	43224	animal	primate
Litton Bionetics, Inc.	43260	tissues	human
Louisville, University of	60902	virus	primate
Meloy Laboratories	43263	virus	murine
Memorial Hospital	43335	tissues	human
Michigan Cancer Foundation	43391	milk	human
Michigan, University of	33224	tissues	human
Microbiological Associates	60914	animal	murine
Microbiological Associates	33288	service	murine, feline
Minnesota, University of	43285	service	biohazards
Montreal Children's Hospital	33377	tissues	human
Naval Biomedical Res. Lab	40201	service	facility support
Naval Biomedical Res. Lab	40200	service	biohazards
Padua, University of	33359	tissues	human
Pfizer, Inc.	33234	virus	animal, human
Southwest Foundation Res. & Ed.	33340	animal	primate
St. Joseph's Hospital	33393	tissues	human
University Laboratories	33222	virus	avian, murine
Wolf Research & Development	43351	service	computer support

TABLE III

ALPHABETICAL LISTING OF CONTRACTS
AND INDEX TO CONTRACT NARRATIVES

CONTRACTOR	FUNCTIONS[1]	PAGE
Aichi Cancer Center (33290)	I (2)	218
Alabama, Univ. of (43394)	I (3); III-A (1)	New[2]
A. Einstein Med. Coll. (33249)	III-A (1)	294
A. Einstein Med. Coll. (33311)	II-A (2); II-B (2); III-A (1, 2); III-B	183
A. Einstein Med. Coll. (43380)	II-B (1)	New
American Cancer Soc. (74152)	Conference	-
Atomic Energy Comm. (43210)	I (3)	219
Atomic Energy Comm. (20208)	I (3); II-B (1, 2); III-A (1); IV-A (1); IV-B (1)	341
Baylor Coll. Med. (43355)	II-B (1)	369
Baylor Univ. (43385)	II-B (1); III-B	New
Baylor Univ. (33257)	I (2, 3); II-A (1, 2); III-A (1)	177
Biolabs, Inc. (22068)	II-A (1); II-B (2); Resources	343
Biotech Res. Labs (43365)	I (1)	New

1/ For description of research activities, see SVCP Convergence Chart and TABLE II.
2/ No narrative provided for newly negotiated contracts.

TABLE III (continued)

CONTRACTOR	FUNCTIONS	PAGE
Boston Hosp. Women (43379)	I (1, 2, 3)	New
Calif. SDPH (43209)	I (3); II-A (1); II-B (1)	289
Calif. Inst. Tech. (43306)	I (3)	New
Calif., Univ. of (33237)	II-A (1); II-B (1); Resources	378
Calif., Univ. of (33242)	I (3); II-A (1, 2)	290
Calif., Univ. of (33293)	II-B (2); III-B	292
Calif., Univ. of (43212)	II-B (2); III-B	355
Calif., Univ. of (43211)	I (1, 2, 3)	222
Calif., Univ. of (43381)	I (3)	New
Calif., Univ. of (43298)	II-B (2)	New
Calif., Univ. of (33253)	II-A (1, 2); II-B (2)	154
Calif., Univ. of (33283)	I (3)	358
Calif., Univ. of (33332)	II-A (1); II-B (1, 2)	356
Center for Disease Cont. (40202)	I (1, 2)	224
Chicago Park District (33271)	Resources	380
Child. Canc. Res. Fdn. (43269)	I (2); II-B (1)	New

TABLE III (continued)

CONTRACTOR	FUNCTIONS	PAGE
Child Res. Center of Mich. (33333)	Resources	381
Children's Hosp. of Phila. (33272)	I (1, 3); II-A (2); III-A (1)	226
Colorado, Univ. of (33400)	Resources	382
Columbia University (33258)	I (3); II-A (2); II-B (2); III-A (1); III-B	178
Connecticut, Univ. of (33221)	Resources	383
Cornell University (02224)	Resources	384
Cornell University (33346)	I (3); II-A (1, 2)	180
Delta Reg. Primate Center (33396)	III-A (1)	261
Dow Chemical Co. (33243)	Resources	145
Duke University (33308)	II-A (2); II-B (2); III-A (1, 2); III-B; Resources	182
Duke University (43395)	I (3); III-A (1)	New
Electronucleonics Labs, Inc. (33355)	Resources	385
Electronucleonics Labs, Inc. (23249)	Resources	386
Electronucleonics Labs, Inc. (43334)	Resources	New
Emory University (33343)	Resources	387
Emory University (22301)	III-A (1)	202

TABLE III (continued)

CONTRACTOR	FUNCTIONS	PAGE
Emory University (43393)	I (3); III-A (1)	New
Flow Labs, Inc. (33201)	Resources	388
Flow Labs, Inc. (33247)	Split into 43387, 43388, 43389 at end of FY	296
(43387)	II-A (1)	
(43388)	I (3); II-A (2); II-B (1, 2); III-A (1, 2); IV-A (1)	
(43389)	I (3); II-A (2); III-A (1)	
Flow Labs, Inc. (33391)	Resources	389
Fort Detrick (20207)	Housekeeping service for 23294 (Litton-Bionetics)	-
George Washington Univ. (23251)	I (2); III-A (1)	230
Georgetown Univ. (50053)	I (1, 2, 3); III-A (1)	156
Georgetown Univ. (33404)	Resources	390
Goodwin Inst. for Canc. Res. (23261)	Resources	391
Harvard Sch. Pub. Health (43218)	Biohazard resource	147
Harvard University (33265)	III-A (2)	299
Harvard University (33390)	I (2, 3); II-A (1); III-A (1)	371
Harvard University (43299)	II-B (1, 2)	New
Hazleton Laboratories, Inc. (33212)	I (3); II-A (1, 2); II-B (1, 2)	301

TABLE III (continued)

CONTRACTOR	FUNCTIONS	PAGE
Health Research, Inc. (43392)	Resources	392
Hebrew University (33342)	I (1, 2, 3); III-A (1)	232
Hebrew University (33310)	I (3); III-A (2); III-B	345
Hoektoen Inst. Med. Res. (43344)	Resources	New
Hopital St. Louis (33365)	II-B (1, 2); III-B	185
Hospital for Sick Children (23266)	Resources	393
Howard University (43287)	I (1, 3)	New
Huntingdon Res. Ctr. (33223)	Resources	394
Illinois, Univ. of (43318)	I (3); II-A (1); II-B (2); III-A (1)	360
Illinois, Univ. of (43345)	Resources	New
Institut du Radium (43219)	II-B (1)	344
Inst. for Med. Res. (33339)	I (1, 2, 3); II-A (2); II-B (2); III-A (1) IV-A (1)	158
Int'l. Agency for Res. on Cancer (43296)	I (1, 2, 3); II-A (1, 2); III-A (1)	234
Int. Assoc. for Comp. Res. on Leukemia (40047)	Conference	-
Jackson Laboratory (33255)	I (3); III-A (1)	303

TABLE III (continued)

CONTRACTOR	FUNCTIONS	PAGE
Jewish Hosp. & Med. Center (43251)	Resources	395
Johns Hopkins Univ. (43330)	I (3); III-A (1)	New
Johns Hopkins Univ. (33337)	I (1, 2, 3)	237
Johns Hopkins Univ. (33245)	I (2); Resources	396
Johns Hopkins Univ. (43266)	I (1, 2); II-A (1, 2); II-B (2); III-A (1, 2); III-B	351
Johns Hopkins Univ. (33345)	I (1, 2, 3); III-A (1)	187
Karolinska Institute (33316)	I (1, 2, 3); II-A (1, 2); III-A (1, 2)	188
Life Sciences (33291)	Resources; II-A (1)	398
Life Sciences (33210)	Resources	397
Life Sciences (33205)	III-A (1, 2); III-B	190
Litton Bionetics, Inc. (43249)	I (1, 3); II-B (1, 2); III-A (1)	346
Litton Bionetics, Inc. (43333)	I (3)	New
Litton Bionetics, Inc. (43252)	I (3)	240
Litton Bionetics, Inc. (43224)	I (3); II-A (1); III-A (1); Resources	399
Litton Bionetics, Inc. (33211)	II-B (2); III-B; III-A (1)	192
Litton Bionetics, Inc. (23294)	II-A (1); III (A)-1; IV-B (1)	271

TABLE III (continued)

CONTRACTOR	FUNCTIONS	PAGE
Litton Bionetics, Inc. (43260)	Resources	401
Louisville, Univ. of (60902)	Resources	402
Mason Research Inst. (33358)	III-A (1)	159
Mass. General Hosp. (33366)	II-B (2); III-A (2)	194
Mass. General Hosp. (43222)	II-B (1)	195
Mass. Inst. Tech. (33348)	I (3); II-B (2); III-B	349
Med. Coll. of Penna. (43319)	II-A (2)	New
Med. Coll. of Wisc. (81010)	I (3); II-A (1); II-B (1)	161
Meloy Labs., Inc. (43207)	I (3); II-A (1, 2); II-B (1, 2); III-A (1, 2); III-B	372
Meloy Labs, Inc. (22020)	II-B (2); IV-A (1)	197
Meloy Labs, Inc. (43223)	I (3); II-A (2); II-B (2); III-A (1)	168
Meloy Labs, Inc. (22306)	Resource for Emory Univ. (22301)	202
Meloy Labs, Inc. (43263)	Resources	403
Meloy Labs, Inc. (43236)	I (3); II-A (1, 2); II-B (1, 2); III-A (1, 2) III-B	352
Memorial Hosp. (N. Y.) (43208)	I (2)	162
Memorial Hosp. (N. Y.) (43335)	Resources	405

TABLE III (continued)

CONTRACTOR	FUNCTIONS	PAGE
Merck & Company, Inc. (12059)	IV-A (1)	198
Miami, Univ. of (33218)	II-A (2); II-B (2)	243
Miami, Univ. of (43358)	II-B (2)	New
Michigan Canc. Fdn. (33347)	I (1, 2, 3); II-A (1, 2); II-B (1, 2); III-A (1)	163
Michigan Canc. Fdn. (43391)	Resources	New
Michigan, Univ. of (33224)	Resources	406
Micro. Assoc. Inc. (60914)	Resources	406
Micro. Assoc. Inc. (33248)	I (3); III-A (1); IV-A (1)	314
Micro. Assoc. Inc. (33288)	Resources	407
Micro. Assoc. Inc. (43240)	I (3); II-A (1, 2); II-B (1, 2); III-A (1); IV-A (1)	305
Micro. Assoc. Inc. (43254)	II-A (1, 2)	311
Minnesota, Univ. of (33357)	I (1, 2, 3); II-B (2); III-A (1)	364
Minnesota, Univ. of (43285)	Resources	149
Montreal Child. Hosp. (33377)	Resources	409
Mt. Sinai Hosp. (43225)	I (2); IV-A (1)	245

TABLE III (continued)

CONTRACTOR	FUNCTIONS	PAGE
Naples, Univ. of (33314)	III-A (1)	200
Naval Biological Res. Labs (40200)	II-A (1), Service	140
Naval Biomedical Res. Labs (40201)	Service	378
Netherlands Canc. Inst. (33368)	I (3); II-A (2); II-B (1, 2); III-B	165
N. Y. Med. College (33398)	I (2); III-A (1)	248
N. Carolina, Univ. of (33336)	II-B (2); III-B	203
Ohio State Univ. (43217)	I (3); II-A (1); III-A (1); IV-A (1)	139
Padua, Univ. of (33359)	Resources	409
Penn. State Univ. (02024)	I (3); II-A (1, 2); II-B (1)	204
Pfizer, Inc. (33239)	I (3); II-A (1, 2)	167
Pfizer, Inc. (33234)	Resources	410
Radiobiological Inst. (43328)	I (3); II-B (1); III-B; IV-A (1)	New
Rockefeller Univ. (33306)	I (3); II-B (1, 2)	206
Rush-Presbyterian Hosp. (33219)	I (3); II-A (1, 2); III-A (1)	208
Salk Institute (43243)	II-B (1); III-B	318
Scripps Clinic & Res. Fdn. (43375)	I (3); II-A (2); II-B (2)	319

TABLE III (continued)

CONTRACTOR	FUNCTIONS	PAGE
Scripps Clinic & Res. Fdn. (33204)	I (3); II-A (2)	252
S. Calif., Univ. of (53500)	I (1, 2, 3); II-A (1, 2); II-B (1, 2); III-A (1); III-B; IV-A (1)	325
S. Calif., Univ. of (43242)	II-B (1, 2); III-B	328
Southwest Fdn. (33340)	Resources	413
Southwest Fdn. (43214)	I (3); II-A (1, 2)	143
St. Joseph's Hosp. (33393)	Resources	412
St. Louis Univ. (43359)	I (3); II-A (1, 2); II-B (2); III-A (1); III-B; IV-B (1)	316
Stanford Univ. (43228)	III-A (1)	New
Stanford Univ. (43244)	I (3); II-B (1); Resources	329
Tel Aviv Univ. (23237)	I (3); II-A (1); II-B (1, 2)	169
Tennessee, Univ. of (43325)	I (3); III-A (1)	New
Texas, Univ. of (33304)	I (1, 2, 3); II-A (1, 2); II-B (1, 2)	210
Texas, Univ. of (33292)	I (3); III-A (1)	254
Texas, Univ. of (43370)	II-B (2)	New
Texas, Univ. of (33301)	I (2); III-A (1); IV-A (1)	256

TABLE III (continued)

CONTRACTOR	FUNCTIONS	PAGE
University Labs, Inc. (33222)	Resources	414
UICC (43292)	III-A (1)	New
Washington, Univ. of (33372)	I (3); III-A (1)	330
Washington, Univ. of (33236)	I (3); III-A (1)	263
Weizmann Institute (43241)	II-B (2)	331
Wisconsin, Univ. of (22022)	I (3); II-A (2); II-B (1,2); III-A (1)	367
Wistar Institute (33250)	I (3); II-A (2)	333
Wolf Research & Dev. (43351)	Service	416

D. Contract Summaries by Segments

1. BIOHAZARD CONTROL AND CONTAINMENT SEGMENT
July 1, 1973 - June 30, 1974

Dr. Alfred Hellman, OADVO, Division of Cancer Cause and Prevention, Chairman
Dr. W. Emmett Barkley, OADVO, Division of Cancer Cause and Prevention, Vice Chairman

The contract programs administered by the Biohazard Control and Containment Segment are designed to identify potential biohazards which may be associated with facilities, equipment and procedures used by virus cancer investigators and to provide guidance and technical assistance to these investigators in order to improve their capacity for performing research with minimum hazards to the laboratory worker and the community and maximum protection against contamination. A major contract effort with the Dow Chemical Company has provided environmental control and laboratory safety services to all contractors of the VCP. These services include conducting surveys of laboratory facilities to identify potential biohazards and to recommend practical corrective actions, designing equipment and developing improved procedures for the safe handling of oncogenic viruses, certifying safety equipment and assisting in the design of laboratory facilities. The equipment certification service has demonstrated that over 60 percent of all laminar flow biological safety cabinets tested to date failed to meet performance specifications and had to be modified in order to provide adequate safety for the user.

A basic safety training course on the principles of biohazard and injury control has been developed through a contract with the University of Minnesota. This course is presented four times annually to laboratory workers engaged in cancer virus research. These courses provide an opportunity to investigators and technicians to learn the fundamentals of laboratory safety including the correct usage of biological safety hoods, personnel protective devices, disinfection and sterilization techniques and the principles of biological, physical, chemical and radiological hazard control.

An evaluation of potential aerosol exposure hazards created by biochemical and biophysical procedures used in virus-tissue culture laboratories has been conducted by the Naval Biological Laboratory. Tests have been performed to quantitate the aerosol output of the blender, sonic homogenizer, pipetting procedures and the zonal centrifuge. Results of these tests demonstrated that the blender, sonic homogenizer and pipetting procedures are capable of producing detectable aerosols which can be inhaled by the laboratory worker.

Basic research studies are in progress to: (1) evaluate the effect of a selected stress situation on induction of viral disease or cancer in situ, (2) investigate the immunological response of laboratory animal hosts to oncornaviral antigens and (3) elucidate the role of endogenous virus infection of laboratory animals. Aerosol exposure of mice to selected environmental insecticides which mimic estrogenic activity induces the

expression of information found in murine leukemia virus. Studies initiated at the Southwest Foundation for Research and Education to determine if oncornaviruses could pass the placental barrier demonstrated the presence of C-type virus particles in the syncytiotrophoblast. This observation is being pursued in detail.

OHIO STATE UNIVERSITY (NO1-CP-43217)

Title: Biohazard Control and Containment in Oncogenic Virus Research

Contractor's Project Director: Dr. D. Yohn

Project Officers (NCI): Dr. A. Hellman
Dr. A. K. Fowler

Objectives:

The principal objective of this program this past year has been to continue the evaluation of prophylactic vaccination of cats against RNA tumor virus oncogenesis. Several regimens and protocols have been employed. For the sake of completeness, this report will summarize the results of all completed protocols as well as those that are still ongoing.

As corollary objectives these studies have provided an opportunity to evaluate the immunologic responses of kittens and adult cats to the various vaccines using several different serologic procedures. These include fluorescent antibody (FA) tests for FOCMA antibodies, complement fixation (CF) and complement fixation inhibition (CFI) tests for antibodies to viral structural antigens and neutralizing antibody (NA) tests to viral membrane antigens. A lymphocyte blast transformation (LBT) assay has been developed to study cell mediated immunity during immunization and oncogenesis.

The final aspect of this program has included pilot studies on the effect of various steroid hormones on infection and/or transformation of various cell cultures by feline sarcoma virus (FSV).

Major Findings:

Vaccination of kittens with killed virus vaccines at weekly intervals for 4 weeks prior to challenge at 35 days of age with a dose of GA-FSV known to induce tumors in 95% of unimmunized kittens has given varying results. Vaccines prepared in Adjuvant 65 have induced no protection and only minimal antibody responses as measured by CFI and NA tests. Vaccines prepared in complete Freunds adjuvant appear to be inducing some degree of protection. Antibody studies are yet to be completed in these animals. To date four kittens developed a persistent neutralizing titer approximately one month after four injections. Two others had only a single serum that was reactive. In another litter kittens readily demonstrated neutralizing antibodies within two weeks after the final injection of vaccine. The variability of these responses in which at best 3/6 produced neutralizing antibodies by the time of virus challenge is interpreted at this point as an unfavorable level of response.

Tests on additional kittens which have been similarly immunized including those immunized with virus emulsified in Freunds complete adjuvant are underway. Sera taken from kittens solidly protected by immunization with inactivated tumor extracts prior to challenge with virus are also being examined for neutralizing antibodies. These tests should be completed within the current contract year.

Results of tests for neutralizing antibodies in seven adult female cats, six of which were immunized during their pregnancy with UV irradiated GA-FSV emulsified in adjuvant 65. These tests show that this immunization regimen induced readily demonstrable levels of neutralizing antibodies in all six pregnant queens by the time they gave birth. These findings may in part account for the moderate protection afforded kittens to GA-FSV from these queens. Of nine kittens from these queens, only four developed tumors which eventually led to their death.

Significance to Biomedical Research and the Program of the Institute:

Development of a vaccine against a type-C RNA virus in a model system such as the cat could provide some rationale as to the potentials for developing such vaccines against human disease if ever a viral induced malignancy can be identified in man.

Proposed Course:

The presence of neutralizing antisera in cats previously immunized will further be determined. In order to permit a greater degree of immunological competency to develop prior to virus challenge, a feline leukemia virus isolated by Dr. Hardy, will be used. This virus will cause disease in adult cats, thereby permitting adequate vaccination regimes to be developed, since other feline tumor virus to date required inoculation into cats during the first week of life.

Date Contract Initiated: June 25, 1965

NAVAL BIOLOGICAL LABORATORY (Y01-CP4-0200)

Title: Aerosol Properties of Potentially Oncogenic Viruses

Contractor's Project Director: Dr. N. Vedros

Project Officers (NCI): Dr. A. Hellman
Dr. A. K. Fowler
Dr. W. E. Barkley

Objectives:

This project involves task-areas as well as providing hood-testing service to certain other NCI contractors. The task areas are: laboratory hazards from aerosols; environmental effects on characteristics of viral aerosols; host-virus interactions; disinfectants.

Major Findings:

1. Aerosol Studies. Aerosols of Rauscher murine leukemia virus (RMLV) were exposed to relative humidities (RD) ranging from 10% to 90% at 26°C. Impinger samples were taken at time intervals through 4 hours post-atomization, and quantification of recovered virus was accomplished using the XC cell assay system.

Survival at conditions of low relative humidity is better than at higher relative humidities. At humidities below 30%, virus could be recovered from aerosols exposed to environmental conditions for 4 hours. The most substantial virus inactivation occurs during the initial drying process leading to the equilibrium state. It appears that the slower this initial drying process is (i.e., at high RH), the more detrimental its effects on the virus.

It is known that various additives (i.e. sugars) can protect aerosolized virus from the effects of relative humidity. All the current studies were performed using standard tissue culture media for aerosolization of the virus. It is also known that certain viruses, when drawn through a vessel maintained at 100% relative humidity, immediately before impinging, show markedly enhanced recovery from the aerosol state. Our initial studies indicate that RMLV does not display this phenomenon.

2. Disinfection Studies. After the initial application of 0.5 ml of each concentration of hypochlorite into 4.5 ml of unpurified poliovirus or Rauscher Murine leukemia virus tissue culture suspension, samples were collected at 10, 20 and 40 minute intervals. The virus samples were rapidly diluted with Hanks Balance salt solution (to avoid the inherent toxicity of hypochlorite for cells) and were then inoculated into either HeLa cell monolayer for plaque assay for poliovirus, or A31 mouse cell monolayer for leukemia virus assay. All experiments were carried out at room temperature.

A preliminary experiment indicated that 0.2 ml of 1000 part per million (PPM) solution of a neutralized hypochlorite (NHC) could be added to a 60 mm in diameter tissue culture dish with 5 ml of medium without killing the contained culture of HeLa cells. Therefore, assay at low dilution levels was possible.

The residual hypochlorite was rapidly decreased after neutralized with KH_2PO_4 to a pH of 7.4 and after 24 hours, only 2% of their initial residual was remaining in the open container. However, the un-neutralized hypochlorite was very stable, even in the open container.

The results of hypochlorite experiment with unpurified poliovirus in tissue culture menstrum indicate that the poliovirus titer decreased gradually with a neutralized hypochlorite solution of 1000 ppm and completely inactivated within 40 minutes upon application. On the other hand, low concentration of hypochlorite (100 ppm) has no effect on poliovirus. However, with higher concentration of neutralized hypochlorite (2500 ppm) poliovirus could be completely inactivated within the first 10 minutes upon application. Furthermore, the data also indicated that the un-neutralized hypochlorite had more effect on poliovirus. When 10% fetal calf serum was added into the virus suspension, the virucidal activity of hypochlorite was decreased.

Neutralized hypochlorite was held in either an open or closed container and samples were withdrawn and used to inactivate poliovirus at 4, 8, 24 and 48 hours after hypochlorite was neutralized with KH_2PO_4, to a pH of 7.4. The data indicated that the poliovirus titer decreased slowly from 1.8×10^8 pfu/ml to 1.4×10^4 pfu/ml, and from 1.8×10^8 to 1.4×10^6 pfu/ml in 40 minutes with a neutralized hypochlorite solution of 1000 ppm of 4 and 8 hour samples, respectively. Moreover, the poliovirus titer decreased more rapidly from 1.8×10^8 to 1.5×10^4 pfu/ml within the first 10 minutes and reduced to $< 5 \times 10^1$ in 20 minutes with a neutralized hypochlorite solution of 2500 ppm of the 8 hours sample. However, there were no virus titers changed in both 24 and 48 hour samples.

Wescodyne and Lysol were also used to inactivate poliovirus in order to compare their virucidal activity. Neither were virucidal.

Rauscher Murine Leukemia virus (RML) was used to test the virucidal activity of hypochlorite. Rauscher leukemia virus was rapidly inactivated by low concentrations of neutralized hypochlorite.

Virus titer was reduced rapidly from 2×10^7 pfu/ml to 2×10^2 pfu/ml in 10 minutes with a neutralized hypochlorite solution of 500 ppm, and completely inactivated in 20 and 40 minutes with 500 and 100 ppm of neutralized hypochlorite, respectively. However, in the presence of fetal calf serum in the leukemia virus suspension, the virus titer decreased gradually and infectious virus was present in the suspension at both the 500 and 100 ppm hypochlorite solution after 40 minutes of inactivation.

Herpes saimiri will next be included in disinfectant tests in preparation for studies of aerosol survival of the latter agent.

3. Laboratory Hazards. Spray factors, useful in determining the probable dosage involved in respiratory exposure to incidental aerosols produced by standard laboratory operations, have been reported previously.

The next step under this task area was to study the extent of inclusion of viral units in airborne particles less than 0.8 µm. This work has been delayed because we have not received the aerosol sampler (centrifuge rotor) that is needed to collect small particles; the unit has been on order for more than 1 year. The item is "hand-made", must be calibrated during final balancing tests, and the manufacturer repeatedly assures us that after a few more tests the rotor will be available.

Significance to Biomedical Research and the Program of the Institute:

An understanding of the survival and clearance rate for virus aerosol will allow the development of a rational and hopefully less expensive means for control of cross infection. Similarly development of mathematical models will permit the more precise evaluation of facilities, on the basis of risk.

Proposed Course:

We propose to continue studies of specific problems in control of laboratory biohazards generated in work with oncogenic viruses and infected tissue cultures. We will study synergistic effects of selected chemicals and viruses on oncogenic susceptibility, and the effect of physiological stress (as related to changes in immune response) on susceptibility. As adjuncts to this work we will study survival of selected airborne viruses in varied environments and optimize methods of assay.

Date Contract Initiated: March 1, 1971

SOUTHWEST FOUNDATION FOR RESEARCH AND EDUCATION (NO1-CP4-3214)

Title: Study of Latent Virus Infection and Transmission

Contractor's Project Director: Dr. R. L. Heberling

Project Officers (NCI): Dr. A. Hellman
Dr. J. Strickland
Dr. A. Fowler

Objectives:

It is the purpose of this contract to study the viral flora of placentas with special emphasis on the occurrence of type-C viruses. Although a number of mammalian species (including the mouse, cat, dog,

horse and cow) are to be surveyed for the presence of type-C viral particles, a major portion of the work will concentrate on baboon and human placentas and fetal tissues derived from these two primate sources.

Major Findings:

The occurrence of a relatively large number of type-C virus particles in the syncytiotrophoblast of all baboon placentas examined, while a few particles were seen in approximately 60% of human placentas. Gs antigen activity and RNA-directed DNA polymerase were demonstrated in baboon placentas where particles were numerous.

With this as a background, studies during the past 6 months have concentrated on attempts to isolate a virus in cell culture in order to carry out definitive studies on its antigenic composition, enzyme activity and pathogenic (oncogenic) potential. Confirmation of the presence of virus particles in cell cultures has been primarily by examination with the electron microscope. Further electron microscope studies have centered on the examination of various placental and fetal tissues in an attempt to define species distribution, the precise locations of viral synthesis within the fetoplacental unit and the point in the gestation period when morphologically distinguishable type-C viral particles are first observed. Initial attempts have also been made to detect viral activity in purified fractions of homogenized placental tissue and cell culture fluids. Seven to 21 day embryo material has been collected and is being processed. Type-C particles have been seen in one 20 day embryo. Previously a 27 day embryo was the earliest placenta studied which was positive. It is of interest that the cytotrophoblast cells of the 20 day embryo have more particles than the syncytiotrophoblast, in opposition to what has been observed in later embryos.

Only a few fetal tissues have been examined to date, but none has been found to contain viral particles.

To date, in excess of 100 cell cultures have been processed for observation on the electron microscope. Approximately 80 of these have been of adequate quality for evaluation. Five preparations have contained what appear to be type-C virus particles. Four of these resulted from the cocultivation of a placenta culture derived from a single baboon with a chimpanzee fetal lung culture or rhesus monkey fetal foreskin. One of the four cultures, a placenta-chimpanzee cocultivation, has been treated with 50 μg/ml IUDR. Several of the placenta-chimps cocultivation cultures show numerous intra- and extracellular particles and are currently releasing significant quantities of type-C particles.

Pools of primate type-C viruses are being prepared for reference materials and the preparation of appropriate antisera. Fluorescein labelled antisera is being made available from Dr. Hellman's laboratory. The sera was prepared in goats and dogs against the M-7 isolate.

Frozen sections of placental tissues are being prepared for use in immunologic techniques such as fluorescent antibody labelling which may allow for the localization of type-C virus antigens, as well as others, within the placenta. Additionally, sera from other baboons are being surveyed for the presence of antibodies to these placental antigens.

Specimens of uterus, ovary, testis, etc. are being collected for examination to determine the origins of type-C viral activity.

A number of placental and fetal tissues (approximately 30) were sent to the Project Officer for this contract (Dr. A. Hellman) for testing in his laboratory and distribution by him to other laboratories. Studies on this material include tests for Gs antigens, RNA-directed DNA polymerase and purification of type-C viral particles from fresh placental tissue. Various cell cultures were also sent for Gs antigen testing.

Significance to Biomedical Research and the Program of the Institute:

The presence of type-C particles in "normal" primate tissue has for the first time been visualized and the agent isolated. The significance of their presence can only be conjectured at this time, however, the fact that they are present in relatively large quantities at a time of endocrinological stimulation further adds significance to the induction of C-particles information by hormones (Hellman and Fowler) and may be significant for propogation of the species. Finding such particles in "normal" tissue permits one now to determine whether they really have any direct cause and effect relationship in inducing malignancy in any primate.

Proposed Course:

All efforts will be directed in this laboratory and that of the project officer to determine the function that these particles have in primates as well as the possible universal presence of such C particles at particular stages of host development.

Date Contract Initiated: June 3, 1971

THE DOW CHEMICAL COMPANY (NO1-CP3-3243)

Title: Research and Development of Biohazard Control and Containment Facilities

Contractor's Project Director: Mr. Cyril B. Henke

Project Officer (NCI): Dr. W. Emmett Barkley

Objectives:

1. In collaboration with the NCI Office of Biohazard and Environmental Control, the contractor performs Biological Safety and Environmental Control surveys of VCP contractor operations to evaluate laboratory practices, safety equipment and facilities and to assess the effect of contractor operations on laboratory safety and environmental quality.

2. The contractor assists the Office of Biohazard and Environmental Control in the preparation and dissemination of safety equipment specifications, facility design criteria, operations guidelines and safety procedures. The contractor also prepares engineering design drawings and specifications for containment systems required to meet specific program needs.

3. The contractor operates a safety equipment certification program to assure safe operation of VCP safety and containment equipment.

4. The contractor assists the Office of Biohazard and Environmental Control in the review of plans and specifications for VCP renovation and new facility construction projects.

Major Findings:

The contractor participated in eight site visits, provided extensive consultation support to VCP contractors, and has reviewed facility design criteria for a number of NCI contractors and grantees. The contractor has assisted the NCI, OB&EC in evaluating laboratory safety problems identified during the eight completed site visits. Individual site visit reports were prepared to provide necessary guidelines to assist contractors in implementing the NCI Minimum Standards of Biological Safety and Environmental Control. A comprehensive survey of Building 37, NIH, was completed. Observed deficiencies were evaluated, listed and presented in a final report to NCI in July. The report emphasized short and long term recommendations for correcting deficiencies and improving their safety and environmental control program.

The contractor, in collaboration with the Division of Research Services, NIH, has prepared two slide-cassette tape packages for distribution to VCP contractor laboratories and other interested investigators. The first presentation entitled, "Effective Use of the Laminar Flow Biological Safety Cabinet" contains 54 slides and a 16-minute tape recording. This packette has been loaned to 167 laboratories. The second package entitled, "Formaldehyde Decontamination of Laminar Flow Biological Safety Cabinets" contains 42 slides and a 13-minute tape recording. So far 90 laboratories have requested and received the second presentation. The contractor arranged a one-day seminar on centrifuge biohazards at the Frederick Cancer Research Center, November 8, 1973.

The equipment certification program has demonstrated that 41 percent of the cabinets tested this past year failed to pass initial leak tests. Cumulative test results indicated an initial failure rate of 57 percent.

Significance to Biomedical Research and the Program of the Institute:

This contract contributes biological safety and environmental control expertise to the VCP. This expertise is used to improve the quality and safety of the cancer research laboratory environment. The contractor functions as an integral part of the NCI Office of Biohazard and Environmental Control.

Proposed Course:

The contractor will continue to provide technical assistance to the VCP contractors on problems of environmental control, personnel safety and product protection. The contractor will participate in approximately ten Safety and Environmental Control surveys. The contractor will review all plans and specifications for renovation and new facility construction projects upon request. All recommendations will be based on compliance with accepted engineering design, biological safety and environmental control practices. The contractor will continue to prepare the slide-cassette tape packages and provide distribution to VCP contractors.

Date Contract Initiated: June 25, 1965

HARVARD SCHOOL OF PUBLIC HEALTH (NO1-CP4-3218)

Title: Cancer and Chronic Disease in Virologic Laboratory Workers

Contractor's Project Director: Dr. T. Mack

Project Officer (NCI): Dr. A. Hellman

Objectives:

1. To evaluate the costs and benefits of several methods of assembling, in retrospect, a roster of virologists, laboratory persons and others with professional exposure to the virological laboratory.

2. To evaluate the feasibility of measuring the past morbidity and mortality in such a cohort.

3. To measure the costs of starting and maintaining an on-going registry of such persons.

4. To evaluate the feasibility of instituting surveillance for chronic diseases among these persons, in order to:

a) Prospectively evaluate the occupational hazards of biological materials in general and of laboratory acquired viruses in particular

b) Most quickly recognize unforeseen disease risks, examine their corrolates, speculate as to their causes, and prudently act to minimize future hazards.

Major Findings:

We are still developing a questionaire to be sent out to selected virology laboratories, until such time findings, significance and proposed course will have to await further development.

Significance to Biomedical Research and the Program of the Institute:

Will have to await further development of the contract.

Proposed Course:

Will have to await further development of the contract.

Date Contract Initiated: July 2, 1973

THE UNIVERSITY OF TEXAS SOUTHWESTERN MEDICAL SCHOOL AT DALLAS (NO1-CP-12135)

Title: Biohazards Information Gathering Center: Surveillance of Laboratory-Acquired Infections and Accidents

Contractor's Project Director: Dr. Malcolm Pike

Project Officer (NCI): Dr. Alfred Hellman

Objectives:

(1) To gather information concerning laboratory-acquired infections and accidents by the use of questionnaires and personal contact;

(2) To collate this information into a readily useable form for determining factors which may affect the occurrence of laboratory acquired infection.

Major Findings:

No progress as yet under this contract, contractor has had difficulty in getting program initiated.

Significance to Biomedical Research and the Program of the Institute:

By becoming aware of the problem, as defined by past experience, it is hoped that work with newer agents, particularly oncogenic viruses, can proceed with a greater chance of protecting the safety of personnel involved.

Proposed Course:

The unused funds from this contract will be used to transfer all historical laboratory acquired infection data to data forms developed by the Center for Disease Control. CDC will maintain in the future a national data bank on laboratory acquired infections.

Date Contract Initiated: May 10, 1971

UNIVERSITY OF MINNESOTA (NO1-CP4-3285)

Title: Development and Presentation of Course in Contamination and Physical Hazard Control

Contractor's Project Director: Dr. Donald Vesley

Project Officer (NCI): Dr. W. Emmett Barkley

Objectives:

The objective of this project is to present four courses on the principles of biohazard and injury control in the biomedical laboratory. Course participants are drawn from personnel from other cancer laboratories associated with the NCI contracts and grants program.

These courses provide an opportunity to scientific investigators and laboratory technicians involved in cancer virus research to learn the fundamentals of laboratory safety including the correct usage of biological safety hoods, the adequacy of protective clothing and other personal protective devices, disinfection and sterilization techniques and the principles of biological, physical, chemical and radiological hazard control.

Major Findings:

Four courses have been presented, three at the University of Minnesota, June 12-15, September 11-14, October 16-19, and one at the NIH from December 11-13, 1973. Approximately 180 cancer scientists and senior laboratory technicians attended the courses presented this past year. A total of approximately 330 investigators and technicians have attended the courses to date.

The contractor has developed a course manual and compiled a "handout packet" of up-to-date reference materials. The course manual includes lecture outlines, references, data tables, and other supporting information for each of the course lectures. Another section of the manual details laboratory exercises and instructions for the laboratory portion of the course. A catalog of safety aids and equipment has also been developed.

The course has succeeded in stimulating the participants to think positively about laboratory safety problems. Follow through efforts by the Office of Biohazard and Environmental Control have demonstrated an increased awareness of laboratory safety objectives among those scientists who have attended the course. Most important, however, has been an increase in laboratory safety practices by many course participants.

Significance to Biomedical Research and the Program of the Institute:

The training program provides a most effective mechanism for disseminating current biological safety information. Course participants benefit directly by learning biological safety and environmental control methods and techniques. This program benefits the institute by increasing the general safety awareness of the laboratory participant and his associates.

Proposed Course:

The contractor will present four courses in 1974, two in June and one each in September and December. Three of the courses will follow the same format as the 1973 courses. Two will be presented in Minneapolis and one at NIH. The second course scheduled for June in Davis, California will be a special session presented to campus safety officers in conjunction with the summer meeting of the Campus Safety Association. The course material will be altered to accomodate the experience and responsibility level of the attendees at this session.

The contractor will reevaluate the course content thoroughly on the basis of evaluations received from the four 1973 courses before plans are finalized for the new series. Handouts and references will be updated as new materials become available. The laboratory sessions particularly will be reviewed and revised as necessary for maximum impact.

Lecturers will be encouraged to update and revise lecture outlines and content after each course. The lectures are being written in a script format for future use in the development of single subject slide-cassette presentations.

Date Contract Initiated: December 10, 1971

SUMMARY REPORT

2. BREAST CANCER VIRUS SEGMENT

July 1, 1973 through June 30, 1974

Radioimmune precipitation assays have been developed for the quantitation of the mouse mammary tumor virus (MMTV) by using MMTV externally labelled with I^{125}. This technique provides a rapid, sensitive assay for intact virus, viral antigens, and antibodies against the virus. The RIA for MTV virion has proven valuable for evaluating MTV produced in culture and for antisera titration. The 50% end point titer of the standard sera is 1:200,000. The MTV-RIA can also be used to assay serum from other species. We have found that antibodies from mice and rats immunized against MTV can inhibit the RIP of MTV.

The structure of MMTV has also been studied. Most of the protein analysis work was performed on sucrose density purified MTV from primary monolayer cultures of BALB/cfC3H tumor cells. The virion was studied in polyacrylamide gel electrophoresis (PAGE) using internal labelling (tritiated amino acid or glucosamine), external labelling with lactoperoxidase iodination and staining (Coomassie blue or PAS).

Twelve polypeptides were reproducibly resolved by the combined methods. Five major polypeptides had estimated molecular weights of 52,000, 36,000, 28,000, 14,000 and 10,000 daltons. Minor polypeptides were observed at 70,000, 60,000, 46,000, 38,000, 30,000, 22,000 and 17,000 daltons. Five of the polypeptides were associated with carbohydrates. They had molecular weights of 70,000, 60,000, 52,000, 36,000 and 10,000 daltons. Three polypeptides, 32,000, 28,000 and 14,000 were present in 1.24 subviral particle while the 28,000 and 14,000 dalton polypeptides were present in 1.27 subviral particle.

In vitro cultivation of mouse mammary epithelial cells gives rise to three dimensional structures referred to as domes. These domes arise within 24 to 48 hours post plating provided 3 conditions are fulfilled. These requirements are: (1) epithelial cells; (2) an initial plating density of 10^6 cells per cm^2; (3) the presence of insulin and cortisol. Domes arise in primary cell cultures of mouse mammary epithelial cells derived from the following sources: tumor, hyperplastic alveolar outgrowths, prelactating mammary tissue, and ductal tissue from virgin females. These cells need not be infected by the mouse mammary tumor virus (MTV) for dome genesis to proceed. However, there appears to be some relationship between the presence of domes and the quantity of the MTV extruded into the culture medium. Because of the relationship of domes to MTV production, the behavior of domes in culture was analyzed by means of sequential photography of diffraction images and time lapse cinematography. The presence of the MTV in the culture fluid seemed to parallel the number of domes present in the culture, suggesting also a cyclic release of MTV. Total virus protein harvested in a 10 day period averaged 3.6 mg.

Studies are in progress to develop quantitative infectivity and transformation assays for Mason-Pfizer monkey virus (M-PMV) and to develop cloned isolates

of M-PMV. The co-cultivation of M-PMV infected cells with human KC cells carrying the RSV genome resulted in production of large numbers of syncytia. The induction of syncytia can be neutralized by incubating the virus with specific antisera. Clones of M-PMV have been isolated and appear to replicate independently of a second virus (Type C) which can also be demonstrated in M-PMV stocks. Transformation of monkey cells, as evidenced by colony formation in semisolid agar, has been demonstrated. M-PMV like particles have been regularly isolated from Rhesus monkey breast biopsies, placentas, and fetuses.

Phospholipase C can be used for preparation of cores from the mouse mammary tumor virus and from human milk particles. The human and mouse cores both contain 60-70S RNA and reverse transcriptase. In addition to offering further evidence of similarity between the human milk particles and the RNA tumor viruses, core isolation obviates certain technical difficulties. Because of their uniquely higher densities, cores, unlike complete virions, band in a region comparatively free of cellular contaminants and minimizes the problems generated by enzyme inhibitors and by the presence of cellular debris found in human milk.

A homology in nucleic acid sequence between the RNA in virus particles from mouse milk and RNA from mouse mammary tumors has been found. Similarly, a homology was reported in nucleic acid sequences between the virus particles from a human milk and RNA from human mammary adenocarcinomas. Studies were done to determine if the ^{3}H-DNA probe synthesized from human milk "cores" is complementary to the RNA of human breast tumors as well as other human tissues. The probe synthesized from the RNA of human milk cores hybridized significantly with the polysomal RNA from human malignant breast tumors; no significant hybridization could be detected with polysomal RNA of human benign breast tumors, normal human breast tissue, human sarcomas, leukemic cells, or "normal" human spleen.

Mason-Pfizer monkey virus (MPMV) was isolated from a spontaneous mammary carcinoma of a rhesus monkey. To determine the origin of MPMV and its relatedness to various human neoplastic tissues, hybridization of ^{3}H-DNA product of MPMV to nucleic acids of various tissues is being studied. Tritiated DNA is synthesized from MP-MV 70 S RNA using purified reverse transcriptase from avian myeloblastosis virus. This ^{3}H-DNA probe hybridizes to the polysomal RNA of approximately two-thirds of human malignant breast tumors examined as analyzed by both cesium sulfate and hydroxyapetite.

The simultaneous detection assay has been utilized to detect particles, in extracts of human breast adenocarcinomas, with a density of 1.16-1.19 g/ml, that contain a 60-70S RNA in association with a reverse transcriptase. Using the detergent sterox, "cores" from these particles are generated that have a density of 1.26-1.27 g/ml and contain a 60-70S RNA in association with a reverse transcriptase. Further studies to characterize these putative viral particles from human malignant breast tumors are in progress. No such particles have been detected in experiments employing benign human breast tumors.

Five out of fifteen human mammary tumors were shown to possess RNA sequences

homologous to MuMTV genome. These RNA sequences hybridized between 18 to 77 percent of the MuMTV DNA probes at C_rt values greater than 10^4. The C_rt curve analysis of these positive tumor RNA's showed very low amounts of MuMTV related RNA ranging from 1 to 8 molecules per cell. Only about 5% mismatching was observed in these MuMTV DNA-human RNA hybrids judging from the differences in Tm.

Several hundred donors participated in the milk collection program. As in the past, family histories of these women were obtained by interview and will be corroborated by rigorous documentation. The detailed analysis of these data will provide the high and low risk profiles to be used in studies correlating breast cancer risk with oncornavirus detection.

To determine the reliability of the simultaneous test for 60-70S RNA and reverse transcriptase and the exogenous template tests for reverse transcriptase for the screening of human milks for RNA tumor viruses, a series of reconstruction experiments where RLV, or AMV was added to human milk were carried out. It was found that using existing protocols, it was difficult to detect reverse transcriptase of the seeded virus in some milks. The reverse transcriptase activity gel profiles from seeded milk tested with the simultaneous test resembled those from virus samples which had RNAse deliberately added at the lysis step to destroy the RNA template.

A recent report indicated that human milks contain cells, primarily derived from mammary epithelium that could be maintained in culture. Milk cell cultures have been tested for reverse transcriptase activity by the simultaneous detection test. Activity was observed in both the cells and in the 1.16 to 1.18 region of the supernatant fluids from such cultures fractionated on 20-50% sucrose gradients. RNA extracted from particles derived from the supernatants of such cultures which band in the 1.16-1.19 gms/cc region of sucrose gradients, was analyzed by velocity sedimentation. While no radioactivity was observed in the 70S region of the gradient, a peak was found with a sedimentation of 35S. The relationship between this and the indication of viral activity in human milk samples is currently under investigation.

A stable cell line of human mammary carcinoma cells has recently been established. These cells were recovered from a pleural effusion of a female with diagnosed malignant mammary adenocarcinoma. The line has been in existence for more than two years. Several different criteria have been employed to establish the human and mammary origin of these cells. These cells were used in an attempt to demonstrate proliferation of a candidate human tumor virus. Results of these studies can be summarized as follows: (a) Buoyant density = 1.7 ($\pm$ 0.01) in sucrose-H_2O, (b) An internal 70S RNA, (c) Reverse transcriptase (RT) which used endogenous 70S RNA as template measured in a simultaneous detection assay, (d) Internal cores with a p = 1.25-1.27 which contains RT activity.

BREAST CANCER VIRUS SEGMENT

Dr. Jeffrey Schlom, OAD, DCCP, Chairman
Dr. Wade Parks, VC, DCCP, Vice Chairman
Dr. Ernest Plata, VLL, DCCP, Executive Secretary

UNIVERSITY OF CALIFORNIA, DAVIS, CALIFORNIA (N01-CP-3-3253)

Title: In Vitro Cultivation of Human and Mouse Mammary Tumor Viruses

Contractor's Project Director: Dr. Robert C. Cardiff

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

Objectives:

1. To study the production of mouse mammary tumor virus (MTV) *in vitro* in order to develop concepts, techniques, and reagents that might be applicable to larger scale production of this virus and of potential human breast cancer viruses.

2. To study human breast tissue under various experimental conditions developed from the mouse mammary model for the presence of possible human mammary tumor viruses.

Major Findings: A radioimmune precipitation assay has been developed for the quantitation of the mouse mammary tumor virus (MMTV) by using MMTV externally labelled with I^{125}. This technique provides a rapid, sensitive assay for intact virus, viral antigens, and antibodies against the virus. Following iodination and dialysis, I^{125} labelled material was found to concentrate after isopycnic centrifugation in sucrose density gradients in the fractions where the MTV virion is usually found. These procedures provided specific activities between 17,000 and 58,000 counts per minute per microgram of total viral protein. Polyacrylamide gel electrophoresis of intact, iodinated MTV revealed that 80-90% of the I^{125} migrated in a single peak. As little as 5.0 mμgms of unlabelled MTV reduced the percent RIP as compared to an uninhibited control RIP.

The specificity of the inhibition was evaluated through the addition of a series of unlabelled antigens. Sucrose density gradient purified C type particles from BALB/c spleen cells, AKR spleen cells and cultures infected with Rauscher leukemia virus, and Moloney sarcoma virus did not inhibit the precipitation of MTV. In a like manner, homogenates of BALB/c lactating mammary gland, defatted BALB/c milk, and fetal calf serum did not inhibit the precipitation reaction.

The RIA for MTV virion has proven valuable for evaluating MTV produced in culture and for antisera titration. The 50% end point titer of the standard sera is 1:200,000. The MTV-RIA can also be used to assay serum from other species. We have found that antibodies from mice and rats immunized against MTV can inhibit the RIP of MTV.

The structure of MMTV has also been studied. Most of the protein analysis work was performed on sucrose density purified MTV from primary monolayer cultures of BALB/cfC3H tumor cells. The virion was studied in polyacrylamide gel electrophoresis (PAGE) using internal labelling (tritiated amino acid or glucosamine), external labelling with lactoperoxidase iodination and staining (Coomassie blue or PAS).

The polypeptides were considered major if they contained 10% or more of the total counts or label and minor if they contained less than 10% of the total. The number of polypeptides were almost identical in all techniques used.

Twelve polypeptides were reproducibly resolved by the combined methods. Five major polypeptides had estimated molecular weights of 52,000, 36,000, 28,000, 14,000 and 10,000 daltons. Minor polypeptides were observed at 70,000, 60,000, 46,000, 38,000, 30,000, 22,000 and 17,000 daltons. Five of the polypeptides were associated with carbohydrates. They had molecular weights of 70,000, 60,000, 52,000, 36,000 and 10,000 daltons.

Treatment of MTV with 1.0% and 2.0% NP-40 gave subviral particles with buoyant densities of 1.24 and 1.27 gms/cc in potassium tartrate density gradients. These particles contained viral RNA as seen when ^{3}H uridine labelled MTV was treated with NP-40. Immunodiffusion tests showed no reaction with antisera made against intact MTV. These subviral particles have been studied using internal labelling (^{3}H amino acids and ^{3}H uridine) and external labelling techniques.

In summary, twelve polypeptides were obtained when MTV grown in tumor tissue cells was studied using SDS-polyacrylamide gel electrophoresis. There were five major polypeptides with molecular weights of 52,000, 36,000, 28,000, 14,000 and 10,000 daltons. The seven minor polypeptides had molecular weights of 70,000, 60,000, 46,000, 38,000, 30,000, 22,000 and 17,000 daltons. Staining with PAS revealed that the 70,000, 52,000, 36,000 and 10,000 daltons polypeptides contained carbohydrate moieties, while ^{3}H glucosamine was found associated with the polypeptides with molecular weights of 70,000, 60,000, 52,000 and 36,000 daltons. Three polypeptides, 32,000, 28,000 and 14,000 were present in the 1.24 subviral particle while the 28,000 and 14,000 dalton polypeptides were present in the 1.27 subviral particle.

In vitro cultivation of mouse mammary epithelial cells gives rise to three dimensional structures referred to as domes. These domes arise within 24 to 48 hours post plating provided 3 conditions are fulfilled. These requirements are: (1) epithelial cells; (2) an initial plating density of 10^6 cells per cm^2; (3) the presence of insulin and cortisol. Domes arise in primary cell cultures of mouse mammary epithelial cells derived from the

following sources: tumor, hyperplastic alveolar outgrowths, prelactating mammary tissue, and ductal tissue from virgin females. These cells need not be infected by the mouse mammary tumor virus (MTV) for dome genesis to preceed. However, there appears to be some relationship between the presence of domes and the quantity of the MTV extruded into the culture medium. Because of the relationship of domes to MTV production, the behavior of domes in culture was analyzed by means of sequential photography of diffraction images and time lapse cinematography.

The presence of the MTV in the culture fluid seemed to parallel the number of domes present in the culture, suggesting also a cyclic release of MTV. The quantity of virus was monitored on a daily or 48 hour basis by determination of total protein by the micro-Lowry method.

Virus can be detected usually by day 4 or 5. Peak virus production appears between day 5 and 25 of culture, with a daily harvest yield between 3 to 25 μg protein per 75 cm^2 flask per 24 hours. Total virus protein per 75 cm^2 flask per 24 hours. Total virus protein harvested in a 10 day period averaged 3.6 mg.

Significance to Biomedical Research and the Program of the Institute: The knowledge and technology gained on the *in vitro* cultivation and structure of MMTV may be directly applicable to a human breast cancer virus.

Proposed Course: The activities described will be continued at the current contract level.

Date Contract Initiated: 01 February 1972

GEORGETOWN UNIVERSITY SCHOOL OF MEDICINE (N01-CP-50053)

Title: Human Breast Cancer Virus Studies

Contractor's Project Director: Dr. William F. Feller

Project Officer (NCI): Dr. Ernest J. Plata

Objectives:

1. The detection of viral markers, such as reverse transcriptase, in human milk and breast tumors to define their association with human breast cancer.

2. The possible propagation of virus-like agents in cell cultures infected or co-cultivated with fractions or cells derived from selected human milks and breast tumors.

Major Findings: Cells of the A204 (human sarcoma) and MC-1 (human milk) established culture lines were shown to agglutinate shortly after exposure to approximately 25% of human milk specimens in a manner reminiscent to the agglutination produced in certain cells exposed to Mason-Pfizer virus. Attempts to repeat the findings with a variety of other available cell lines were not successful. Preliminary characterization showed the agglutination factor to be soluble after centrifugation at 10,000 x G for 1 hour, stable at 37°C and -80°C. It does not appear to be an antibody of the ABD or HL-A systems. No association between the agglutination patterns and the incidence of breast disease was detected to date.

Cell culture studies resulted in the establishment and characterization of the MC-1 cell line derived from the co-cultivation of cells obtained from a specimen of human milk with monolayers of human foreskin cells (Flow 7000). MC-1 cells have characteristics of human macrophages.

This program has also been in operation to obtain milk from women with and without family or personal history of breast cancer. Specimens were tested by the simultaneous detection test for reverse transcriptase (RT) and 60-70S RNA which might be indicative of virus-like activity. To date, no correlation has been shown between the findings in milk and the clinical status or history of the donors. Since human milk contains substances which are inhibitory or interfere with the tests described, this contractor developed a method of ultracentrifugation of clarified milk through a cesium chloride density gradient solution which removed at least part of the inhibitory activity present in milk. This advance failed to resolve the question of relationship of RT activity and clinical history of the donor.

Significance to Biomedical Research and the Program of the Institute: The search for viral markers in human milk and breast tumors is important for the definition of the significance of the reported biochemical milk particles in human breast cancer. The demonstration of biological activity and/or viral characteristic such as infectivity and replication of these agents will facilitate tremendously further studies concerning the etiology of human breast cancer.

Proposed Course: Work in this laboratory and others has shown that epidemiological studies with reverse transcriptase in human milk are at best premature at this time. Cell culture studies of milk and human tumors are being carried out in a variety of laboratories throughout this program. Based on these considerations, this contract will terminate on 31 August, 1974.

Date Contract Initiated: 19 November 1964

INSTITUTE FOR MEDICAL RESEARCH (N01-CP-3-3339)

Title: Studies of Human Milk and Mammary Tumors

Contractor's Project Director: Dr. Dan H. Moore

Project Officer (NCI): Dr. Ernest J. Plata

Objectives: To determine, through a multi-disciplinary program, the significance of virus-type particles resembling oncogenic RNA viruses present in some human milks and breast tumors.

Major Findings: Five out of fifteen human mammary tumors were shown to possess RNA sequences homologous to MuMTV genome. These RNA sequences hybridized between 18 to 77 percent of the MuMTV DNA probes at C_rt values greater than 10^4. The C_rt curve analysis of these positive tumor RNAs showed very low amounts of MuMTV related RNA ranging from 1 to 8 molecules per cell. Only about 5% mismatching was observed in these MuMTV DNA-human RNA hybrids judging from the differences in Tm.

MTV replication and mammary tumorigenesis were suppressed in C57BL mice vaccinated with killed and challanged with live RIII MuMTV. The immunity shown was principally, if not wholly, a cellular immune response.

A radioimmunoassay for MuMTV with I^{125} labeled whole virions was developed. It was sensitive for the detection of MuMTV in RIII milk at a milk dilution of 1:6000 and an antibody dilution of 1:800.

With freeze cleaning, etching, and critical point drying techniques for electron microscopy it was shown that the surface of MTV has a coating which apparently covers and embeds the surface "spikes". This coating although not yet chemically defined, consist of highly regular arrays of subunits.

Studies to demonstrate correlation between particles observed in the EM and reverse transcriptase (RT) activity have shown no correlation to date. In general, milks containing the MS-1 particle contain RT activity. Reconstruction experiments by adding mouse RIII virus to human milk showed that RT activity was inhibited. In addition, some milks effect extensive destruction of MMTV as seen by EM. No correlation was found between the loss of morphologic integrity with the loss of RT activity and infectivity. Attempts have been made to obviate some of the problems by modifying the assay. These techniques have resulted in 100 to 1000 fold increase in the sensitivity of detection of RT activity of RIII MMTV as compared with the previous method. With the new technique, 74% of human milk specimens were positive for RT activity. However, there is still no correlation between RT activity and particle content in human milk or disease status of the patient.

It has been found that approximately 25% of human female serum neutralize MMTV by infectivity tests in mice. In this series, no correlation with the cancer status of the patient was shown.

In the past year a total of 110 human breast tumors have been explanted. The life span of breast tumor cells in suspension was never extended beyond the tenth month. A search for 70S RNA and reverse transcriptase in primary cultures and co-cultures of breast tumors did not give clear evidence of an oncogenic virus. One hundred seventy specimens have been searched by EM for the presence of budding virus particles without positive findings; however, many virus-like particles are found in high-speed pellets of culture supernatants. A serum substitute supplemented with amniotic fluid and prolactin has been developed that appears to stimulate cell multiplication in primary cultures of human breast tumor cells.

Significance to Biomedical Research and the Program of the Institute: This approach to the human breast cancer virus problem is one of using technology developed in the MMTV system to determine whether a comparable viral agent can be demonstrated in human breast cancer. This contractor was selected initially because the Principal Investigator has had long experience with, and has contributed significantly to the development of the mouse model system. Exploratory studies on human materials led to strong evidence for a comparable human virus and resulted in the establishment of this multi-disciplinary effort to follow-up these leads.

Proposed Course: The potentially significant findings reported will be confirmed and extended. It is of great importance that additional data be generated to support the observations of possible hybridization of MuMTV probes with RNA of human breast tumors. The immunological studies also warrant consolidation. It is expected that the next period will be scientifically productive and may lead subsequently to possible applications of proven techniques to human systems.

Date Contract Initiated: 28 June 1968

MASON RESEARCH INSTITUTE (N01-CP-3-3358)

Title: Study on the Role of Hormonal Factors on Induction of Breast Tumors

Contractor's Project Directors: Dr. Arthur E. Bogden

Project Officer (NCI): Dr. Jeffrey Schlom

Objectives: To evaluate the oncogenic potential of two viruses isolated from mammary adenocarcinomas, MPMV from a rhesus monkey and R-35 from a Spraque-Dawley rat, and determine the roles of hormones, especially those which induce mammary hyperplasia and lactation, in pathogenesis of mammary cancer caused by these viruses.

Major Findings: There are presently in-house 25 female and 4 male rhesus, 8 female cyno and 1 male bonnet inoculated neonatally with M-PMV; and 44 mature M-PMV inoculated and control female rhesus, 12 mature M-PMV inoculated cyno, bonnet and owl monkeys and 1 male rhesus breeder. There are also 3 juvenile (1 male, 2 female) rhesus born in-house to M-PMV inoculated mothers. The age range of the neonatally M-PMV inoculated animals is from 8 months to 3.5 years of age. As of this date, no evidence of palpable mammary tumors has been found in any primate under test. To warrant long term holding of the rhesus colony, a concentrated effort has been initiated during this contract period directed at demonstrating M-PMV infection and/or antigen expression in virus inoculated and un-inoculated monkeys. In collaboration with two other VCP laboratories immunological tests for virus antigens, reverse transcriptase determinations, DNA and RNA probes for hybridization studies, as well as a tissue culture plaque assay for detecting infectious virus, are being performed on the blood and tissue of selected animals from various experimental groups. Several tissues from each monkey have been sent to the above investigators and the remainder used for histopathology and freeze preservation.

R-35 MTV was inoculated subcutaneously into neonatal GFRC:SPF(SD) female rats, a Sprague-Dawley derived stock negative for known rodent virus contamination, to determine its mammary tumorigenic activity in the species of origin. During a 20 month observation period, the total percent tumor incidence was 29.5 in the R-35 MTV inoculated population and 18.5 in the control population. Although the incidence of benign tumors in both control (16.7%) and virus inoculated populations (18.7%) was similar, the 10.8% incidence of malignant tumors (primarily mammary adenocarcinomas) in the R-35 MTV inoculated population was six times greater than the control incidence of only 1.8%. Malignant tumors were first detected in the virus inoculated animals between 4 and 5 months of age as compared with 9 to 13 months in the control group, whereas the onset of benign tumors in control and virus inoculated animals were similar. Malignant tumors, 90 percent of which were mammary neoplasms of epithelial origin, occurred earlier than the benign. Significantly, the rate of increase in malignant tumor incidence plateaued after 250 days of age, or at a population incidence of approximately 30 percent. No leukemias were found over a 400 day observation period.

An attempt was made to induce a synergistic effect and accelerate mammary tumorigenesis by combining 17β-Estradiol pellet implantation with total body X-irradiation. It was found that X-irradiated estradiol pellet implanted populations had significantly fewer tumors (33% incidence than the non-estrogen treated, X-irradiated populations (67% incidence), indicating a tumor-inhibiting effect by estradiol that was totally unexpected. Estradiol treated, X-irradiated populations not only had a fewer number of tumors over an equivalent time span, but there was a definite lag in the onset of such tumors as compared to non-estrogen treated populations.

Significance to Biomedical Research and the Program of the Institute: This project is part of a program to determine if viruses are related to breast cancer in other species besides mice, including monkeys and humans. Animal studies are necessary to develop methods and reagents for the search for viruses in human cancer patients. This will contribute to VCP objectives by providing substantial support, or lack of it, to the general idea that a type of cancer of great clinical significance in humans is caused by viruses. The leads involved must be investigated and evaluated as soon as possible.

Proposed Course: Completion of the survey described to detect evidence of M-PMV infection prior to or independent from tumorigenicity. This will lead to data on which to base decision concerning continued long-terms holding and observation of these animals. Evaluation of the results obtained with R-35 MTV as the experimental periods are completed and if warranted extend certain confirmatory studies in genetically and virologically defined rats.

Date Contract Initiated: 9 June 1970

MEDICAL COLLEGE OF WISCONSIN (N01-CP8-1010)

Title: Hormone Effects on Virus Production in Breast Cancer

Contractor's Project Director: Dr. Roland A. Pattillo

Project Officer (NCI): Dr. Ernest J. Plata

Objectives: To study the effects of human hormones on possible oncogenic virus production in human breast cancers in vitro. The hormones to be studied include the lactogenic hormone and biologically active estrogenic and progestational steroids.

Major Findings: Estrogen metabolism was identified in primary human breast cancer in short term culture and in an established long term human breast tumor line (HBT-3). Conversion of ^{3}H-estrone to ^{3}H-estradiol was documented in infiltrating ductal carcinoma. Because of previous findings that estradiol stimulates luminal secretions in the aveoli of breast tumor lobules, it is suggested that estrone, the major estrogen in post menopausal females may act as a biogenetic steroid precursor or pre-hormone for the biologically active hormone, estradiol. Stimulation of virus production in hormonally manipulated tumor cultures was sought since the work of others has shown that mammary tumor virus replication appears to be related to insulin and hydrocortisone associated cellular organization. No evidence of viral synthesis was detected by reverse transcriptase assays. Fetal breast cell strains were established. Parabiotic cultures of primary breast cancer with these neonatal cells monitored by reverse transcriptase assays and sucrose density gradients demonstrated inconclusive increases in radioactivity with the synthetic template poly rA·oligo dT.

Milk from lactating mothers operated for mammary carcinoma in the opposite breast was inoculated on fetal breast cells in culture. Phase microscopic monitoring revealed lipid uptake by these cells. Continued observations of these cultures has shown no transformation, growth stimulation or other indication of viral infection.

Breast cancers of various histologic types also have been co-cultivated with female and male fetal breast cells. An established cell line from an infiltrating ductal adenocarcinoma has been developed and is presently being characterized. No evidence of viral replication has been detected to date by—EM or enzymatic studies.

Significance to Biomedical Research and the Program of the Institute: The understanding of the gene regulatory functions of hormones and their subsequent influence on virus expression is of paramount importance to oncogenic virology. It is likely that the most important model for breast cancer in humans is the MTV system; hormonal influence in viral expression has been clearly demonstrated in mice and that same influence is fairly clear concerning the growth of breast tumors in the humans. Further understanding in these areas could be translated into improved treatment and diagnosis as well as improved understanding of the pathogenesis of breast cancer.

Proposed Course: The progress of this contract has been limited because of the complexity of the systems studied and perhaps prematurity in the choice of human systems for experimentation. This contract will terminate on 31 May 1974.

Date Contract Initiated: 19 September 1963

MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASE (N01-CP4-3208)

Title: Collection of Breast Cancer Specimens

Contractor's Project Director: Dr. Herbert F. Oettgen

Project Officer (NCI): Dr. Ernest J. Plata

Objectives: Procurement of serum specimens from the following defined population groups as a part of a collaborative effort to determine whether candidate viruses isolated from human or animal sources are related etiologically to human breast cancer.

Basic defined population: Women entering Memorial Hospital, New York City, for first diagnosis of any breast disease.

Test group: Women whose lesions prove to be malignant as determined by biopsy.

Control groups:

a. Women whose lesions are found on biopsy to be benign proliferative reactions or reactions suspected as being preneoplastic in nature.

b. Women whose lesions are considered to be unrelated to neoplasia.

Major Findings: From May, 1972 to December, 1973 the contractor collected over 650 blood samples. The serum was separated and stored in small aliquots. Questionnaires pertaining to family history were completed. Serums were collected from 301 patients with malignant conditions of the breast and from 330 patients with benign conditions of the breast. Some additional specimens are awaiting histological confirmation of diagnosis. Specimens continue to be collected at a rate of approximately 50 per month.

Significance to Biomedical Research and the Program of the Institute: Since viral agents suspected of causing cancer in man cannot be tested directly in human subjects, it is necessary to establish etiological relationship indirectly through immunoepidemiological 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 high frequency, and in larger amounts, in sera of women with breast cancer as compared with appropriate controls.

Proposed Course: Since the objectives of this contract have been largely fulfilled, the collection phase of this effort will be concluded on 24 June 1974. The serum collection will be stored and monitored by the Breast Cancer Virus Segment of this program.

Date Contract Initiated: 23 June 1971

MICHIGAN CANCER FOUNDATION (N01-CP-3-3347)

Title: Studies on High Risk Breast Cancer Families

Contractor's Project Director: Dr. M. Brennan

Project Officer (NCI): Dr. Jeffrey Schlom

Objectives: This program, aimed at the identification and characterization of that sub-population at high risk to breast cancer, may be divided into two areas: 1. A determination of the presence and the biological significance of oncornaviruses in human milk, aimed at both elucidating an

etiological role for these agents and the development of practical methods for the exploitation of their presence as a correlate of high risk to the disease. 2. The development and utilization in vitro systems for the cultivation of candidate human breast cancer viruses.

Major Findings:

1. Acquisition of milk specimens: During the first five months of this contract year, 452 primary donors participated in this milk collection program. As in the past, family histories of these women were obtained by interview and will be corroborated by rigorous documentation. The detailed analysis of these data being conducted as part of the breast cancer epidemological program of the Michigan Cancer Foundation, will provide the high and low risk profiles to be used in studies correlating breast cancer risk with oncornavirus detection.

2. Biochemical detection of virions in milk: To determine the reliability of the simultaneous test for 60-70S RNA and reverse transcriptase and the exogenous template tests for reverse transcriptase for the screening of human milks for RNA tumor viruses, a series of reconstruction experiments where RLV, or AMV was added to human milk were carried out. It was found that using existing protocols, it was difficult to detect reverse transcriptase of the seeded virus in the great majority of such milks although occasional samples did exhibit some activity. The reverse transcriptase activity gel profiles from seeded milk tested with the simultaneous test resembled those from virus samples which had RNAse deliberately added at the lysis step to destroy the RNA template.

3. Cultivation of cells from human milk: A recent report indicated that human milks contain cells, primarily derived from mammary epithelium, that could be maintained in culture. The contractor has confirmed and extended these findings.

Milk cell cultures have been tested for reverse transcriptase activity by the simultaneous detection test of Schlom and Spiegelman. Activity was observed in both the cells and in the 1.16 to 1.18 region of the supernatant fluids from such cultures fractionated on 20-50% sucrose gradients.

RNA extracted from particles derived from the supernatants of such cultures which band in the 1.16-1.19 gms/cc region of sucrose gradients, was analyzed by velocity sedimentation. While no radioactivity was observed in the 70S region of the gradient, a peak was found with a dedimentation of 35S. The relationship between this and the indication of viral activity in human milk samples is currently under investigation.

4. Cultivation of a human mammary cell line of malignant origin: Cell culture studies - A stable cell line of human mammary carcinoma cells has recently been established. These cells were recovered from a pleural effusion of a female with diagnosed malignant mammary adenocarcinoma. The line has been in existence for more than two years.

Several different criteria have been employed to establish the human and mammary origin of these cells

These cells were used in an attempt to demonstrate proliferation of a candidate human tumor virus. Results of these studies can be summarized as follows:

1. Particles with the following biochemical and biophysical characteristics are replicated _de novo_ in MCF-7 cells.

    a. Buoyant density = 1.17 ($\pm$0.01) in sucrose-H_2O.

    b. An internal 70S RNA.

    c. Reverse transcriptase (RT) which used endogenous 70S RNA as template measured in a simultaneous detection assay.

    d. Internal cores with a ρ = 1.25 - 1.27 which contains RT activity.

Significance to Biomedical Research and the Program of the Institute: These studies concern the isolation and characterization of a potential human breast cancer virus.

Proposed Course: The activities described will be continued at the current level.

Date Contract Initiated: 20 June 1971

NETHERLANDS CANCER INSTITUTE (N01-CP-3-3368)

Title: Immunogenetic Studies on Breast Cancer and Leukemia

Contractor's Project Director: Dr. L.M. Boot

Project Officer (NCI): Dr. Walter E. Heston

Objectives: To study the immunogenetics of mammary tumors and MTV transmission, as well as that of leukemia and MuLV, to determine the presence and function of specific genes for transmission, MTV replication, histocompatibility, and immune response. In addition, a small effort has been directed towards the search for oncornavirus in man and other animals.

Major Findings: The development of early mammary tumors in the mouse is under control of one dominant gene. This was shown by correlating the expression of MTV-gs and the ML antigen, a cell surface antigen induced by MTV, in crosses of a variety of selected strains.

Through the very sensitive radioimmunoassay, it was possible to classify mouse strains into those which yielded strongly positive milk (GR, C3H, BALB/c and (Balb/c x C3H)$_{f}$1), negative milk (MTV-free BALB/c and C57Bl), and intermediate (C3Hc). This technique permitted the study of the C3H$_{f}$-MTV genetics in normal rather than tumorous mice.

Mice of congenic strains of the C57Bl/BO strain with different haplotypes were foster nursed on (C3Hc x 020)$_{f}$1 hybrid female mice. Their tumor incidence showed remarkable differences. The haplotypes H-2^{b} and H-2^{m} were highly resistant, those of H-2^{f}, H-2^{d}, H-2^{i} and H-2^{a} were highly susceptible with other haplotypes showing intermediate susceptibility. Thus, through recombinant experiments it appears that the D-end of the H-2 locus determines susceptibility, the subloci $2D^{b}$ and $2D^{g}$ resistance, and $2D^{d}$ and $2D^{f}$, high susceptibility.

Virological studies showed that the oncornavirus reported by Gelerblom et al did not cross-react with MTV or MuLV but cross-reacted with Mason Pfizer MV by virtue of two common antigens (16,000 and 28,000 Daltons respectively) found in both viruses. Additionally, virus-like particles with type C characteristics were detected by EM in 14 out of 15 cell cultures obtained from different cattle with hyperlymphocytosis. Nine detailed reports of these findings were submitted for publication during this contract period.

Significance to Biomedical Research and the Program of the Institute: This type of genetic study concerning the host's susceptibility and capacity for internal control of oncogenic virus expression and infectivity is of crucial importance to the program of this institute. The results obtained thus far, begin to delineate a variety of factors, in addition to the presence of an oncogenic virus, which contribute to or modify the pathogenesis of mammary cancer of leukemia. The continued search for oncogenic viruses that may be of human origin or that could be potentially infectious to humans is also essential to the program. The findings described contribute to the investigations of possible etiological agents of human cancer, as well as to greater understanding of the fundamental biology of cancer.

Proposed Course: The contractor proposes to continue his studies concerning the genetics of MTV and MuLV expression, transmittability, susceptibility and their relationship to histocompatibility and immune response. Studies concerning possible oncornaviruses of man will continue at approximately the same minimal level.

Date Contract Initiated: 28 June 1972

PFIZER, INCORPORATED (N01-CP-3-3239)

Title: Viral Studies of Animal and Human Breast Cancer

Contractor's Project Director: Dr. Sami A. Mayyasi - Dr. Mumtez Ahmed

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

Objectives: To develop quantitative infectivity and transformation assays for Mason-Pfizer monkey virus (M-PMV). To develop cloned isolates of M-PMV and to assess the role of any other virus present in M-PMV studies.

Major Findings: The co-cultivation of M-PMV infected cells with human KC cells carrying the RSV genome resulted in production of large numbers of syncytia within 48-96 hours. Evidently a specific interaction, syncytia do not form when 118 MG cells (control KC cells free of RSV) or when XC cells (rat with RSV) are co-cultivated with M-PMV cells. A dose response is observed with respect to the M-PMV inoculum added to the test cells and the number of syncytial phaques formed following co-cultivation with KC cells. Syncytia will also form when KC cells are exposed to M-PMV cell lysates or cell-free virus inocula. The induction of syncytia can be neutralized by incubating the virus with specific antisera.

Clones of M-PMV have been isolated and appear to replicate independently of a second virus (C type) which can also be demonstrated in M-PMV stocks. Transformation of monkey cells, as evidenced by colony formation in semisolid agar, has been demonstrated.

M-PMV like particles have been regularly isolated from Rhesus monkey breast biopsies, placentas, and fetuses.

Significance to Biomedical Research and the Program of the Institute: A determination of the role of M-PMV in breast carcinoma of monkeys is of obvious significance in assessing the role of virus in the human disease.

Proposed Course: Cloning experiments will be completed so that quantitative studies on the biological properties of M-PMV and its relationship to other virus present in M-PMV stocks can be initiated. Syncytium formations by M-PMV will be reformed into a single quantitative assay for this virus. Properties of M-PMV transformed cells isolated from semisolid agar colonies will be investigated.

Date Contract Initiated: 28 June 1967

MELOY LABORATORIES, INC. (N01-CP-4-3223)

Title: Molecular Studies of Human and Animal Cancer with Emphasis on Mammary Adenocarcinoma

Contractor's Project Director: Dr. John Verna

Project Officer (NCI): Dr. Jeffrey Schlom

Objectives: This program is aimed at the use of techniques in molecular biology to determine a viral involvement in human mammary carcinoma. The murine mammary tumor virus system as well as other animal models are employed to help in the development of new technology as well as for model systems. The program is divided into four major areas of interest: (a) Evidence for an RNA Tumor Virus in Human Milk, (b) Molecular Studies of Mason-Pfizer Monkey Virus, (c) Cell Cultures Studies of Murine and Human Mammary Carcinomas, (d) Biochemical Evidence for an RNA Tumor Virus in Human Malignant Adenocarcinomas.

Major Findings: Phospholipase C can be used for preparation of cores from the mouse mammary tumor virus and from human milk particles. The human and mouse cores both contain 60-70S RNA and reverse transcriptase. In addition to offering further evidence of similarity between the human milk particles and the RNA tumor viruses, core isolation obviates certain technical difficulties. Because of their uniquely higher densities, cores, unlike complete virions, band in a region comparatively free of cellular contaminants. This minimized the problems generated by enzyme inhibitors and by the presence of cellular debris found in human milk. Under these circumstances, the assays for particles become more sensitive and certain.

A homology in nucleic acid sequence between the RNA in virus particles from mouse milk and RNA from mouse mammary tumors has been found. Similarly, a homology was reported in nucleic acid sequences between the virus particles from a human milk and RNA from human mammary adenocarcinomas. The contractor has undertaken studies to determine if the ^{3}H-DNA probe synthesized from human milk "cores" is complementary to the RNA of human breast tumors as well as other human tissues. The tritium labeled DNA probe complementary to the RNA of the human milk particles is synthesized via an endogenous reaction using cores from the human milk particles. The ^{3}H-DNA probe synthesized from the RNA of human milk cores hybridized significantly with the polysomal RNA from human malignant breast tumors. No significant hybridization could be detected if the same probe was hybridized to the polysomal RNA of human benign breast tumors, normal human breast tissue, human sarcomas, leukemic cells, or "normal" human spleen.

Mason-Pfizer monkey virus (MPMV) was isolated from a spontaneous mammary carcinoma of a rhesus monkey. To determine the origin of MPMV and its relatedness to various human neoplastic tissues, hybridization of ^{3}H-DNA product of MPMV to nucleic acids of various tissues is being studied.

Tritiated DNA is synthesized from MP-MV 70S RNA using purified reverse transcriptase from avian myeloblastosis virus. This ^{3}H-DNA probe hybridizes to the polysomal RNA of approximately two-thirds of human malignant breast tumors examined as analyzed by both cesium sulfate and hydroxyapetite.

The contractor has shown that MPMV RNA has a molecular weight of approximately 8 x 10^6 when analyzed by polyacrylamide gel electrophoresis. Short term harvests of MPMV yield a 60-70S RNA which when denatured by formamide (40%) or by heat (80°, 2.5') yields heterogeneous RNA species with molecular weights ranging from 1.5-3 x 10^6 (25-25S). Longer harvests yield smaller RNA subunits when denatured.

The simultaneous detection assay has been utilized here to detect particles, in extracts of human breast adenocarcinomas, with a density of 1.16-1.19 g/ml, that contain a 60-70S RNA in association with a reverse transcriptase. Using the detergent sterox, "cores" from these particles are generated that have a density of 1.26-1.27 g/ml and contain a 60-70S RNA in association with a reverse transcriptase. Further studies to characterize these putative viral particles from human malignant breast tumors are in progress. No such particles have been detected in experiments employing benign human breast tumors.

Significance to Biomedical Research and the Program of the Institute: These studies are aimed at the isolation and characterization of a putative breast cancer virus from human milk and malignant breast tumors.

Proposed Course: The activities described will be continued at the current level.

Date Contract Initiated: 20 July 1973

TEL AVIV UNIVERSITY (N01-CP-2-3237)

Title: Isolation, Purification and Propagation of B type Particles from Human Milk in Israel

Contractor's Project Director: Dr. Jafa Keydar

Project Officer (NCI): Dr. Wade Parks

Objectives: To isolate and grow possible oncorna virions from the milk of breast cancer patients or from human adenocarcinomas in human embryonic tissue culture cells; to develop methods for the assay and enhancement of production of this "virus"; and to continue characterization studies of the "virions" produced in the human cell cultures.

Major Findings: Nine human embryonic cell lines have been established which produce particles with 70S RNA and reverse transcriptase activity, after infection was accomplished with either milk from breast cancer patients or through co-cultivation with human mammary adenocarcinomas. Although these human cell lines are stable, "virus" production fluctuates and proceeds at very low levels. The murine model system was studied to establish methods of detection, assay procedures and finally culture conditions which would maximize mammary tumor virus (MuMTV) production. Employing [^{3}H]-uridine incorporation into particles banding at densities of 1.15 to 1.19 sucrose solutions, these investigators observed that MuMTV synthesis was enhanced in sparsely seeded cultures, at low serum concentrations, at relatively high pH, and in the presence of hydrocortisone.

An established ascites cell line, originally derived from a spontaneous mouse mammary carcinoma was observed to produce relatively large amounts of viruses. Electron-microscopy, isopycnic banding in sucrose, RNA directed DNA polymerase activity, nucleic acid hybridization, and serological methods have shown that these are mostly type B viruses. Some evidence suggest that small amounts of type C virus particles are produced simultaneously.

Significance to Biomedical Research and the Program of the Institute: The evidence of possible infectious virus-like material in human milk obtained from breast cancer patients in this contract is very relevant to the NCI program. This findings must be confirmed and extended to determine whether these "viruses" have any association with human cancer. The development of a murine cell line producing mostly type B virus in large quantities *in vitro* also serves to accelerate other studies concerning the biology of this type of viruses.

Proposed Course: To continue the characterization of "viruses" and the virus-producing human embryo cell lines using the procedures developed last year in the murine model systems.

Date Contract Initiated: 23 March 1972

SUMMARY REPORT

3. DEVELOPMENTAL RESEARCH SEGMENT
July 1, 1973 - June 30, 1974

The mission of the Developmental Research Segment is the elucidation of the association and presumed etiological relationship of RNA and DNA viruses to human neoplasia leading to the development of measures for the prevention or control of virally-induced neoplasms in man. To meet this objective collaborative contract research projects include studies on: seroepidemiology, screening of human neoplasms for the presence of virus or virus genetic expression; factors which influence virus genetic expression; molecular processes involved in virus replication and cell transformation and humoral and cellular host immune responses to viral and virally-induced tumor cell antigens.

Collaborative contract research projects are administered through the Office of the Chief, Viral Biology Branch. The Chief, VBB, as Chairman of this Segment, arranges for the review of solicited and unsolicited research proposals pertinent to the mission of the Segment. Reviews are conducted for relevance, priority, and need within the Virus Cancer Program by regular meetings of the Program Segment Chairmen and Vice Chairmen and for scientific merit by the Segment Working Group. The voting members of the Working Group include seven scientists from non-governmental institutions and two from government laboratories other than NCI.

Projects within the Segment are primarily devoted to investigations bearing upon the role of viruses in the etiology of certain malignancies in humans and the prevention or control of such neoplasms. Current research projects may be assigned to three major areas of investigation: (1) determination of the association of viruses with, and their presumptive causal relationship to, human neoplasia; (2) factors influencing virus genetic expression in cells; and (3) approaches to the development and evaluation of measures for the prevention or control of virus-induced neoplasia. The viruses under study include members of the herpesvirus group and viruses of the RNA tumor virus group.

Attention to the possible role of herpesviruses in oncogenesis was aroused by the isolation of a member of this group, the EB virus, from cells cultured from African Burkitt's lymphoma. Subsequently, certain animal herpesviruses were shown to induce lymphoma or leukemia in a number of different animal species. Serological studies on patients with cervical carcinoma implicated herpes simplex virus type 2 (HSV-2) as a possible cause of this neoplasm. Further study of HSV-2 inactivated by exposure to ultraviolet light demonstrated its capability to cause malignant change in cultured hamster embryo cells. Investigations initiated in these several areas have continued in the collaborative contract research program.

At the Karolinska Institute, the relationships between the EB virus and the host lymphocyte have been under study. The virus genome was found to be

linearly integrated into some, but not all, chromosomes of the infected lymphocyte. A nuclear antigen, EBNA, induced by the virus associates exclusively with chromatin fibrils in interphase nuclei, and with chromosomes in metaphase. Lymphoblastoid cell lines containing the virus genome may be sensitive or resistant to the superinfection by EB virus. Hybrids between sensitive and resistant cells showed sensitivity to be dominant. The virus transforms human cord blood cells in vitro. This transformation is inhibited by antibody to EB virus-induced cell membrane antigens. In patients with Burkitt's lymphoma, the circulating antigen-antibody complexes are due to the release of material containing the membrane antigens and not to free virus. The tumor in the patient contains few cells in which the virus-induced early antigens and capsid antigens are demonstrated by immunofluorescence tests probably because these cells are eliminated by opsonization followed by phagocytosis. However, similar tests show the presence of EBNA in most of the tumor cells. The presence of from 5 to 113 EB virus genome equivalents per cell of 24 of 26 Burkitt's tumors from patients in Kenya was demonstrated at the University of North Carolina. The high degree of association of EB virus with nasopharyngeal carcinoma tissue obtained from African patients is demonstrated by the detection of from 5 to 85 virus genome equivalents per cell in 32 of 38 specimens. The unique association of virus with tumor cells is evidenced by the absence of detectable levels of EB virus DNA in the peripheral blood leukocytes of African patients with Burkitt's lymphoma or nasopharyngeal carcinoma. Surprisingly, not one of 4 American patients with a diagnosis of Burkitt's lymphoma contained detectable EB virus DNA in tumor tissue.

Established cultured lines of lymphocytic cells which were characterized as having B-lymphocyte origin contained from 5 to 510 EB virus genomes per cell whereas those lymphocytic lines with T-cell characteristics did not contain virus DNA. The T-cell lines do not appear to be susceptible to infection by EB virus. Investigators at the Pennsylvania State University have isolated an intracellular inhibitor capable of interfering with the replication of EB virus. The inhibitor is not interferon.

Among nonhuman primates, the marmoset has been the most sensitive to tumor development following infection of a number of different tumor viruses. Experiments at the Rush Presbyterian St. Lukes Medical Center in which several EB virus containing cell lines were inoculated into marmosets but produced no disease contrasted with trials made with the EB virus-infected marmoset cell line, B95-8, of Shope and Miller which does induce lymphoma in this primate species. This observation raises questions concerning possible strain differences between the EB viruses involved or, alternatively, the presence of, or modification by, another agent. Indeed, studies on African Burkitt's tumor tissues conducted at Columbia University had shown that these tissues contained nucleotide sequences with some homology with DNA probes prepared from the RNA extracted from the Rauscher murine leukemia virus.

To gain further understanding of the relationship between herpesvirus and human lymphoma, studies have been underway on similar virus-lymphoma systems in animals. The availability of a line of specific pathogen free (LSI-SPF) chickens reared under high containment at Life Sciences, Inc., permitted studies on the interaction between the Marek's disease herpesvirus (MDHV) and

RNA viruses of the avian leukosis group (ALV). In contrast to conventional chickens, the LSI-SPF birds exposed to a MDHV monocontaminated environment for 8 weeks did not develop tumors or mortality characteristic of Marek's disease. Enhancement of tumorigenesis and mortality occurred in both SPF and conventional birds concurrently infected with ALV (RAV2) and MDHV. Two strains of MDHV were investigated. One produces a pattern of chronic disease with cytologic changes characteristic of Marek's disease in both SPF and conventional line chickens. The other produces typical Marek's disease in SPF birds characterized by a high early mortality and a limited gross tumor response. The acute pattern of disease is modified upon co-infection with ALV (RAV2) and by co-infection with the acute and chronic MDHV strains apparently by interference with the lethal effect produced by acute strain MDHV. Concurrent inoculation of the SPF line birds with ALV (RAV1) and MDHV increased mortality to 69 percent over a 49 day period as compared with 16 percent and 6 percent in chickens inoculated with MDHV or RAV1 respectively.

Investigations on the role of herpesviruses in the cause of neoplasia in humans are directed to: (1) demonstrate the degree to which serological evidence of infection correlates with neoplasia; (2) demonstrate the presence of the virus or virus genome within tumor cells; (3) determine oncogenic properties of virus strains in cell culture systems; (4) determine oncogenic properties in nonhuman primates. This pattern of research has been continued in current research contracts studying the relationship of herpes simplex virus type 1 and type 2 to carcinomas in humans.

The production at the University of Naples of a complement-fixing (CF), sedimentable non-virion antigen, induced early in the cycle of infection of guinea pig cells by HSV-1 and HSV-2 permitted tests of human sera for the presence of specifically reacting antibodies. The results of initial trials were highly suggestive of a relationship between the presence of antibodies to the HSV-2 induced non-virion antigen and cancer of the genital tract. These observations were repeated and verified by further study at the Frederick Cancer Research Center. The results of the CF tests completed on 202 serum samples from individuals without cancer and from patients with cancer in 29 different areas of the body showed that only those sera from patients with advanced carcinoma of the lip, mouth, oropharynx, nasopharynx, kidney, urinary bladder, prostate, cervix uteri, and vulva, comprising 56 cases in all, gave a positive CF reaction. No antibodies reactive with the non-virion antigens were detected in serum donors without cancer, with current or recurrent HSV-1 and HSV-2 infections or with other cancers. None of 4 women with early neoplastic changes of the cervix uteri gave positive reactions. Sera from 7 patients with carcinoma of the lip or oropharynx reacted only with HSV-1 non-virion antigen. Appropriate controls were included to demonstrate the specificity of the reaction.

With the use of antibodies to herpesvirus non-virion antigens, complement-fixing reactivity was shown for soluble cell membrane antigens separated from lip and cervical carcinomas but not for similar extracts of cells from normal vaginal tissue or intestinal carcinoma. Human serum specimens coded and supplied by Baylor University were tested against separated CF-reactive

components of non-virion antigens of HSV-1 and HSV-2. Positive CF reactions were found in 87, 90, and 62 percent respectively of sera from patients with squamous cell carcinoma of cervix, larynx and other head and neck areas. Only 8 percent of patients with other tumors and 6 percent of normal donors had similarly reactive antibody whereas the titers of HSV neutralizing antibodies were similar in patients and controls.

Similar relationships between an early HSV-2 induced antigen and CF antibodies in the sera of cervical cancer patients were observed at Johns Hopkins University. Sera from 91 percent of patients with invasive cervical carcinoma and 9 percent of matched controls were positive. Positive reactions were also obtained with sera from 68 percent of cases of carcinoma *in situ* and 35 percent of cases of cervical atypia. The early antigen (AG-4) is a soluble surface antigen made between 4 and 16 hours after infection of HEp2 cells with HSV-2.

Biopsy specimens of cervical carcinoma from Taiwanese patients were examined at the University of North Carolina for the presence of HSV-2 DNA. Reconstruction studies of DNA renaturation kinetics analysis indicated that 0.1 genome of HSV-2 per cell could be detected when 2000 μg of cellular DNA are tested. No evidence of HSV-2 DNA at a level of 0.1 to 0.2 genome virus equivalents per cell was detected in any of 5 tumor specimens examined.

In tests made at the Pennsylvania State University, 18 percent of HSV-1 and 52 percent of HSV-2 strains examined after inactivation with UV light were capable of morphologically transforming hamster embryo fibroblasts in culture. A number of established cell lines grew as tumors when inoculated into hamsters. Photodynamic inactivation of HSV also yielded transforming virus. Preliminary results suggest conversion of human cells held at elevated temperature. Evidence of transforming capability has been extended to include cytomegalovirus. The transformed cells were shown to contain virus-specific antigens.

The cebus monkey was selected as an experimental nonhuman primate for tests to determine whether HSV-2 would induce cervical neoplasia *in vivo*. Of the 164 animals examined prior to genital infections by virus, two showed mild dysplasia but were negative on re-examination. Similarly, one animal of 48 which received control preparations showed mild cervical dysplasia and was also negative on re-examination. Five animals which received HSV-2 genitally 1 to 2 1/2 years previously and demonstrated laboratory evidence of infection have shown persistent mild to severe cervical dysplasia.

Another approach to detect the presence of the HSV-2 genome in cancer cells was initiated at Baylor University. Temperature sensitive (ts) mutants of the virus for marker rescue provided suggestive evidence of the presence of a complementing HSV-2 genome in some cloned lines of hamster cells transformed by HSV-2.

Alteration in the biochemical composition of cells rendered neoplastic by viruses were determined. Increased synthesis of a galactoprotein cell component followed transformation of rat cells by MSV. A block in the

synthesis of complex fucosylglycosphingolipids with a concomitant build-up of simpler ones was associated with cell transformation by RNA sarcoma virus and with HSV induced malignancy in hamster cells. The altered synthesis was unrelated to virus production.

Demonstration of the relationship of RNA viruses to human neoplasms was sought through attempts to isolate representative viruses from specimens of selected malignancies, through studies on possible relationships of animal viruses to human neoplasia, and through search for evidences of virus genetic information or virus gene expression in tumor specimens.

Particular emphasis has been placed upon human sarcomas and leukemias as most likely to be the result of virus activity. Measures which have been successfully applied to induce production of endogenous virus in animal cells have not stimulated release of biologically active virus from human sarcomas examined at the Rockefeller University. Similar studies at the University of Texas provided suggestive evidence of viral expression. Morphological changes which occurred in co-cultivated cells of human osteosarcoma and leukemia were accompanied by the appearance of antigen reactive in immunofluorescence tests with osteosarcoma patients' sera previously absorbed to remove heterophile reactivity. Electron microscopy, serological tests and biochemical tests have repeatedly yielded suggestive evidence of the presence of virus information in the tumor cells but virus recovery remains elusive.

The DNA probes produced on the RNA templates extracted from known tumor viruses by the action of viral reverse transcriptase were applied to further study of human neoplasms at Columbia University. At least a portion of the RNA hybridizing with the DNA probes detected in human mammary carcinoma, leukemia, and Burkitt's lymphoma exists as 70S RNA associated with an RNA-instructed DNA polymerase. The particulate elements from human leukemia cells which contain this RNA and DNA polymerase were used to synthesize single-stranded tritium labeled DNA probes. Such probes clearly demonstrated the presence of DNA sequences in leukemic blood cells which were absent in normal human blood cells. Similar technology demonstrated that probes prepared from particles separated from human brain tumors did not cross hybridize with genetic material from other human neoplasms. Probes from cancer of lung and colon were also unique. On the other hand, over half of the sequences of the DNA product of the endogenous reaction using particulates separated from human acute myeloid leukemia cells was shown at Litton-Bionetics laboratories to hybridize to simian sarcoma virus-1. Differing patterns of hybridization to RNA of different animal leukemia viruses substantially supported the relationship of the RNA in the leukemia cell particulates to the simian tumor virus RNA. The long poly (A) stretches characterizing RNA tumor viruses were shown at the Massachusetts General Hospital to be located at the 3'-OH end of AMV 35S RNA. Poly (A) was not covalently integrated in other regions of the 35S strands.

Elucidation of the molecular events in viral nucleic acid replication is being attempted at the Albert Einstein College of Medicine. Observations on Rous sarcoma virus infection of chick cells showed that a covalent hybrid

structure involving DNA and Rous virus RNA is localized in the nuclei within 2 hours after infection. The source of the DNA in the hybrid structures is unknown. The nuclei of myeloblasts infected with AMV catalyze transcription in vitro, yielding RNA products which hybridize with AMV DNA transcript probes. Similar experiments were made with chromatin from myeloblasts and purified thymic RNA polymerase II.

Purified proteins and glycoproteins of the Rauscher leukemia virus prepared at the Albert Einstein College of Medicine were each shown to carry type, group and interspecies antigenic determinants. The glycopeptides contained a previously undescribed interspecies determinant of mammalian oncogenic type C RNA viruses. Similar lack of specificity for type or group determinants was noted in avian virus polypeptides purified at the Duke University Medical Center. Studies with avian viruses demonstrated that the expression of the virus genome in various host-virus relationships is not uniform; not all the polypeptides are expressed in some cases. Human malignant cells have been examined for the possible presence of antigen components related to the purified polypeptides of known viruses without success. Antigenic components specific for human chronic lymphocytic and acute myelocytic leukemia (AML) have been isolated and their specificity for the leukemia has been established. Along these lines, a leukemia-associated nuclear antigen (LANA) was detected by anticomplement immunofluorescence tests conducted at the Karolinska Institute. The antigen is predominantly associated with blast cells of AML.

At the Massachusetts General Hospital, the graft versus host reaction (GVHR) was shown to be highly effective in activating murine leukemia virus (MuLV) with subsequent development of leukemias in mice. Further observations have shown that MuLV is activated from recipient lymphoid cells responding to foreign skin graft antigens in sites where lymphoid blastogenesis is maximum. Interferon treatment prevented this activation of virus and current studies suggest that interferon may also prevent GVH-virus-induced leukemogenesis.

The augmentation of tumor transplant antigens (TTA) by infection with vesicular stomatitis virus (VSV) was shown to stimulate the immune system of animals to more intensive response against the TTA. Experiments at Meloy Laboratories showed that the immunity induced in animals by the augmented antigens stimulated tumor rejection. The TTA augmentation phenomenon is dependent upon infection of tumor cells by VSV. However, effective immunogenicity is retained within sub-cellular fractions and following VSV inactivation.

DEVELOPMENTAL RESEARCH PROGRAM SEGMENT

Dr. Robert A. Manaker, VBB, Division of Cancer Cause and Prevention, Chairman
Dr. Michael A. Chirigos, Associate Chief, VBB, Division of Cancer Cause and Prevention, Vice Chairman

BAYLOR COLLEGE OF MEDICINE (NO1-CP3-3257)

Title: Studies on Viruses as Related to Cancer

Contractor's Project Director: Dr. Joseph L. Melnick

Project Officers (NCI): Dr. Robert A. Manaker
Dr. Michael A. Chirigos

Objectives: To determine the relationship of viruses to selected neoplasias and their significance in the neoplastic process.

Major Findings: Antibodies to separated CF-reactive components of non-virion antigens of herpes simplex virus type 1 (HSV-1) and herpes simplex virus type 2 (HSV-2) were found in 87, 90, and 62% respectively of sera from patients with squamous cell carcinoma of cervix, larynx and other head and neck areas. Only 8% of patients with other tumors and 6% of normals had similarly reactive antibody. Titers of neutralized antibodies to HSV were similar in patients and controls.

Marker rescue with temperature sensitive (ts) mutants of HSV-2 suggest the presence of a complementing HSV-2 genome in some cloned lines of HSV-2 (UV inactivated) infected hamster cells. Antiserum to the C4a antigen, an early non-structural polypeptide induced by HSV, is the only reagent thus far found to react strongly by immunofluorescence with antigen in HSV-transformed hamster cells.

Alterations in the biochemical composition of cells rendered neoplastic by viruses were determined. In normal cells, the synthesis of glucoprotein-like materials from galactose is predominant. This synthesis in HSV-infected cells is replaced by increased synthesis of a galactoprotein component. Since there was no alteration of the nucleotide sugars, UDP-galactose and UDP-glucose, as a result of HSV infection, the site of alteration lies somewhere between the nucleotide sugar precursor and the finished product, perhaps at the glucosyltransferase stage. Increased synthesis of a galactoprotein component follows MSV transformation of rat cells, but the biochemical basis appears different from that in HSV-infected cells (may be due to an epimerase choke).

All oncogenic RNA virus transformed cells exhibit a block in the synthesis of complex fucosylglycosphingolipids with a concomitant build-up of simpler ones. The altered FSL synthesis is associated with transformation rather than with virus production (and has been extended to include oncogenic HSV-hamster transformed cells).

Studies on the metabolism of 2 deoxy-D-glucose (dglc) into the glycolipids and glycoproteins of transformed cells were pursued to probe the surface structure of transformed cells and to evaluate the possible use of dglc as an inhibitor of transformation.

Dglc inhibits infectivity of HSV-1 and 2. The major glycopeptide (C5) of each of four strains of HSV-1 is shifted from 123,000 daltons to 85,000. C5 only shifts slightly to 115,000-120,000 daltons for each of four strains of HSV-2. In MSV transformed cells the dglc is incorporated at a greatly reduced level into one of the two major glycolipids found in normal cells whereas the pattern of incorporation into normal or untransformed MuLV infected cells is the same.

Twenty-two ts mutants of HSV-1 representing 15 complementation groups and eight ts mutants of HSV-2 representing seven complementation groups are being characterized. Twenty-five potential ts mutants of H. saimiri were isolated and stocks of selected stable mutants are being prepared for in vivo study to select attenuated "vaccine" strains.

No signs of malignancy were observed in monkeys under study for possible co-carcinogenic effect of hepatitis B antigen and N-nitrosodiethylamine after 10-15 months observation.

Significance to Biomedical Research and the Program of the Institute:

Serological and sero-epidemiological evidence obtained by controlled studies on patients with squamous cell carcinomas implicate genital and oral strains of herpesvirus as factors in the genesis of these malignancies. Intensive study of the biochemical, genetic, and immunological aspects of the herpesviruses associated with these neoplasms provides fundamental information which may be invaluable as the role of the herpesviruses in oncogenesis is further developed.

Proposed Course: In order to solve the problem of the causal relationships of herpesvirus 1 and 2 to human carcinomas, the following objectives are being emphasized by the contractor: (a) detection of herpesvirus gene functions in human carcinomas by complementation and marker rescue studies; (b) search for virus specific antibodies and antigens in human tissues with purified specific virion and nonvirion antigens of herpesviruses; (c) insights into cell transformation and genetics by temperature sensitive and UV mutants of herpesviruses; and, (d) mechanism and definition of virus-induced alterations of cell membranes and function.

Date Contract Initiated: June 27, 1963

COLUMBIA UNIVERSITY (NO1-CP3-3258)

Title: Replication of Oncogenic Viruses and Its Relation to Human Cancer

Contractor's Project Director: Dr. Sol Spiegelman

Project Officers (NCI): Dr. Robert A. Manaker
Dr. Maurice L. Guss

Objectives: This project is directed toward the elucidation of the molecular biology of RNA tumor viruses and the mechanisms by which they induce oncogenic transformation of infected cells. This knowledge is applied to the determination of the viral etiology of human neoplasms and ultimately to development of effective control measures for human cancers.

Major Findings: The initial observations detecting viral specific RNA in human tumors by molecular hybridization have now been extended to answer the following questions: 1) How large is the RNA being detected? 2) Is it associated with a RNA-instructed DNA polymerase? 3) Are the two found in a structure possessing a known physical characteristic of an RNA tumor virus? In order to answer these questions an experimental procedure has been developed that permits the simultaneous detection of 70S RNA and reverse transcriptase activity successfully applied to human leukemic cells, human breast carcinomas and Burkitt's lymphomas. In each of these malignancies, the data obtained demonstrates that at least a portion of the RNA found exists in the form of 70S RNA associated with an RNA-instructed DNA polymerase in a particle having a density between 1.16 and 1.19 gm/ml.

The particulate elements containing 70S RNA and reverse transcriptase identified in human leukemic cells have been used to synthesize single-stranded ^{3}H-DNA in endogenous reverse transcriptase reactions. This probe was used in DNA:DNA hybridizations to detect complementary sequences in human normal and leukemic white blood cell nuclear DNA. The data clearly demonstrate the presence of DNA sequences in the leukemic genome not present in the normal genome, suggesting that there is not an omnipresent DNA segment coding for malignancy.

DNA:DNA hybridization technologies were developed to identify the natural host of RNA tumor viruses, based on the observations that they possess homology to the DNA of their non-infected natural hosts. The methodology has been applied to RD-114 virus and the data demonstrate that it is feline, and not human, in origin.

Further significant work has included a study of the biological and molecular basis of murine mammary neoplasia as a model for human breast cancer and the demonstration of polyadenylic acid sequences in virus-like particles in human milk.

Nucleotide sequences in Burkitt's lymphoma and Hodgkin's disease tissues were demonstrated to possess some homology with RNA from Rauscher leukemia virus but none with RNAs from mouse mammary tumor virus or avian myeloblastosis virus. This provides some evidence that EBV is probably not the sole factor in Burkitt's lymphoma. Similar studies of the Marek's disease model system revealed the presence of type C virus information, indicating that at least a herpesvirus and an oncornavirus may be required for development of typical Marek's disease in chickens.

Probes prepared from brain tumor particles did not cross hybridize with genetic material of other human neoplasias (breast cancer, sarcomas, leukemias). In similar fashion, lung cancer and colon cancer probes were unique. These studies may lead to a useful serological test for detection of specific organ site tumors.

The DNA:DNA hybridization studies performed in human leukemias indicating the presence of particle-related sequences in leukemic nuclear DNA not present in normal leukocytes will be extended to determine whether: 1) the leukemia-specific DNA sequences are present in all types of leukemia; 2) the leukemia-specific sequences are present in all organs of leukemic patients; 3) the leukemia-specific sequences are identical in all patients with leukemia; and 4) leukemia-specific sequences are present in long-term remission cases. Similar techniques will be applied to human lymphomas, including Burkitt's disease.

Significance to Biomedical Research and the Program of the Institute:

A systematic molecular biological study is being pursued to determine the role of viruses in the genesis of human cancer. Studies demonstrated the presence in human cancers of particulate materials possessing characteristics unique to the known animal RNA tumor viruses. New approaches to the study of virus-cell relationships have been devised. The contractor has developed data which are highly provocative and his pioneering advances provide a foundation for future investigation leading to an understanding of the virus-cell relationship as it applies to human cancer.

Proposed Course: Molecular probing of human neoplastic tissue for evidence of the presence of oncogenic RNA viruses will continue. Similar procedures will be applied to "normal" human tissues. The reverse transcriptases of oncogenic RNA viruses and of neoplastic tissues will be purified and characterized. The existence of RNA nucleotide sequences in particulate fractions of human cells which are homologous to the RNA of animal viruses requires further investigation to determine the significance of the observations made.

Date Contract Initiated: October 29, 1969

CORNELL UNIVERSITY (NO1-CP3-3346)

Title: Application of Feline Virus Systems in the Study of Viral Relationships to Human Neoplasia

Contractor's Project Director: Dr. Charles Rickard

Project Officers (NCI): Dr. Michael A. Chirigos
Dr. Robert A. Manaker

Objectives: To investigate the possibility of natural infection of humans by feline viruses and to recover putative human oncogenic viruses from established tumor cell lines and tumor biopsies using feline reagents developed by the contractor.

Major Findings: Attempts are being made to rescue transforming virus from human sarcoma cells by exposure of these cells in a monolayer tissue culture to either endogenous or exogenous FeLV. The resulting pseudotype virus should have a distinctive human species specific antigen and a serologically unique RDDP. Purified reagents have been prepared for this study.

Preliminary work has shown that natural infections in cats and experimental infections in dogs by FeLV results in serum antibody detectable in FA tests. Since children may be more susceptible to FeLV than adults, sera of children who may have been infected by FeLV, leukemic children, their siblings, and close associates are being tested by IIF using unfixed feline or human cells.

Two populations of cats have been selected and segregated for breeding, on the basis of expression of endogenous type C virus, in order to provide a source of somewhat genetically defined experimental animals. Detailed studies of the biological and oncogenic properties of the endogenous cat virus are being conducted.

Nine spontaneous feline mammary tumors have served as the source of inocula for 86 newborn or fetal kittens. No neoplasms have been induced in 23 kittens which have reached ages of 4-16 months. No type B particles have been observed by EM although typical type C particles were observed in 4 of 9 spontaneous mammary tumors and appear to be conventional FeLV.

Significance to Biomedical Research and the Program of the Institute:

The cat offers several advantages for the study of viral relationships to oncogenesis. It is susceptible to horizontal infection by three sub-types of RNA leukemia virus, it provides a natural population for study, cat viruses reproduce in cells of species other than the cat, including humans, and the cat possesses an endogenous type C virus differing from the cat viruses already characterized. Since the cat is a common household pet and its type C viruses are infectious for human tissue, intensive study to determine whether its tumor viruses pose a threat to humans, particularly young children, is necessary.

Proposed Course: The experimental systems developed in the cat will be applied to search for and attempt recovery of human oncogenic viruses. Studies will continue to be conducted to determine whether any relationship exists between the endogenous and the exogenous type C viruses of the cat and neoplasia in children. At present a mammary tumor virus is known only in the mouse. Investigations will be made to determine whether a mammary tumor virus can be recovered from the cat. To assist in immunological approaches to cancer control, attempts will be made to obtain carcinogen-induced tumors in the cat and to determine whether viruses are a co-factor in genesis of such tumors.

Date Contract Initiated: June 23, 1965

DUKE UNIVERSITY MEDICAL CENTER (NO1-CP3-3308)

Title: Expression of the RNA Tumor Virus Genome in Animal and Human Malignant Cells

Contractor's Project Director: Dr. Dani P. Bolognesi

Project Officers (NCI): Dr. Peter Fischinger
Dr. Maurice L. Guss

Objectives: To characterize the structural components of animal RNA tumor virus particles and develop serological probes to detect virus gene products in animal and human malignancies.

Major Findings: The term group specific (gs) antigen for some of the avian and mammalian polypeptides is clearly a misnomer. An example illustrating this relates to an avian polypeptide thought to be group specific (p19), and one thought to be type specific (gp85). Type determinants are also present in p19 while group determinants are present as well in gp85. Clearly, the terms type, group and interspecies are coarse approximations and as the analyses become more critical, a whole spectrum of reactivities associated with these polypeptides may be anticipated.

Analyses of patterns for the four major polypeptides of AMV have revealed no common peptides among these proteins within the same virus. However, peptide patterns of related viruses (AMV-B, PR-RSV-C) are quite similar. It is of interest that for p19, distinct peptides can be discerned which may relate to the type and group serological specificities exhibited by the p19 fraction. These fragments are currently being isolated and analyzed in more detail.

Tryptic peptide maps of MuLV p30 and FeLV p30 clearly indicate the marked difference between the proteins, but some similar peptides, possibly related to the interspecies region, may be resolved. These are currently being investigated in detail. Recent results with SSV-1 p30 are indicative of a protein quite distinct from p30 of MuLV and FeLV.

It is clear from studies with avian viruses that the expression of the virus genome in various host-virus relationships is not uniform. Not all the polypeptides are expressed in some cases. One polypeptide appears to be ubiquitous in the avian system (p15) and may represent a host protein. Furthermore, using complex antisera which react primarily with p27 and p19, some of the Chf chicken lines have tested positive for gs.

Studies employing humoral cytotoxicity have revealed that there is a strong likelihood that MuLV p30 is expressed at the cell surface even in the case where actual particles are not produced. The reaction is complement dependent and can be abolished by absorption of the antiserum with the

purified polypeptide. Antiserum against FeLV p30 likewise detects the interspecies determinant of MuLV p30 on the surface of murine cells and is also strongly cytotoxic. Furthermore, these antisera are also cytotoxic to methylcholanthrene (MCA) induced rat tumor cells.

The salient features concerning infectivity of AMV cores are: 1) cores are infectious for both genetically susceptible and resistant cells; 2) virus originating from core infected cells is identical to that from which the cores were derived; 3) in contrast to viruses, infectivity of cores is not enhanced by polycation; and 4) inactivation by temperature of cores and virus is clearly different.

Molecules bearing antigenicities specific for human leukemia cells have been isolated. Chronic lymphocytic leukemia (CLL) and acute myelocytic leukemia (AML) antigens have been isolated and shown to be highly specific for the leukemia cell type from which they have been derived. They are not present in normal leukocytes and are distinct from the various HL-A tissue antigens. Antisera are being prepared currently and will represent immensely powerful tools for immunodiagnosis and immunotherapy of human leukemia.

Significance to Biomedical Research and the Program of the Institute:

The virus genome is expressed by translation of message into protein products. This project was initiated to characterize the major protein antigens of selected RNA tumor viruses, prepare immune sera to identify these antigens in infected cells, and to utilize these materials to determine basic molecular events in the process of cellular transformation. The knowledge gained may be used to develop sensitive methods for detection of virus activity in human cells and to provide a basis for therapy by blocking translational events at the sub-cellular level to prevent cell transformation.

Proposed Course: The characterization of the structural protein components of the mammalian RNA tumor viruses will be expanded to include B-type particles. A bank of highly specific immune sera against the intraspecies specific determinants of the mammalian viruses will be developed for use in the study of translational events related to the process of cell transformation and in the search for protein products of viral activity in human tumors.

Date Contract Initiated: April 19, 1971

ALBERT EINSTEIN COLLEGE OF MEDICINE (NO1-CP3-3311)

Title: Research Studies of the Molecular Biology of Oncogenic Viruses and Malignant Transformation

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

Project Officers (NCI): Dr. Robert A. Manaker
Dr. Maurice L. Guss

Objectives: To determine the molecular events involved in the adsorption and penetration of RNA tumor viruses into host cells, in viral nucleic acid replication, and in malignant transformation of cells.

Major Findings: Two Rauscher leukemia virus (RLV) polypeptides with apparent molecular weights of 69,000 and 71,000 have been purified and characterized (gp 69, 71). These polypeptides contained a previously undescribed interspecies antigenic determinant of mammalian oncogenic type C RNA viruses as was demonstrated by the reaction of the murine antigen with anti-feline leukemia virus (FeLV) serum. Radioimmunoassay analysis showed that both polypeptides were precipitated by the anti-FeLV serum, indicating that they each contained the interspecies determinant or that they were closely associated and coprecipitated. The gp 69, 71 interspecies antigen was distinguished from the known p30 interspecies antigen and the virus reverse transcriptase (RDDP) by protein purification, physical properties, and immunological analysis. The new antigen was not detected by competition radioimmunoassay in uninfected mouse cells or mouse cells productively infected with vesicular stomatitis virus (VSV).

The antigenic determinants of two purified protein constituents of mammalian C-type RNA viruses, p30 and gp70 were examined by competition radioimmunoassay. By the appropriate choice of antiserum and competing proteins it was possible to distinguish type-specific , group-specific and interspecies determinants. Both of the viral constitutents were found to contain each of these classes of antigens. The results suggested that the majority of the determinants of the major structural protein were group-specific, 5% to 30% were interspecies, and a small fraction were type-specific. In the case of the envelope glycopeptides, the chief determinants were type- and group-specific, and a small fraction were interspecies.

Compared with several other enveloped viruses, purified virions of frog virus 3 (FV3) contained a relatively high activity of a protein kinase which catalyzed the phosphorylation of endogenous polypeptides or added substrate proteins. Virions also contained a phosphoprotein phosphatase activity which released phosphate covalently linked to proteins. It was possible to select reaction conditions where turnover of protein phosphoesters was minimal, as the phosphatase required Mn^{2+} ions for activity whereas the protein kinase was active in the presence of Mg^{2+} ions. Electrophoretic studies in polyacrylamide gels containing SDS indicated that at least 10 of the virion polypeptides were phosphorylated in the *in vitro* protein kinase reaction. Characterization of these phosphoproteins demonstrated that the phosphate was incorporated predominantly in a phosphoester linkage with serine residues. The protein kinase was solublilized by disrupting purified virions with a nonionic detergent in a high-ionic-strength buffer and was separated from many of the virion substrate proteins by zonal centrifugation in glycerol gradients. The partially purified protein kinase would phosphorylate polypeptides of many different animal viruses, and maximal activity was not dependent on added cyclic nucleotides. These properties distinguished the virion protein kinase from a well characterized

cyclic AMP-dependent protein kinase which phosphorylated viral proteins only to a small extent.

The formation of covalent hybrid structures involving Rous sarcoma virus (RSV) RNA has been detected in nuclei of infected chick cells. This hybrid is detected within two hours postinfection and is localized to the nuclei. The source of the DNA in covalent hybrid structures is under investigation.

In vitro transcription catalyzed by nuclei isolated from myeloblasts of AMV infected chicks yields RNA products which hybridize with AMV·DNA transcript probes. Similar experiments have been carried out with chromatin isolated from myeloblasts and purified RNA polymerase II of thymus (or *E. coli* RNA polymerase). Thus *in vitro* RNA products can be obtained which should be identical to AMV·RNA sequences.

Significance to Biomedical Research and the Program of the Institute:

The knowledge gained of molecular mechanisms and products in tumor virus-cell relationships, the factors involved in the repression and derepression of virus gene expression, and the intracellular events associated with malignant transformation may be used to develop sensitive methods for detection of virus or virus activity in human cells and for devising a basis for therapy by blocking translational events at the subcellular level during cell transformation.

Proposed Course: Purification of the structural proteins of FLV and the Woolly monkey virus will continue. Characterization of the proteins will emphasize analysis of their antigenic properties and genetic origin. The viral coded or induced proteins will be used as probes for viral gene expression. The techniques developed in studies on murine and primate RNA viruses will be used for detection and characterization of putative viral structural proteins in human malignant tissue. Studies will be initiated to purify and analyze virion proteins of herpes simplex virus types 1 and 2 in order to provide specific viral proteins for virus detection and radioimmunoassay of human serum. Studies will continue in order to achieve a purified *in vitro* reverse transcriptase system for replication of tumor virus DNA. For control of viral gene expression, the enzymatic mechanism for transcription of viral DNA integrated into the host chromosome will be characterized.

Date Contract Initiated: April 26, 1971

HOPITAL ST. LOUIS (NO1-CP3-3365)

Title: Molecular Virological Studies on Human Leukemia

Contractor's Project Director: Dr. M. Boiron

Project Officers (NCI): Dr. George Todaro
Dr. Robert A. Manaker

Objectives: To search for viral DNA sequences in human leukemic cells and for expression of the RNA expression of an integrated genome in these cells using murine leukemia and sarcoma viruses grown in human and in mouse cells as a source of the synthetic DNA probe.

Major Findings: Optimal conditions for the endogenous RNA-directed DNA polymerase activity of the murine sarcoma virus (MSV-MLV), produced in the $78A_1$ line in order to obtain a DNA product representing a complete transcript of the 70S RNA genome, were established. The DNA product was analyzed by velocity and equilibrium gradient centrifugation, gel electrophoresis, hydroxyapatite chromatography and sensitivity towards single-stranded specific nuclease. The extent of transcription of viral 70S RNA was measured by ^3H product DNA-labeled 70S RNA hybridization. Results indicated that the single-stranded DNA was obtained in high yield under these conditions and represented a complete transcript of the viral genome since a three fold excess of product DNA was sufficient to anneal the 70S RNA completely. In contrast double-stranded DNA was not a good transcript of the viral genome. Therefore, single-stranded DNA could be used as a probe for the detection of virus specific sequences in various cells. The results obtained in analyzing double-stranded DNA seriously brought into question the validity of using this DNA as a probe in reassociation kinetics studies.

The detection of integrated viral DNA sequences in leukemic cells was hampered by the fact that double-stranded DNA represented only a very limited proportion of the viral genome, and was considered useless in reassociation kinetics studies. This important limitation led to the use of single-stranded DNA as a probe for the detection of RNA tumor virus-specific sequences in cell DNA. The kinetics of association of this labeled single-stranded DNA product to unlabeled sheared and denatured cell DNA from $78A_1$ cells was studied. A relatively good percentage of single-stranded DNA (28%) could be annealed to cell DNA. This technique required large amounts of cellular material but was specific to virus-related DNA sequences since larger amounts of sheared and denatured salmon sperm DNA could not anneal to complementary viral DNA.

Murine sarcoma virus was adapted to growth in an heteroploid human cell line JIII in order to get sufficient amounts of this virus (Hu-MSV) for biochemical studies. These will be compared to those made with MSV produced in murine cells. The transforming component (MSV) disappeared after early passages but the non-transforming component (MLV) was continuously produced although its titer remained low.

Significance to Biomedical Research and the Program of the Institute:

Data have been reported demonstrating the presence of nucleotide sequences in human leukemia cells homologous to sequences in RNA tumor virus RNA. An intensive study of human leukemia and Hodgkin's disease cells under this project is expected to provide information expanding on these observations

to determine the nature of the common nucleotide sequences, their distribution in patients' cells, and the relationship between the presence of the common sequences in virions and the species and nature of the cells in which the virus is grown.

Proposed Course: The original and reproducible techniques developed will be applied to the investigation of as many human leukemias as possible.

Date Contract Initiated: June 28, 1972

JOHNS HOPKINS UNIVERSITY (NO1-CP3-3345)

Title: Herpesvirus Antigens and Virions in Neoplastic Cells from Cervical Carcinoma

Contractor's Project Director: Dr. Laure Aurelian

Project Officers (NCI): Dr. Gary R. Pearson
Dr. Charles W. Boone

Objectives: The immediate objective of this project is the identification of herpes simplex virus type 2 (HSV-2) antigens and virions in neoplastic cells. The ultimate objective is development of evidence for or against HSV-2 as a factor in the etiology of carcinoma of the human uterine cervix.

Major Findings: A microquantitative complement fixation test has been used to study the presence of complement fixing antibody to an early (4 hours) HSV-2 antigen (AG-4) in sera from 61 patients with cervical cancer and 61 control women matched for age, race and socio econmic class. Ninety-one percent of sera from patients with invasive cervical carcinoma are positive for AG-4 antibody as compared to 9% of the controls. Similar differences are not observed between cases and controls with respect to neutralizing antibody to HSV-2. Antibody to AG-4 shows the progression expected for the development of cervical carcinoma, suggesting it may be of diagnostic significance. Thus, of 20 women with cervical atypia, 7 (35%) had antibody to AG-4 as compared to 13 (68%) of the 19 women with carcinoma in situ and 20 (91%) of the 22 women with invasive carcinoma. Antibody to AG-4 correlates with the presence and extent of the tumor suggesting it may be of prognostic significance and reflect active tumor growth. Thus, of 24 women with treated invasive carcinoma and no evidence of recurrence not one had antibody to AG-4, recurrent neoplasia was associated in two patients with persistence of AG-4 antibody. Some of the properties of AG-4 antigen were studied. Preliminary data indicate that it is made between 4 and 16 hours after infection, it does not block the neutralizing activity of human and rabbit anti-HSV-2 sera and is not correlated with presence of virions. Furthermore, it is soluble and appears to differ from the nonvirion antigen of Sabin and Tarro. Preliminary studies by anti-complement immunofluoresence have indicated that AG-4 is a surface antigen, contributing materially to the interpretation of its role in oncogenesis.

Biologic and immunologic properties of the S-1 variant of HSV-2 isolated from cervical tumor cells of S332 G were compared to those of the G prototype. The observed differences further confirm the original conclusion that S-1 is a distinct virus and did not result from the contamination of S332 G cells with the G virus.

Two additional cervical cancer cell cultures were established from biopsies of invasive cervical cancer. Staining of the cells with human and rabbit sera containing neutralizing antibody to HSV-2 revealed the presence in 2% of these cells of membrane fluorescence. The staining appears to be herpesvirus specific as it is not obtained with a human serum without antibody to either herpesvirus type. Intracellular antigens are not discerned in these cells and virus replication cannot be induced by BUDR, IUDR, diethylstilbestrol and medium of high pH. Virions are not observed by electron microscopy. The study on the persistence and expression of the HSV-2 genome in these cells is now in progress.

HSV-2 isolates obtained in Yugoslavia were compared to American prototypes. The results indicate: (i) the Yugoslav variants differ from the American ones in antigenic properties and (ii) one isolate obtained from the cervix causes giant cells, unlike the vulvar isolates.

Significance to Biomedical Research and the Program of the Institute:

Considerable data has been acquired demonstrating an association between HSV-2 infection and carcinoma of the uterine cervix. In humans, it is extremely difficult to conclusively show that virus associated with neoplasms is a factor in oncogenesis and not simply a passenger. Studies conducted under this project are expected to provide some of the data required to reach a decision regarding the role of this virus in oncogenesis.

Proposed Course: Further studies will be made to determine the presence of "early" HSV-2 antigens in cervical tumors, the prevalence of antibodies to this antigen in patients, and the prognostic significance of these antibodies. Cervical tumor cell cultures will be analyzed for expression of persisting HSV-2 genome or of a fraction of a genome. A search will be made for HSV-2 specific antigens on the surface of tumor cells, biopsied, exfoliated, or cultured *in vitro*.

Date Contract Initiated: May 5, 1971

KAROLINSKA INSTITUTE (NO1-CP3-3316)

Title: Studies on the Significance of Herpes-type Viruses and RNA Viruses in the Etiology of Some Human Cancers

Contractor's Project Director: Dr. George Klein

Project Officers (NCI): Dr. Charles W. Boone
Dr. Gary R. Pearson

Objectives: To elucidate EB virus-cell-host interactions, mechanisms of cell-mediated anti-tumor immune reactions, and regulations of type C virus expression in defined systems.

Major Findings: EBV studies showed the virus genome to be linearly integrated into some, but not all, chromosomes of the cell. EBNA, the EBV induced nuclear antigen, was found to associate exclusively with chromatin fibriles in interphase nuclei and with chromosomes in metaphase. Hybrids between cells sensitive and cells resistant to superinfection by EBV showed the sensitivity to be a dominant trait. One T-cell line and two B cell lines were found to be free of EBNA and EBV genome. Tests for EBV in different neoplasms showed that high titers to EBV do not necessarily indicate association of virus with tumor cells. Antibody to EBV-induced MA inhibited transformation of cord blood cells. Further confirmation was obtained of the relationship between high antibody titer to EA and a poor prognosis in BL patients. Circulating antigen-antibody complexes in BL patients are due to the release of MA containing material by EBV carrying cells and not to release of free virus. In the human patient, EA and VCA positive cells are most likely eliminated by opsonization followed by phagocytosis. The sensitivity of target cells to killer T-cells appeared to be a characteristic of each line. Sensitivity may depend on a "blast cell associated" antigen since three EBV genome free lines were also killed. This could be related to HLA rearrangements as suggested by anomalous typing results obtained on established lymphoblastoid lines.

Studies on immunological aspects of human sarcomas and leukemias were continued. The peripheral lymphocytes drawn from 10 of 17 sarcoma cases were stimulated by autologous mitomycin-treated tumor cells and by KCl extracts of the tumor tissue. The reactivity of cells from lymph nodes draining the tumor is often specifically paralyzed against autochthonous tumor cells in patients whose peripheral nodes are reactive. Other experiments suggest these paralyzed lymph node cells carry an accumulation of tumor derived antigen.

Using the anticomplement fluorescence method, a leukemia-associated nuclear antigen (LANA) was detected, predominantly in blast cells of AML. This antigen is not related to EBNA and is demonstrated with autochthonous or allogeneic sera tested against smears of AML blast cells. It was not found in CML or CLL.

In collaboration with other investigators, work was continued on herpesviruses oncogenic in animals and on RNA tumor viruses in mice as models for observations on human materials.

Significance to Biomedical Research and the Program of the Institute:

Investigations under this project are directed to two areas of importance to overall program. First, the recognition that certain herpesviruses induce neoplasms in animals and that EB virus and herpes simplex virus type

2 are associated with human neoplasms requires intensive study to provide a better understanding of the host-virus relationship for this group of agents. Data acquired under this project contributes to assessment of the role of herpesviruses in the causation of human neoplasms. Second, the analysis of the immunological responses of the host to tumor cell surface antigens provides basic information important in approaches to control of tumor development. The project is strongly oriented to human neoplasia, utilizing defined animal systems as required for progress in understanding the fundamental mechanisms involved.

Proposed Course: This project will continue without change.

Date Contract Initiated: April 9, 1968

LIFE SCIENCES, INC. (NO1-CP3-3205)

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

Contractor's Project Director: Dr. Jack Frankel

Project Officers (NCI): Dr. Gary R. Pearson
Dr. Michael A. Chirigos

Objectives: To determine the exact nature of the role of the herpesvirus associated with Marek's disease in the etiology of this disease and the elucidation of the mechanisms of interaction between herpes and viruses in tumorigenesis using specific pathogen free avian hosts.

Major Findings: Studies were carried out to determine whether similar interactions between avian leukosis virus (ALV) and Marek's disease herpes virus (MDHV) occur in vivo as well as in vitro, and the effect on pathogenesis. For this purpose, isolator-derived, barrier-sustained chickens (LSI-SPF) were used since MDHV or ALV infection of this line by contact-exposure for eight weeks did not result in tumors or mortality characteristic of Marek's disease (MD) or lymphoid leukosis. Enhancement of tumorigenesis and mortality occurred when LSI-SPF and conventional chickens were concurrently infected with ALV (RAV-2) and MDHV by contact-exposure.

Examination of serum samples for MDHV neutralizing antibody revealed that, following incubation with MDHV, some of the sera significantly enhanced focus formation in chicken kidney cell (CKC) cultures. The serum enhancing factor (SEF) was originally detected in four week serum samples from untreated conventional and LSI-SPF chickens reared in a facility containing similar birds and that had been previously inoculated with MDHV and RAV-2. Five weeks after contact-exposure to both viruses, conventional sera enhanced focus formation by an average of 308%. Of interest is the fact that uninoculated chickens reared in the RAV-2 monocontaminated

environment exhibited SEF. Following peaking of SEF between four and six weeks, neutralizing antibodies were detected or the chickens died. All LSI-SPF chickens which were surviving at 9 and 10 weeks exhibited neutralizing antibody.

The acute strain of MDHV did not cause the production of foci in chick embryo fibroblast (CEF) cultures and produced high, early mortality with limited gross tumor response when injected into LSI-SPF chickens. The chronic strain of MDHV induced cytologic changes characteristic of MDHV in CEF cultures, and produced low mortality with a delayed, high level of tumor involvement in LSI-SPF birds. The response was comparable to Marek's disease as observed within 4-6 weeks after inoculation of chronic MDHV in conventional chickens. The mortality response of LSI-SPF chickens inoculated with both acute and chronic viruses shows interference with the lethal effect produced by acute MDHV in birds initially inoculated with chronic MDHV. At necropsy, no differences in gross tumor response were apparent between experimental and control groups of chickens when examined after three, four and five weeks. However, of the surviving chickens that were necropsied at eight weeks, the group which had been inoculated with chronic MDHV showed a high level of visceral tumor involvement, as well as large solid tumors in several cases. The highest early mortality was observed among groups of LSI-SPF chickens in which MD-antigen was observed less frequently in feather follicle epithelium.

Concurrent inoculation of LSI-SPF chickens with RAV-1 and MDHV resulted in a marked increase in mortality during 49 days of observation. The mortality incidence among dually-infected birds was 69% compared with 16% and 6% in chickens inoculated with MDHV or RAV-1, respectively. MDHV neutralizing antibodies were detected after 35 days in the group infected with MDHV alone, but were not demonstrated at this time in birds inoculated with MDHV + RAV-1.

Recent studies have shown a difference in the spectrum of cell cultures in which acute and chronic MDHV replicate. Cell-free chronic MDHV produced foci in CKC, chicken embryo fibroblast (CEF), duck embryo fibroblast (DEF), chicken embryo brain (CEB) and chicken embryo eye (CEE) cultures. Contrary to expectation, acute MDHV did not produce foci in any culture except CKC. Neither cells nor supernatant fluids from acute MDHV-inoculated CEF, DEF or DEB cultures contained MD-antigen. Viral DNA has not been demonstrated in CEF cultures inoculated with acute MDHV. On the other hand, MD-antigen (immunodiffusion) and viral DNA were detected in CEF and DEF cultures inoculated with chronic MDHV, and CKC cultures inoculated with acute MDHV. MDHV was rescued from CEB and CEE cells inoculated with acute MDHV by co-cultivation with susceptible CKC cultures, even though the virus-inoculated CEB and CEE cells did not exhibit cytologic changes characteristic of MDHV infection.

Significance to Biomedical Research and the Program of the Institute:

In comparison to the RNA tumor viruses, comparatively little is known concerning the role of herpesviruses in oncogenesis. Certain herpesviruses have been implicated in the etiology of carcinoma, lymphoma and leukemia in

different species of animal and other viruses of this group have been shown to be strongly associated with neoplasia in man. This project provides opportunity to acquire information on one herpesvirus in relation to a malignant disease which may aid in understanding the role of herpesviruses in oncogenic processes in man.

Proposed Course: Studies on the interaction between MDHV and other viruses as this relates to the disease process will be continued.

Date Contract Initiated: November 1, 1968

LITTON-BIONETICS, INC. (NO1-CP3-3211)

Title: Studies on Molecular Events Leading to Transformation by RNA Oncogenic Viruses

Contractor's Project Director: Dr. Alan Wu

Project Officers (NCI): Dr. Robert Gallo
Mr. J. T. Lewin

Objectives: To elucidate the molecular mechanisms by which RNA tumor viruses transform normal cells into malignant cells.

Major Findings: The "virus-like" particles contained in the particulate fraction derived from human leukemic cells was used to synthesize DNA on an RNA template using an RNA primer. Over half of these DNA sequences hybridize to animal tumor virus RNA (SSV-1). The pattern of hybridization to RNA from different leukemia viruses leaves little doubt that the human DNA is synthesized from an RNA related to primate type C RNA tumor virus RNA.

The virus-related reverse transcriptase (RDDP) from human acute myeloblastic and lymphocytic leukemic blood cells was found to prefer RNA from dtrA over dtdA and is immunologically similar to the RDDP from Gibbon ape leukemia virus and SSV-1.

The particulate fraction which contains RDDP also contains a ribonuclease H (RNase H) activity. This is separable by ion-exchange chromatagraphy, thus differing from the pattern observed with AMV. The RNase H molecular weight is estimated at 70,000 daltons and is an endonuclease that uses circular hybrid polymer substrates as efficiently as the linear ones. Additionally, the degradation products contained a higher proportion of tetraribonucleotides.

Hybridization of labeled viral RNA from Rauscher leukemia virus and SSV-1 to an excess of nuclear human DNA has failed to reveal DNA sequences present in leukemic cells which are absent in normal cells. Cytoplasmic DNA from leukemic cells of one patient appears to be unusually rich in viral related

sequences; this is being extended to cytoplasmic DNA from other leukemic patients and to DNA from normal cells.

DNA was identified as the only demonstrable template of the RNA-dependent, i.e., RNase-sensitive DNA polymerase, activity of the high speed pellet of PHA-stimulated normal human lymphocytes. Using identical methods the results with human leukemic leucocytes indicate that RNA serves as template.

Cell lines have been established from human leukemic leucocytes and are being examined for: 1) viral genetic information in the nuclear DNA; 2) viral-specific RNA; and 3) endogenous RDDP by biochemical and immunological criteria. Positive results have been obtained for two cell lines on one or more of these tests.

The measurement of genetic relatedness using viral-synthesized DNA and viral RNA has been extended to include primate, feline, and avian viruses. The divergence of viruses from two different animals parallels the divergence of the host animals. The results indicate that RNA tumor virus information, or a portion of it, originated from the host cell. It also suggests that human RNA tumor virus information is related to animal RNA tumor virus information in the same way that humans are related to the natural hosts of the animal RNA tumor viruses. This was verified using the DNA synthesized endogenously by viral-like particles of human leukemic cells.

It was demonstrated that virus induction may be enhanced by dexamethasone in several systems. Cells producing a high titer of virus or cells infected with a high titer of virus were not stimulated significantly by dexamethasone, while cells producing a low titer of virus or infected with a low titer of virus were stimulated 3 to 20 fold by dexamethasone.

Significance to Biomedical Research and the Program of the Institute:

It is known that RNA tumor virus genomes within cells may be repressed and in some instances may be defective. Molecular biological methods are valuable to probe cells for evidences of virus expression. Under this project, criteria were defined to differentiate between purified tumor virus RDDP activity and the activity of the purified major DNA polymerases of normal cells. The RDDP activity in particulate fractions separated from leukemic human cells was purified, characterized, and found to resemble the virus enzyme. This work contributes to the search for evidence of viral expression in tumor cells and provides a basis for studies on the inhibition of viral function in the neoplastic process.

Proposed Course: Studies will continue to further define the nature of the virus-like activity expressed in human leukemic cells.

Date Contract Initiated: September 1, 1972

MASSACHUSETTS GENERAL HOSPITAL (N01-CP3-3366)

Title: Characterization of Nucleic Acids of the Avian Myeloblastosis Virus

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

Project Officers (NCI): Dr. George Vande Woude
Dr. Maurice L. Guss

Objectives: The primary objective is the determination of the possible molecular mechanisms by which oncogenic viruses change the metabolism of susceptible host cells. In order to define this system, long range biochemical studies on the RNAs of AMV have been initiated and involve the chemical characterization of the nucleotide structure of viral RNAs and biochemical functions of the viral RNA fractions as measured by their activity in conjunction with RT from the virus and the ability of the 4S RNA fractions to accept amino acids.

Major Findings: The high molecular weight RNA of the avian myeloblastosis virus ends in an adenosine residue at the 3'-OH terminus. It was determined that the 3'-OH end of the 35S RNA consisted of a poly adenylic acid segment at least 30 residues long. Therefore, if there are subunits of the 70S RNA complex consisting of two or more distinct 35S sequences, they all terminate in a poly(A) sequence. No internally located poly(A) segments were found. This is the first report of such findings. The 3'-OH poly(A) segment therefore may serve in future sequencing operations as a convenient marker for the 3' terminus of the 35S molecule, and as a very useful site for hydrogen bonding of a primer dT segment and for constructing a DNA complementary strand using labeled deoxynucleoside triphosphates. Such a labeled oligodeoxynucleotide would then be amenable to sequencing operations.

At least one half of the "70S-associated" AMV 4S RNA is composed of transfer RNA. The minor base constituency of this 70S-associated 4S RNA has been determined to differ quantitatively in important particulars from the minor base patterns of total AMV virion tRNA, and of the mixed total tRNA's of chick liver and myeloblasts. The 70S-associated 4S RNA also esterifies certain amino acids. These observations have helped lay the groundwork for the very recent unpublished observations of others that a specific 4S RNA serves as a primer for the transcription of DNA from the 70S RNA of Rous sarcoma virus.

Using the sensitive procedure for minor base detection developed by the Randeraths evidence has been found for the presence of small amounts of minor bases in the 35S RNA of AMV. These observations need further scrutiny, however, in order to eliminate the possibility of artifacts or of continued non-covalent association of small amounts of tRNA to this high molecular weight RNA fraction.

Progress is being made on improvements of the earlier Yu-Zamecnik-Gilham periodate-amine method for sequencing RNA, which will render the procedure more quantitative during each elimination step, and more sensitive by virtue of using radioactive amines. Chemical procedures have been developed

for labeling the 3'-OH and 5'P ends of high molecular weight RNA with P^{32} of high specific activities.

An antigenic protein is produced by Hodgkin's disease tumor nodules after growth in tissue culture. This antigen is not found in a variety of other human cells in culture. A particulate component is released into the medium, which can be pelleted at high centrifugal forces, banded in sucrose gradients at a peak between 1.15 and 1.21 sp. g., and labeled with ^{3}H-TTP or ^{3}H-UTP. There is no cross reaction of this component against a variety of known animal viral antibodies.

Significance to Biomedical Research and the Program of the Institute:

This project was initiated in the expectation that elucidation of the nucleotide sequences of the 70S RNA of AMV and possibly other oncogenic RNA viruses might reveal segments active in specific functions and as binding sites of viral polymerases or inhibitors, thereby increasing our knowledge of transcription processes. Elucidation of the differences in t-RNA encapsulated in virions and those present in normal and infected cells might show how virus infection demonstrates translation processes.

Proposed Course: Studies will continue on the analyses and the acromolecular sequencing of large molecular weight RNA of AMV and on the analyses of the minor base components of the transfer RNA of AMV.

Date Contract Initiated: June 29, 1971

MASSACHUSETTS GENERAL HOSPITAL (NO1-CP4-3222)

Title: Activation of Oncogenic Viruses and Induction of Cancer by Immunologic and Non-immunologic Methods

Contractor's Project Director: Dr. Paul H. Black

Project Officers (NCI): Dr. Michael A. Chirigos
Mr. J. T. Lewin

Objectives: To determine the relationships between chronic allogeneic disease, immunosuppression, and interferon inducers on the activation of covert infections by oncogenic RNA viruses.

Major Findings: Interferon treatment prevented virus activation in the graft versus host reaction (GVHR). As an extension of these studies, one half of a large group of GVH mice were treated with interferon, the other half not. Although these studies are not yet completed, it is apparent that inteferon can also prevent GVH-virus-induced oncogenesis; several animals in the non-interferon group have developed lymphomas whereas none in the interferon group has.

Interferon-containing preparations have modest immunosuppressive effects *in vivo*. These effects correlate well with *in vitro* studies demonstrating inhibitory effects of interferon preparations on mitogen-induced lymphocyte blastogenesis. Since interferon preparations have only partially been purified, it is not known whether the same molecule has both anti-viral and immunosuppressive capability. However, studies to date suggest that the anti-cellular and anti-viral properties are inseparable, and that effects on cell DNA synthesis increase in parallel with anti-viral effects as the purity of the interferon preparation increases. Interferon may well be a lymphokine that serves as a feedback inhibitor of lymphocyte function; it is produced by lymphocytes undergoing blastogenic transformation, but once present in sufficient quantity, it may inhibit further cellular proliferation. Since rejection of skin allografts is dependent on lymphocyte-mediated immunity, the effects of interferon are probably a result of its lymphocytic inhibitory properties. Although interferon, by itself, had only modest effects on graft rejection, in combination with other agents it may well prove to be a clinically useful agent. Many patients undergoing immunosuppressive chemotherapy are extraordinarily susceptible to viral infections, and the majority of the viral agents implicated in these infections are sensitive to the actions of interferon. An additional potential bonus of interferon treatment in such patients is its established inhibitory effect against tumors not known to be associated with viruses. Thus, interferon has three properties which suggest that in patients, such as transplant recipients, it may be a helpful agent for adjunctive immunosuppression. These properties are its capacities to 1) immunosuppress, 2) inhibit viruses and 3) inhibit tumor growth. In non-transplant patients with mild autoimmune types of reactions, interferon by itself might be sufficient immunosuppressive therapy.

In studies on the site of activation of leukemia virus following skin transplantation, virus first became detectable in regional nodes and spleens between 1-2 weeks after grafting. Thereafter, virus multiplied to high titers in spleens, but was never detectable in skin graft sites, thymuses or tail segments. These studies strongly suggest that MuLV is activated from recipient lymphoid cells responding to foreign skin graft antigens in sites where lymphoid blastogenesis is maximum.

Significance to Biomedical Research and the Program of the Institute:

This project contributes new information concerning immunological factors involved in the activation of covert virus and its influence on the development of virally-induced cancer.

Proposed Course: The present studies on the interactions of immunostimulation, immunosuppression, and leukemia virus activation in the murine model systems will be extended. Studies are planned to expand these observations to include similar situations in man.

Date Contract Initiated: September 15, 1971

MELOY LABORATORIES (NO1-CP2-2020)

Title: Cell Biology Facility: Mechanisms of the Immune Response to Squamous Cell Carcinoma, Adenocarcinoma, and Fibrosarcoma in the Mouse and Experimental Immunotherapy

Contractor's Project Director: Dr. Kenneth Blackman

Project Officers (NCI): Dr. Charles W. Boone
Mr. J. T. Lewin

Objectives: To elucidate the mechanisms of specific tolerance to tumor exhibited by the cellular immune system in a tumor-bearing animal, to develop improved *in vitro* and *in vivo* assays for detecting tumor specific antigens and antibodies, and to develop systems of immunotherapy.

Major Findings: The augmentation of tumor transplantation antigens (TTA) by vesicular stomatitis virus (VSV) was further studied. An homogenate of VSV infected E4 tumor cells was inoculated into the foot pads of mice that had been injected two days earlier with one million tumor cells. Nine of 25 tumors so treated regressed, whereas there were no regressions in the control groups. This experiment is encouraging in that tumor cells that had been growing for two days underwent regression after treatment.

Efforts are now being concentrated on using a Moloney virus induced ascites lymphoma of strain A mice as the basic model system to study the VSV-TTA augmentation effect. Frozen stocks of VSV infected cultured lymphoma cells are being routinely prepared. The lymphoma system was selected because of its potential applicability in developing models for the treatment of human lymphomas and leukemias.

Conclusive evidence has been obtained that the virus antigen acts as a helper antigen to stimulate the immune system to a more intensive response against the TTA. The virus must infect the tumor cells. Simply mixing egg-grown virus with a tumor cell homogenate does not result in augmented immunogenicity.

Tumor necrotic material from two types of tumors, E4, and LSQ, were radioiodinated by a modification of the routine iodine monochloride method and extensively dialyzed to remove all unreacted iodine. As a control, normal mouse liver was prepared in the same way. In the first group of experiments, ^{125}I-tumor necrotic material was injected intraperitoneally in normal syngeneic mice and daily tail blood samples were taken to determine rate of appearance in blood and percent of nondialyzable material. The amount of radioactive material in the blood is maximal at 1-5 hours and decreased to 10% of this amount in three days. The amount of nondialyzable material varies with the source of tumor necrotic material, but reaches a steady maximum in approximately two days. In a second group of experiments, mice injected with ^{125}I-TMN were exsanguinated at 2, 3 and 7 days post-injection and the serum proteins studied by Sephadex G-200 chromatography, paper and cellulose acetate electrophoresis, and ammonium sulfate salt fractionation. These studies indicate that the molecular weight of

tumor derived proteins varies with the source of tumor although electrophoretic mobilities are similar. The major molecular weight peaks are comparable to globulins (150,000), albumin (68,000) and small molecules (5,000 or less); the major peak in the electrophoretic pattern is in the albumin region with a small peak in the gamma globulin region. Major molecular weight fractions of these serum proteins (separated on Sephadex G-200) have been prepared and lyophilyzed and await further study.

Significance to Biomedical Research and the Program of the Institute:

Prior infection of tumor cells with influenza virus to augment the immunogenicity of specific tumor transplantation antigens in cell-free fractions of tumor cell homogenates provides a method whereby a patient could be immunized without the risk associated with X-irradiated viable cells. Research on the mechanisms of the immune response to tumor could provide methods for manipulation of the immune system to benefit the cancer patient both in diagnosis and in therapy.

Proposed Course: The relationship of cell surface structure to host response to tumor will be continued.

Date Contract Initiated: August 20, 1971

MERCK AND COMPANY, INC. (NO1-CP1-2059)

Title: Research on Oncogenic and Potentially Oncogenic Viruses, Virus Production and Vaccine Development

Contractor's Project Director: Dr. Maurice Hilleman

Project Officers (NCI): Dr. Robert A. Manaker
Dr. Michael A. Chirigos
Mr. J. Thomas Lewin

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

Major Findings: C-type RNA virus vaccine against the leukemia-sarcoma virus complex of cats is being developed as a model to establish technical guidelines for application to man at such time as human type C virus(es) become available. Major areas of accomplishment during the current contract report period were: 1) continued routine production of concentrated purified feline leukemia virus (FLV), 2) formalin inactivation studies with FLV, 3) development of a vaccine assay model system utilizing FSV in young adult cats, 4) study of the immune response to FLV vaccine after single and multiple doses in cats, 5) establishment of immunological assay procedures for detecting humoral immunity (neutralizing and cytotoxic antibodies) and cellular immunity (lymphocytic cytotoxicity), and 6) the

continued production of SPF cats.

Herpes simplex type 2 (HSV-2) was selected as the virus of choice for herpesvirus vaccine development because it has been implicated as an etiologic agent in human cervical carcinoma, it can be propagated and quantitated readily by conventional procedures and susceptible laboratory animals are available for testing vaccine protective efficacy. Efforts are being directed toward developing three types of vaccine: 1) purified viral subunit vaccine (first priority), 2) inactivated virus vaccine by conventional methods, and 3) live attenuated virus vaccine by conventional procedures. During the current contract report period, the major accomplishments were 1) improvements in large volume virus production methodology resulting in a 20-fold increase in the average yield of infectious virus, and 2) the initiation of studies directly concerned with the development of a glycoprotein viral subunit vaccine.

Work related to the approach of immunization with homologous or autochthonous tumor cell antigens administered before or during the tumor latent period or after tumor detection but prior to overwhelming metastases included: 1) the preparation and assay of tumor cell antigens for protective efficacy in two animal-tumor model systems (adenovirus type 12 in hamsters and CBA mice), 2) the study of stimulation of host cell-mediated immunity with lipid conjugated antigen, and 3) the establishment of two new animal-tumor model systems (L_2C guinea pig leukemia in strain 2 guinea pigs and H. saimiri in marmosets).

Significance to Biomedical Research and the Program of the Institute:

If viruses are essential in the genesis of some human cancers, prophylaxis by vaccines to prevent or minimize infection must be evaluated. Similarly, the practical value of vaccination by tumor cell or fetal antigens must be determined. Although greatest benefit would be expected by prevention of viral infections transmitted horizontally, vaccines could be effective in control of tumor development where vertically transmitted virus genetic information is not expressed in the production of proteins until later stages in the life of the host. Viruses may act as essential co-factors in the development of neoplasms, and immunization against such secondary agents could prevent the disease. This project provides for the evaluation of immunological approaches to cancer control to determine feasibility for use in humans.

Proposed Course: The contractor will continue efforts on the evaluation of viral vaccines. Studies on immunological control of RNA virus-induced tumors will use the feline sarcoma/leukemia system and the L_2C guinea pig system. Herpesvirus type 2, which is associated with human cervical carcinoma, and Herpesvirus saimiri, which induced lymphoma and leukemia in lower primates, are systems selected for investigation of measures for control of DNA virus-related oncogenesis.

Date Contract Initiated: March 1, 1971

UNIVERSITY OF NAPLES (N01-CP3-3314)

Title: Studies of Non-Virion Antigens of Herpes Simplex Virus

Contractor's Project Director: Dr. Giulio Tarro

Project Officers (NCI): Dr. Charles W. Boone
Dr. Michael A. Chirigos

Objectives: The determination of the presence of non-virion antigens of herpes simplex virus in tumors of the urogenital tract and of antibody to nonvirion antigens in the sera of cancer patients. These studies should provide more evidence concerning the association of herpesvirus type 2 with tumors of the genitourinary system.

Major Findings: The results of complement fixation tests on 202 serum samples from individuals without cancer and from patients with cancer in 29 different areas of the body showed that only those sera from patients with advanced cancers of the lip, mouth, oropharynx, nasopharynx, kidney, urinary bladder, prostate, cervix uteri, and vulva (56 cases in all) gave a positive specific reaction with the non-virion antigens induced by the oral strain (HSV-1) and the genital strain (HSV-2) of the DNA herpes simplex viruses. None of 57 persons without cancer, including 10 cases of current and 18 cases of recurrent HSV-1 and HSV-2 infections, had serum antibodies reacting with the non-virion antigen. Negative results were observed with sera from 81 patients with other cancers, including cancer of the gum, tongue, tonsil, salivary gland, accessory sinus, epiglottis, lung-bronchus, stomach, colon, breast, corpus uteri, ovary, testis, liver, thyroid, Wilm's embryonal kidney, melanoma, Hodgkin's disease, acute lymphocytic leukemia, and acute myelocytic leukemia. None of four women with early malignant changes in the cervix uteri gave positive serological results. Sera from seven patients with advanced cancer of the lip or oropharynx reacted only with HSV-1 non-virion antigen. The reactivity of sera from thirteen women with advanced cervical carcinoma and one woman with advanced cancer of the vulva was indicative of an association with HSV-2. Sera positive in tests against the herpesvirus non-virion antigens did not react with antigens in cells harvested at different times after infection by vaccinia virus. Furthermore, absorption of positive sera with trypsinized, uninfected human embryonic kidney cells did not affect the titer of the antibodies to HSV-1 and HSV-2 nonvirion antigens.

Of nine HSV-1 strains (from lip, mouth, throat, cornea or brain) only five produced enough nonvirion antigen to be detected by complement fixation with specially prepared, virion-absorbed, type-1 guinea pig antisera, while the remaining four strains produced only enough of the same antigen to induce specific antibody in hyperimmunized guinea pigs. While the type 1 virion antiserum used reacted equally well by complement fixation with the type 1 and type 2 strains, the type 1 nonvirion antisera failed to react with nonvirion antigens produced by three type 2 (genital) strains. However, type 2 nonvirion antiserum reacted equally well with the three type 2 and four type 1 nonvirion antigens that were tested. It appears, therefore, that while HSV-1 codes only for type 1 nonvirion antigen, HSV-2

codes not only for an immunologically distinct type 2 nonvirion antigen but also for enough type 1 nonvirion antigen to stimulate antibody production for it. HSV-2 nonvirion antigen exhibited the same properties as type 1, i.e., its activity was lost on storage at 4°C for 15 days, it was sedimented by centrifugation at 33,360Xg for one hour, and the maximum concentration was found three hours after infection of cultured guinea pig kidney cells, but at 24 hours in HEp2 and rabbit kidney cell culture. Sera from patients with genital lesions caused by HSV-2, as well as from randomly selected adults, failed to react with either type 2 or type 1 nonvirion antigens. Accordingly, the basic information is now available to permit the use of these nonvirion antigens to investigate the possible role of the herpes simplex viruses in the etiology of certain human cancers.

With the use of antibody to herpesvirus nonvirion antigens, complement fixing reactivity has been shown for soluble cell membrane antigens separated from lip and cervical carcinomas but not for similar extracts of cells from normal vaginal tissue or intestinal carcinoma. Neither the serum obtained from the guinea pig before hyperimmunization with the herpesvirus nonvirion antigen nor the antiserum of guinea pigs immunized with comparable uninfected cell extracts reacted with these tumor soluble membrane antigens. Since the above soluble membrane antigens could be specific markers for the presence of virus genome within the tumor cells, the findings could support an etiological role of herpesvirus in selected human malignancies.

Significance to Biomedical Research and the Program of the Institute:

Present evidence indicates that the HSV-2 genome is largely repressed in cervical carcinoma cells. Some virus gene expression is expected if this virus is a necessary factor in the development of this neoplasm. These studies have shown that the sera from patients with cervical carcinoma contained antibodies to HSV-2 induced nonvirion antigen whereas in cancer-free persons with HSV-2 neutralizing antibodies in their sera, no antibodies to nonvirion antigens were detected. This suggests continuing stimulation of the cancer patient's immune system with virus-induced nonvirion antigen produced in the tumor cells, a situation apparently different from the virus-cell relationship in cancer-free persons. Further studies are required to determine the significance of these observations with respect to the virus-tumor relationship.

Proposed Course: The investigation of the immune status of cancer and control populations with respect to the nonvirion antigens induced by HSV-1 and HSV-2 will be expanded to better define the virus-tumor relationship.

Date Contract Initiated: April 9, 1971

NINDS/NCI COLLABORATIVE RESEARCH PROJECT
EMORY UNIVERSITY (NO1-NS2-2301); MELOY LABORATORIES (NO1-NS2-2306)

Title: Collaborative Project on the Oncogenic Potential of Herpesvirus in Primates

Contractor's Project Directors: Dr. Andre Nahmias, Emory University
Dr. John Verna, Meloy Laboratories

Project Officers (NINDS): Dr. John Sever
Dr. William T. London

Project Officers (NCI): Dr. Robert A. Manaker
Dr. Gary R. Pearson

Objectives: To determine whether intravaginal infection of the Cebus monkey by herpes simplex virus type 2 (HSV-2) will induce neoplasia of the uterine cervix.

Major Findings: Pap smears obtained from the cervix of female Cebus monkeys demonstrate similar cellular features as those observed in humans. Of the 164 animals examined prior to inoculation with virus or control tissue culture preparations, two have shown mild dysplasia but were negative on re-examination. Similarly, one animal out of 48 monkeys which received control non-virus-containing preparations showed mild cervical dysplasia and was also negative on re-examination. Five animals which received HSV-2 genitally 1 to 2 1/2 years previously and demonstrated laboratory evidence of viral infection, have shown mild to persistent severe cervical dysplasia.

Prior histological studies of the cervix of Cebus monkeys and the preliminary observations in Atlanta had revealed that the monkey cervix differs in several respects from the human cervix: 1) the squamous epithelium of the monkey cervix consists of a much thinner layer of cells (5 to 6), as compared to that of the human cervix, which is 15-16 cell layers deep. This means that the vasculature in the monkey cervix, with the hairpin capillaries, should be more prominent by colposcopic examination in monkeys than in humans, 2) the squamous epithelium in the monkey occupies about one-third of the endocervical canal, whereas in humans it usually extends only to the cervical os. This suggests that if the major site of cervical anaplasia in monkeys is similar to what has been noted in humans, i.e. at the junction of the endocervix and ectocervix, it is important to look within the cervical os of the monkey, 3) unlike the human cervix, which is relatively smooth, the monkey cervix shows many folds of squamous epithelium and histologically demonstrates a greater amount of subepithelial papilli. Colposcopic examinations have been made on 28 animals. Negative colposcopic findings reflect a normal appearing vasculature. Positive colposcopic findings denote the presence of atypical vasculature, which in humans would indicate cervical cancer. However, because of the thinner cervical squamous epithelium of the monkey, more experience is required before such observations in monkeys can be correlated with cancer. Nevertheless, the correlation between results obtained with cytological and colposcopic examinations was good. Three animals demonstrated atypical

vasculature and cervical dysplasia on Pap examination. In one animal colposcopy revealed a whitish epithelium covering the cervix, so that the vasculature could not be observed. One animal with atypical vasculature had a normal Pap examination. However, sampling errors may not make it possible, on occasion, to detect abnormal cervical cells.

Two large batches of HSV-2 strain BEN (titers 10^6 PFU/0.2 ml) and two of noninfected tissue culture control material have been prepared. All the animals have been tested virologically, serologically and cytologically prior to and periodically after inoculation.

Significance to Biomedical Research and the Program of the Institute:

This project utilizes an experimental lower primate to contribute data to establish a causal relationship between a herpesvirus infecting human genitalia and the induction of cervical carcinoma. The demonstration of an etiological relationship to carcinoma in the primate could provide basic information leading to control measures for humans.

Proposed Course: This project is expected to continue for at least three years following primary infection of animals.

Date Contract Initiated: March 1, 1972

UNIVERSITY OF NORTH CAROLINA (NO1-CP3-3336)

Title: Molecular Studies on Herpes-type Viruses of Potential Oncogenicity

Contractor's Project Director: Dr. Joseph Pagano

Project Officers (NCI): Dr. Maurice L. Guss
Dr. Robert A. Manaker

Objectives: To define at the molecular level the virus-cell relationships for herpes simplex virus type 2 (HSV-2) and human cervical carcinoma and Epstein-Barr virus (EBV) and Burkitt's lymphoma (BL) and nasopharyngeal carcinoma (NPC).

Major Findings: Several batches of HV-2 have been prepared in roller-bottle cultures of HEp-2 cells. Following purification of virus, high molecular weight viral DNA is extracted with pronase and SDS and purified by two cycles of isopycnic centrifugation. The *in vitro* method of labelling EBV DNA by repair synthesis with Kornberg DNA polymerase I has been adapted to HV-2 DNA. The specific activities obtained in several batches ranges from $1x10^6$ to 3.8×10^6 cpm/ug.

Reconstruction studies of DNA renaturation kinetics analysis indicated that 0.1 genome of HV-2 is detectable when 2000 ug of cellular DNA are tested. No host cell (HEp-2) DNA sequences were detected in *in vitro* labeled HV-2

DNA. Five biopsy specimens from Taiwanese patients with cervical carcinoma were examined and failed to show any evidence of HV-2 DNA at a level of 0.1 to 0.2 genome per cell on the average if the whole genome was represented.

Using the cRNA-DNA hybridization technique on membrane filters to quantitate viral DNA in malignant tissue, 24 of 26 Burkitt tissues from Kenya were shown to contain 113 to 5 genome equivalents per cell on the average. Both negative tissues were also negative by the EBNA test. In 32 of 38 specimens of African NPC, 85 to 5 genome equivalents were measured. Tumor tissue from NPC patients in Taiwan and Tunis were also shown to contain EBV DNA. The homologous DNA in positive specimens bore at least 95% identity to viral DNA of HR1K origin. In situ hybridization tests are being conducted to determine the type of cells which contain the viral DNA. Peripheral blood leukocytes from African patients with BL or NPC do not contain EBV DNA. None of 19 tumors of American origin nor of eight from four American Burkitt's lymphoma patients contained detectable EBV DNA.

All lymphocytic cell lines tested to date which have the characterization of B-lymphocyte origin and which were established from peripheral blood contained EBV DNA in amounts ranging from 510 to 5 genome equivalents per cell. The T-cell lymphocytic cell lines, MOLT and one established by Moore and Minowada did not contain EBV DNA. The MOLT line does not appear to be susceptible to infection with high concentration of EBV.

Significance to Biomedical Research and the Program of the Institute:

The studies accomplished verify the close relationship between EB virus and cells in BL biopsies. The continuing refinement by the contractor of the molecular methods provide useful tools in the study of viral associations with tumor cells where repression of virus genetic expression masks the presence of infection.

Proposed Course: The molecular aspects of the relationship between HSV-2 and cervical carcinoma will be emphasized.

Date Contract Initiated: April 25, 1972

PENNSYLVANIA STATE UNIVERSITY (NO1-CPO-2024)

Title: Studies on the Oncogenic Potential of Defective Human Viruses

Contractor's Project Director: Dr. Fred Rapp

Project Officers (NCI): Dr. Robert A. Manaker
Dr. Michael A. Chirigos

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

Major Findings: New isolates of herpes simplex virus type 1 (HSV-1) and type 2 (HSV-2) were screened for transforming ability; the tests revealed that 18% of HSV-1 and 52% of HSV-2 strains examined were capable of transforming hamster embryo fibroblasts (HEF) after the viruses were inactivated with ultraviolet light. A number of the established cell lines induced tumors when inoculated into newborn hamsters. In addition, preliminary results suggest conversion of human cells held at elevated temperature. The 3T3 mouse cells appear to be a relatively sensitive target for transforming experiments. The newer technique of inactivating virus photodynamically has revealed that some strains previously thought to be nontransforming can morphologically convert hamster cells under appropriate conditions.

Studies with cytomegalovirus (CMV) have yielded strong evidence that this agent can induce host DNA synthesis under permissive and nonpermissive conditions. Cells transformed by CMV were shown to contain virus-specific antigens, especially at the cell surface, by a variety of immunologic methods. They do not appear to exhibit tumor-specific transplantation agents, a finding similar to that previously made with HSV-transformed cells. An additional antigen now detected in some HSV-transformed cell lines is the specific glycoprotein, CP-1. As this antigen elicits cross-neutralizing antibodies against HSV-1 and HSV-2, their occurence in tumor-bearing animals is probably a response to continued synthesis of CP-1.

Inhibitors have been isolated from the herpes transformed cells that block replication of HSV in permissive cells. It has been demonstrated that the inhibitors under scrutiny are not interferon but their molecular activity is unknown. A similar inhibitor, capable of interfering with the replication of the Epstein-Barr virus (EBV) and CMV has also been isolated.

Additional somatic cell hybrids were developed incorporating mouse, human, and EBV lines. The results with a variety of hybrids are consistent with the hypothesis that synthesis of the EBV intranuclear antigen (EBNA) is associated with the chromosome carrying the EBV genome and that the cell harboring that genome controls expression of selected virus functions.

Significance to Biomedical Research and the Program of the Institute:

This project was established to determine whether viruses producing common diseases in man might, under appropriate conditions, establish a chronic cellular infection culminating in neoplastic transformation. The results obtained with herpesviruses commonly afflicting humans suggest that these viruses have oncogenic properties that may be expressed in humans. The failure to find all strains of HSV-1 or HSV-2 to possess similar transforming properties in addition to information reported by other laboratories suggests that strain differences among these two herpesviruses do exist and must be considered in studies to determine their relationship to oncogenesis in man.

Proposed Course: Studies will continue to determine the nature of the relationship established between virus and the transformed cells.

Date Contract Initiated: October 27, 1969

ROCKEFELLER UNIVERSITY (NO1-CP3-3306)

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

Contractor's Project Director: Dr. Hidesaburo Hanafusa

Project Officers (NCI): Dr. Charles W. Boone
Dr. Robert A. Manaker

Objectives: To apply methods used successfully to isolate viruses from covert infections in animals in an attempt to isolate viruses from human cancers and to characterize the viruses so recovered.

Major Findings: During the past year 23 solid tumors, mostly sarcomas, have been examined for the presence of oncogenic viral agents. No biologically active virus was demonstrated; occasionally virus-like particles have been detected but none of them was reproducibly confirmed. Since viral etiology of human sarcoma and leukemia has been proposed by molecular hybridization studies, attempts have been made to detect viral products in the tumor specimens using the simultaneous detection method. Out of 20 samples tested six gave positive results. Since the application of this technique in combination with other techniques is important, the test will be made in the early phase of examination of tumor specimens. The positive specimens will be given closer attention by other methods.

One of the tumor cells (T-59) derived from a chondrosarcoma grew relatively well and was available for various tests. Morphologically these cells are refractile, spindle-shaped or round forms. They form colonies in soft agar, though the development is relatively slow. The saturation density is not particularly high. Electron microscopically, cytoplasmic granules containing mucopolysaccharides were observed in T-59 cells, indicating that these cells retained the activity of the original tissues. Further characterization concerning the phenotypic expression must be performed to determine whether or not they are malignant cells. An attempt to produce tumors in newborn hamsters was not successful.

The presence of virus-like particles in various cell lines of human origin has been reported. The characterization of these virus particles is important, since they could be viruses indigenous to the human cells and might serve as a good helper virus for the possibly defective human tumor viruses. Thirteen established human cell lines, four lines of human fetal fibroblasts, and Vero cells were examined. Cultures were treated with IUDR and were subjected to tests which included the detection of radioactive virus in sucrose gradient and detection of the polymerase positive particles

in culture fluids. In contrast to published results, most of the cultures were negative for the virus particles. Radioisotope labeled virus was found only in the culture fluid of Detroit-98, with and without IUDR. Particles were also found by electron microscopy. However, they were negative in the polymerase assay. Thus these particles appear to be either a virus other than RNA tumor virus or a polymerase negative virus. A polymerase positive virus was found in cultures of J111 following treatment with IUDR. The characterization of the product of the polymerase reaction is being performed. Virus particles have also been seen in Vero cells by electron microscopy, but could not be demonstrated in other tests. Further characterization is being done. Since most of these cell lines easily produce colonies in soft agar, clones of cells have been made. The possibility still remains that some of the clones selected in the rapid colony formation could express more virus-related functions. An analysis of these clonal lines will be made.

Since the beginning of this project 79 specimens have been examined without a single positive. However, only a few tumors were derived from young patients. It is conceivable that most tumor specimens derived from older patients may have developed slowly under circumstances where various immunological reactions could have taken place or treatment with various drugs may have occurred.

The maintenance of growing tumor cells in reasonable numbers is very crucial for meaningful studies, since normal fibroblastic cells can easily overgrow the more slowly growing tumor cells. A trial to apply initial selection of tumor cells by plating cells in soft agar directly from tumors has not been successful. It is very important, therefore, to develop criteria for the identification of tumor cells. The discovery of a specific protease in transformed cells will be useful for this purpose and may be helpful in determining survival of tumor cells on transfer.

Significance to Biomedical Research and the Program of the Institute:

Since virally-induced sarcomas occur in mice, rats, chickens, and some nonhuman primates, the human may be no exception. Methods developed in animal systems which permit recovery of RNA tumor virus from covert infections are being applied systematically to human sarcoma and leukemia tissues in attempts to demonstrate similar viral associations in the human neoplasmas.

Proposed Course: The research effort will continue as planned using the culture techniques successfully applied to maintain neoplastic cells in continuous culture for intensive study.

Date Contract Initiated: April 27, 1971

RUSH-PRESBYTERIAN-ST. LUKE'S MEDICAL CENTER (NO1-CP3-3219)

Title: Studies of Tumor Viruses in Small Primates

Contractor's Project Director: Dr. Friedrich Deinhardt

Project Officers (NCI): Dr. Robert A. Manaker
Dr. Michael A. Chirigos

Objectives: To study selected viruses and virus-induced neoplasia in marmosets.

Major Findings: Cross-reacting cell membrane antigens were identified on marmoset cells transformed by Rous sarcoma virus, feline sarcoma virus, and simian sarcoma virus (SSV-1). The complement-dependent serum cytotoxicity test was used for these studies. Additionally, marmoset cells could be transformed by the murine sarcoma virus, the polyoma virus, and the SV-40 virus.

SSV-1 was capable of inducing brain tumors (gliomas) by intracerebral inoculation of neonatal white-lipped marmosets.

Studies with herpesvirus ateles demonstrated that this species has a tropism for T-cells in vivo similar to the T-cell tropism of herpesvirus saimiri in susceptible hosts.

Although marmosets inoculated with several Epstein-Barr virus (EBV)-transformed cell lines have shown no evidence of disease, the B95-8 line of Shope and Miller induced lymphoma in marmosets. Hybridization studies with tritiated EBV DNA indicate approximately 20 to 10 genomes per B95-8 cell.

Marmosets inoculated with the Colburn strain of cytomegalovirus did not develop overt disease but became sero-positive.

Differences were observed in lymphocytotoxicity assays against allogeneic, cultured breast cancer cells and control fibroblasts between patients with slowly progressive disease and both those with rapidly progressive disease and controls.

Significance to Biomedical Research and the Program of the Institute:

Restrictions in the host range of viruses require that a primate animal be available for study of viruses isolated from human tumors. The marmoset has been proven to be responsive to the oncogenic activity of viruses of lower animals and of other primates. This project has contributed considerable information, not only on oncogenic RNA viruses, but has been contributing substantially to understanding of the role of herpesviruses in oncogenic processes.

Proposed Course: Emphasis will be placed on the RNA-and herpesviruses associated with neoplasia in nonhuman primates and viruses recovered from human neoplasms as these became available.

Date Contract Initiated: March 15, 1962

RUTGERS UNIVERSITY (NO1-CP1-2077)

Title: Studies on Genetic Acquisition of Oncogenic Potential by Nononcogenic RNA Viruses

Contractor's Project Director: Dr. Robert W. Simpson

Project Officers (NCI): Dr. Michael A. Chirigos
Dr. John W. Pearson

Objectives: To determine whether a nononcogenic RNA animal virus can acquire tumor-producing or cell-transforming capability as a consequence of host-induced genetic modification of the viral RNA, by intracellular persistence of incomplete but functionally active viral genetic material, or by induced mutation.

Major Findings: Moloney MSC-M mouse tumor cells underwent marked polykaryocytosis when infected with highly cytolytic vesicular stomatitis virus (VSV) or mutants thereof, resulting in the formation of giant, syncytial spherules. The phenomenon was multiplicity dependent. MSC-M cells originally infected at nonpermissive temperature with a double conditional lethal mutant of VSV which is temperature-sensitive (ts) and host-restricted (hr) in certain cells were spared from cytopathic effects and showed no shedding of VSV when shifted to permissive temperature. Evidence for persistence of VSV genomes in these cells could not be demonstrated by testing for resistance to superinfection with VSV, treatment with chemicals capable of activating oncornaviruses, by exposure of cells to chemically-inactivated VSV or by immunofluorescent monitoring for viral antigens. MSC-M cells productively infected with VSV yielded viral progeny which was strongly neutralized (0.01%) survival) by VSV antibody but not Moloney virus antibody (Fisher rat serum) using either avian CEF or murine L cells for the plaque assay. The failure to detect VSV pseudotypes or phenotypically mixed virus could be due to a low potency of the Moloney antiserum used. Infection of MSC-M cells with the arbovirus, Guaroa, resulted in cell destruction and no apparent change in the plaque characteristics of the infecting virus when assayed in CEF cells.

A line of guinea pig embryo (GPE) cells was established by a nonproductive infection with the small plaque, temperature-sensitive mutant, VSV hrl/ts, at nonpermissive temperature. Despite the fact that the GPE/hr 1 cells showed no virus shedding when shifted to permissive temperature 19 days after the original infection, superinfection of these cells with the VSV hr 1/ts mutant gave rise to a viral population showing wild-type phenotype for the ts, hr and plaque size markers. These markers were stable following single passage in normal GPE cells. After several cell generations, GPE cells originally infected at nonpermissive temperature with WSN influenza ts

mutants failed to show persistence of viral genomes based on negative effects of superinfection with complementing mutants (nonpermissive temperature) or attempts to activate infections by treatment with appropriate chemicals (BUDR, DMSO, etc.) after shift to permissive temperatures.

Significance to Biomedical Research and the Program of the Institute:

Comparatively little is known of viral interactions or the long-term effects of genetically-modified viruses with respect to oncogenic processes. This project was initiated to acquire information in this area.

Proposed Course: This project terminated on December 31, 1973.

Date Contract Initiated: February 15, 1971

UNIVERSITY OF TEXAS (NO1-CP3-3304)

Title: Studies on the Relationship of Viruses to Human Neoplasia

Contractor's Project Director: Dr. Leon Dmochowski

Project Officers (NCI): Dr. Gary R. Pearson
Dr. Robert A. Manaker

Objectives: To pursue a systematic study of selected human patients with neoplastic disease to establish the association of viruses with their cancers.

Major Findings: Spontaneous cell transformation has been observed in cultures derived from six human neoplasms. The transformed cells gave positive cytoplasmic and perinuclear fluorescence with sera of a majority of patients with different types of neoplasia. Absorption tests indicated that this fluorescence reaction was due to tumor antigens. In attempts at induction of virus production, 53 co-cultures of cells derived from human osteosarcomas, with cells from bone marrow or peripheral blood of patients with different types of leukemia were studied in short and long-term cultures. Morphological changes were observed in seven of 13 long-term co-cultures, indicative of transformation resembling that induced by RNA tumor viruses. This transformation was accompanied by the appearance of a new antigen, as shown by fixed immunofluorescence tests with sera from patients with osteosarcoma. Absorption of these sera with heterophile and Forssman-like substances, whole human embryo cells, and osteosarcoma cells demonstrated the reaction to be due to a tumor antigen(s) present in the transforming cells. Electron microscope examination of the transformed cells has failed to reveal type C particles.

Transformed cells derived from a culture of human fibrosarcoma which transformed following treatment with bone marrow aspirate from a patient with acute lymphocytic leukemia grow progressively in immunosuppressed mice

and produce high molecular weight (HMW) RNA. The transformed cells following growth in immunosuppressed mice and re-establishment in tissue culture have revealed the presence of typical type C virus particles. Medium from these cultures contained 68S RNA. The cells have been found to be of human origin by karyotype and by mixed hemadsorption tests with anti-human cell sera.

The simultaneous detection assay has been used to detect fast-sedimenting DNA species in concentrated tissue culture fluid from spontaneously transformed cells from the bone marrow of a patient with acute lymphocytic leukemia and from bone marrow cells of a patient with melanoma. Employing the exogenous poly (rA):poly (dA) template preference assay to test for the presence of RT activity, particulate components in high-speed pellets of culture fluids from 25 selected human cell cultures failed to give positive results. In subsequent tests on animal cell cultures known to be releasing RNA tumor viruses, two of 10 cultures also failed to give positive results when high-speed pellets were tested. However, upon partial purification of the fluids by isopycnic centrifugation, these two cultures gave positive results. This suggested that certain cell cultures might be releasing inhibitory substances, such as nucleases, into the medium. A series of studies directed toward the detection and characterization of HMW RNA species released into the culture fluid by human neoplastic cells has been carried out. A number of human tumor cell cultures were found to release particulate components containing HMW RNA species with sedimentation coefficients similar to the genomic RNA or RNA tumor viruses. Molecular nucleic acid hybridization tests have demonstrated that a nucleotide sequence homology exists between the RNA genome of RD-114 and Crandell virus.

Sera of mice bearing spontaneous mammary cancer have been found to contain antibodies to cells of their own and homologous mammary cancers, as shown by mixed hemadsorption and immunofluorescence tests. Absorption of such sera with a variety of materials has shown that sera of mice bearing spontaneous mammary cancer contain antibodies to a complex of viral, tumor, and heterophile antigens of mouse mammary tumor cells. Twenty-six percent of sera from normal mice contained similar types of antibodies compared with 44% of sera of mice with spontaneous mammary cancer. Studies utilizing the immunoperoxidase technique have demonstrated in sera of mice bearing spontaneous mammary cancer and in sera of tumor-free, but of older age (nine months) mice, the presence of antibodies against budding and type V virus particles in the tumor cells, but not type A particles, a precursor of type V particles. Suitable absorption experiments have shown that this reaction is specific for mouse mammary tumor virus. Similar studies appear to indicate the presence in the sera of some patients with breast cancer of antibodies labeling with peroxidase mouse mammary tumor virus particles. These studies strongly indicate the association with human breast cancer of a virus antigenically related to mouse mammary tumor virus.

Recent studies have demonstrated in some human breast cancers the presence of particles resembling type C particles, intracisternal type C particles, and particles resembling type H particles originally observed in hamster tumors and then found in some mouse mammary tumors.

Significance to Biomedical Research and the Program of the Institute:

Current program requires intensive, systematic investigations on selected human neoplasms to detect viral association with the disease process.

Proposed Course: This project will terminate on October 31, 1974.

Date Contract Initiated: March 19, 1965

SUMMARY REPORT

4. IMMUNOLOGY-EPIDEMIOLOGY SEGMENT

July 1, 1973 to June 30, 1974

During this fiscal year, the Immunology-Epidemiology Segment concentrated most of its efforts on developing programs related to the viral etiology and control of human leukemia, lymphoma, breast cancer and sarcoma. Seventeen contracts participated in these immunological and epidemiological studies throughout the entire fiscal year; an additional four contracts were initiated and a fifth one was transferred to the segment in the second half of the fiscal year. Completion of workscope and/or the decreased emphasis on tumor immunology due to the establishment of the Tumor Immunology Program in the Division of Cancer Biology and Diagnosis led to the phasing out of four I-E Segment contracts and the transfer of two others to the Tumor Immunology Program.

A major emphasis on the role of herpesviruses in human lymphoma was reflected by the participation of eleven I-E Segment contracts in this program area. Under the supervision of the International Agency for Research on Cancer (IARC), a prospective study continued to obtain sera from healthy African children prior to the onset of Burkitt's lymphoma (BL). Four cases were detected in which serum was available for study prior to the onset of disease and subsequent to diagnosis, making it possible to study whether EBV sero-negativity was a prerequisite to the development of BL. The role of malaria in the etiology of BL, formerly investigated by Makerere University, is now being studied by IARC. The seroepidemiology studies performed by IARC are complemented by a contract at Children's Hospital of Philadelphia, where new serological techniques [including measurement of neutralizing antibodies to EBV and complement-dependent fluorescent antibodies to EB nuclear antigens (EBNA)] were applied to longitudinal studies on patients with cancer and controls in an attempt to determine which antibodies correlate with the stage of disease. Further documentation of the relationship between high antibody titers to the early antigen and poor prognosis was obtained in BL and nasopharyngeal carcinoma (NPC). In addition, screening of cell lines for EBNA demonstrated that at least one lymphoblast culture derived from a patient with Hodgkin's disease was free of EBV. Antibody to EBNA was routinely studied in human sera; tests indicated that this antibody appears late in the course of infectious mononucleosis in most cases and thereafter appears to persist for life. The titration of EBV-associated antibodies, therefore, may now be applied to specific cases of infectious mononucleosis, and the diagnosis may be made more readily even if pre-disease sera is not available. Attempts to purify the early antigen and other EBV-related antigens were initiated at the Medical College of Pennsylvania, and studies performed by TRW on the EBV-associated soluble antigens indicated that the "S" antigen is not identical in BL and NPC lines. Assays measuring cell-mediated immunity to EBV-related antigens were carried o[illegible] at George Washington University where it was shown that lymphoma patients commonly developed *in vivo* and *in vitro* evidence of immunity to antigens on lymphoid

cells obtained from lymphoma patients but not on cells derived from normal individuals.

Epidemiological approaches to the viral etiology of Hodgkin's disease were conducted by the National Center for Disease Control (NCDC) and Hebrew University. At NCDC, an evaluation of infectious mononucleosis as a premalignant disease was initiated by identifying approximately 7,000 cases of infectious mononucleosis with matched controls at selected U.S. colleges. Information has been collected on approximately 1,800 case-control sets at the University of Nebraska, Georgia Tech., and the University of California in Santa Barbara, and data are presently being collected for an additional 2,100 cases at the University of California at Berkeley and at Yale University. Studies to detect inter-personal transmission of leukemia and lymphoma, especially Hodgkin's disease, are being performed using Connecticut Tumor Registry data, and involve identification of cases of leukemia and lymphoma among present and former teachers and students in Connecticut high schools. Similar studies are being carried out in Israel where, in addition, evaluation of the migration patterns in determining the age and histological subtype of lymphoma is possible because of the availability of a complete population registry and cancer registry for this defined geographic area. Two new contracts emphasizing studies on the viral etiology of Hodgkin's disease were developed with the International Union against Cancer (UICC), which will concentrate on aspects related to the early onset of Hodgkin's disease in children in Colombia, and the Aichi Cancer Center, which will investigate the reasons for the apparently low incidence of Hodgkin's disease in young adults in Japan in comparison to the incidence in Japanese who have moved to Hawaii.

An animal model for induction of lymphoma by a naturally-occurring horizontally transmitted herpesvirus was initiated at the Delta Regional Primate Center, where successful chemotherapeutic immunosuppression of squirrel monkeys made it possible to determine whether *Herpesvirus saimiri* can induce tumors in a natural host.

The viral etiology of acute leukemia was suggested by immunological studies at Johns Hopkins University, where an antibody was identified in normal individuals that appeared to be cytotoxic for human lymphoblasts. *In vitro* assays for cell-mediated immunity also indicated that relatives of leukemia patients and laboratory investigators had greater immunity against tumor-associated antigens than random controls, thus providing evidence for an environmental agent associated with the etiology of human acute leukemia. Identification of leukemia clusters, with an emphasis on multiple case families, was pursued by NCDC. This group provided VCP investigators with sera for identification of relevant antibodies and supplied lymphocytes for HLA typing to detect patterns of leukemia susceptibility. Leukemia viruses, particularly agents reported to transform donor bone marrow cells given to irradiated leukemic transplant recipients, were sought at the University of Washington. A canine model system was successfully developed during the past contract year, making the study of a possible viral transformation of donor cells a feasible one. Two contracts (Scripps Clinic and Research Foundation and Mt. Sinai School of Medicine) evaluated spontaneous leukemia in mice as a model for humans. At Scripps, antigen-antibody complexes in the kidneys of

leukemic mice were purified and studies were initiated to identify similar complexes in the kidneys of humans with neoplastic disease. Using kidneys from African patients with BL as a model, the contractors successfully isolated antigen-antibody complexes relevant to EBV, proving that the methodology is successfully operating. The Scripps group is also studying kidneys provided by the investigators at Mt. Sinai who are able to cure AKR mice of spontaneous leukemia. This successful chemoimmunotherapy, which is able to provide an excellent model for the cure of human leukemia, is studying the role of antiviral immunity in the successful control of the disease in order to provide additional information regarding reinduction of the disease as a possible obstacle to long-term control. Another approach to chemo-immunotherapy was developed at the University of Texas, M.D. Anderson Hospital where immunization of leukemia and solid tumor patients with a formalinized RNA virus (Rauscher leukemia virus) demonstrated that cancer patients were not tolerant to oncogenic viruses. Serum was generated from these studies which will now make it possible to further analyze the specificity of previously reported cross-reactivity between oncogenic animal RNA viruses and human leukemic cells.

Relevant studies on the relationship of fetal and embryonic antigens present on or within virus-induced cancer cells were pursued by the Atomic Energy Commission. Using a variety of *in vivo* and *in vitro* techniques in hamsters, characterization of these antigens was attempted. To date it has been demonstrated that CMI directed against SV40 targets is present within four weeks after initial infection of newborn hamsters with SV40. These affected cells are coated *in vitro* with soluble or reversibly bound inhibitors presumably preventing action *in vivo*. Studies aimed at distinguishing between TSTA and embryonic antigens were carried out, and it was concluded that antibody directed against TSTA is not present in the course of virus tumor induction in the hamster model.

Cellular immunity in breast cancer was intensively studied by four I-E Segment contracts. At New York Medical College, evidence for a viral etiology of human breast cancer was demonstrated by the migration inhibition assay, which showed that the migration of leukocytes in breast cancer patients was commonly inhibited by RIII mouse milk containing MTV, but not by RIII/F mouse milk which does not contain MTV. Similar studies using cryostat sections of breast tumors as antigens showed that small tumors were antigenic, and breast cancer patients with small tumors were able to mount an immune response against MTV and human breast cancer associated antigens, whereas patients with advanced disease were unable to respond to breast cancer related antigens. At George Washington University, specific fractions of breast tumors demonstrated delayed hypersensitivity in breast cancer patients which appeared to correlate with stage of disease. Studies of these extracts are now underway to determine if these apparently tumor-specific antigens contain viral information. Lymphocyte transformation tests were successfully applied to longitudinal studies in mice with breast cancer at the University of Miami. An MTV-free breast cancer system was developed using CGRL mice, and immunity to virus-associated antigens was distinguished from immunity to non-viral breast cancer associated antigens. The investigators at the University of Miami also showed that the immune response was maximal when the tumor size was

small, comparable to the findings of the New York Medical College group in humans; progressive disease was associated with a fall in tumor-specific immunity. Of interest was a rebound immunological response after surgery which was present even when sham surgery was performed and the tumor was not resected. Stimulation of the immune response to tumor- and virus-associated antigens in mice was initiated at the Mt. Sinai Medical School. Studies are under way to determine whether it is possible to improve the immune response and bring about control of virus-induced breast tumors using neuraminidase treated tumor cells, an approach that was successful for the control of virus-induced spontaneous leukemia.

Human sarcoma studies were emphasized at a second M.D. Anderson University of Texas contract where evidence for an oncogenic virus was detected in a cell line derived from a rhabdomyosarcoma patient. In addition to a piling up of cells and focus formation, transfer of the focus forming principle to other cell lines was demonstrated. Immunologic evidence for a viral etiology in human sarcoma was obtained with the apparent demonstration of cell-mediated immunity and serum blocking factors in the blood of normal humans that appeared to be specifically directed against tumor cell lines. These latter findings were supported by three other I-E Segment contracts: Johns Hopkins University, UCLA, and Litton Bionetics.

More general studies on the identification of the precancerous state using laboratory procedures was undertaken by four I-E Segment contracts. At IARC, the absence of two HLA loci was associated with a predisposition to NPC, and family studies indicated that this predisposition was inherited rather than acquired as a part of the disease. Supportive evidence for a genetic relationship to cancer identifiable by HLA typing was obtained by investigators at the University of California in Los Angeles, where a full set of HLA antigens was associated with normal old age and the absence of two HLA antigens was associated with the early onset of cancer. The skin fibroblast transformation test, which has been reported to detect an increase in susceptibility to cancer in studies on Down's syndrome, Fanconi's anemia, xeroderma pigmentosum, and familial cancer, was pursued by a new I-E Segment contract at Biotech Laboratories.

Evidence for elevated antibodies to EBV indicating susceptibility to cancer was provided by studies at IARC and Litton Bionetics. IARC's sero-epidemiological studies showed that normal Chinese have very high antibodies to the EBV-associated "S" antigen. The finding of these high titers in normal Cantonese Chinese, the group most likely to develop NPC, may be an important link between EBV and NPC. This is particularly significant since patients with NPC maintain high levels of antibody to the soluble CF antigen even when their tumors are in complete remission. At Litton Bionetics, normal individuals within multiple case cancer families were shown to have higher antibodies to the EBV-associated viral capsid antigen and early antigen. The assays noted above (fibroblast transformation, CMI, EBV titration, and HLA typing) will now be applied to additional studies on cancer families identified by IARC, NCDC, UICC, and other clinical groups.

Other activities of the Segment: Proceedings of the International Symposium on Human Tumors Associated with Herpes Viruses sponsored by the I-E Segment was published in Cancer Research.

IMMUNOLOGY-EPIDEMIOLOGY SEGMENT

Dr. Paul Levine, VLLB, DCCP, Chairman
Dr. Gary Pearson, VLLB, DCCP, Vice Chairman
Dr. Clarice Gaylord, VLLB, DCCP, Executive Secretary

AICHI CANCER CENTER RESEARCH INSTITUTE (N01-CP3-3290)

Title: Immunologic and Epidemiologic Studies on Cancer Patients in Japan

Contractor's Project Director: Dr. Yohei Ito

Project Officer (NCI): Dr. Tadao Aoki

Objectives: (1) To investigate the role of herpes and type C RNA viruses as possible etiological agent(s) for certain human neoplastic diseases: (2) to carry out comparative studies on the immunology and epidemiology of certain types of cancer in different areas of Asia, particularly in Japan; (3) to apply various immunological approaches in order to detect tumor-specific and virus-specific antigens in patients with cancer in Asia; and (4) to continue collection of cancerous specimens from Orientals.

Major Findings: Efforts to culture, maintain, and procure cells from cancerous tissues of human origin were continued. These included cells of the THE (Transformed Human Embryo) series, Hodgkin's cells AICHI-4, NC-37, nasopharyngeal carcinoma, hyperplastic tonsils, and SiHa cells from carcinoma of the uterus. These cultures were supplied to other laboratories as instructed by the VCP. Procurement of human embryos and cultivation of embryonic cells were also continued, and the number of embryos processed since the initiation of the project was close to 100. Production of Rauscher leukemia virus (RLV) was carried out, employing an in vitro system, and over 200 mg of RLV was produced. These materials were used for the reverse transcriptase studies.

Immunological and seroepidemiological studies were continued both on DNA viruses, including herpes type virus (HTV), and on type C RNA tumor virus (RLV). In the herpes area, the main project was to assay the EBV antibody (VCA) titer of serum specimens collected from 822 atomic bomb casualties in Hiroshima and Nagasaki. Little abnormality was observed in the distribution pattern of the EBV antibody titer among this group as compared with normals. Immunological studies on the type C RNA virus were carried out to establish an experimental model system which could hopefully be applicable to the human system. During such studies, the presence of embryonic antigen(s) in the Rauscher leukemia cells was demonstrated.

Studies on reverse transcriptase (RT) were continued. The isolation and purification of RT was attempted repeatedly from AMV and RLV. The effective conditions for stabilizing the enzyme were determined. RLV-RT was purified on DEAE-cellulose and on Sephadex G-200. The most interesting finding was the isolation of high molecular weight RT (480,000 daltons) from RLV, which split into smaller particles (135,000 and 70,000 daltons) under certain conditions.

Cooperative activities with other laboratories was continued. The AICHI-4 cells, an established cell line from Hodgkin's disease, was sent to Dr. H. Kaplan's laboratory at Stanford. Six lines of human cell cultures established in the contractor's laboratory were sent to Drs. Deinhardt and Nonoyama through the VCP. Contact was resumed with researchers in other Asiatic countries to faciliate the procurement of human cancerous tissues in the future.

Significance to Biomedical Research and the Program of the Institute: This project represents a prime source of tissues and sera from a non-Caucasian population in the Far East. These materials, along with the contractor's studies on immunology and epidemiology, are of considerable value in obtaining data which are complementary to observations made on Caucasian populations concerning antibody distribution to viral antigens and the possible involvement of viruses in human cancer. The relationship of genetics to the viral etiology of cancer is extremely important and can best be worked out in different racial groups at different risks for specific tumors.

Proposed Course: Collection of human neoplastic tissues, cellular materials, and serum specimens from Asiatic cancer patients as well as their healthy normal controls will be continued, particularly from Japanese. These experimental materials will be maintained and will be distributed through the VCP. Studies will also be carried out employing these materials and specimens to check for possible presence of EBV and type C viral genomes. The search for RT activity in human neoplastic tissues and studies on basic aspects of the purified enzyme will be continued. Studies will be undertaken on the immunology and epidemiology of various types of cancers. These will be carried out in close collaboration with research workers at the NCI.

Date Contract Initiated: May 1, 1973

ATOMIC ENERGY COMMISSION (Y01-CP4-3210)

Title: Studies on the Relationship of Fetal Antigens to the Etiology and Control of Cancer

Contractor's Project Director: Dr. Joseph H. Coggin, Jr.

Project Officer (NCI): Dr. Gary Pearson

Objectives: The overall objective of this contract is to define the potential usefulness of fetal or embryonic antigens present on virus-induced cancer cells for cancer detection and control. The specific objectives involve research designed: (1) to define the humoral and cellular immune responses to fetal antigens with particular emphasis on cytostatic factor, (2) to determine the relationship between fetal antigens and virus-induced tumor-specific transplantation antigens through the application of a variety of *in vitro* and *in vivo* techniques, (3) to conduct pilot studies to evaluate the efficacy of using fetal antigens to prevent spontaneous neoplasms, and (4) to evaluate

male and female response differences to direct fetal immunization and female antisera responses developed during pregnancy.

Major Findings: The character and quantity of antibodies and cellular mediated immune reactivities to SV40 and adenovirus tumors of hamsters which are known to exhibit fetal antigens *in vivo* and *in vitro* are being characterized in a primary, autochthonous tumor model system. Neonatal, syngeneic hamsters infected at birth with oncornaviruses are tested throughout the course of tumor development for antibody produced against tumor-associated surface antigens. These include both tumor-specific transplantation antigens and fetal antigens. Three assays are presently in use, including the *in vitro* assay for cytostatic (C) immunoglobulin, antibody detected by the radioimmunoassay, and by immunofluorescence. C antibody against fetal antigen, which was shown to be IgG, is detected by two to three weeks post-infection with tumor virus, and is present throughout the latent period of 3-6 months prior to SV40 induced tumor appearance. Several weeks prior to tumor development at the subcutaneous site, C antibody titers fall and tumor-bearing animals never have detectable circulating C antibody. The antibody, bound to surface antigen, seems to be responsible for the slow development of the tumors when the tumor cell focus contains only a restricted number of cells (to about 2-1/2 months). Current work centers on efforts to establish firmly that the reactive, cytostatic antibody is indeed directed against fetal antigens.

Cellular immunity (CMI) directed against SV40-induced fetal antigens and tumor specific antigens is under evaluation. To date it has been demonstrated conclusively that CMI directed against SV40 target cells *in vitro* is present within four weeks after initial infection of newborn hamsters with SV40. These effector cells are "coated" *in vivo* with soluble, reversibly bound inhibitors presumably preventing their cytotoxic action *in vivo*. Current work centers on defining whether these inhibitory substances represent fetal antigen released from the growing tumor mass. Pursuant to this objective soluble antigen materials isolated from tumors cultivated *in vitro* or from the serum of pretumor and tumor-bearing and pregnant hamsters are being evaluated for their ability (1) to neutralize (abrogate) tumor-specific cytotoxicity directed against SV40 target cells by LNC's from animals immunized as adults with SV40, and (2) to abrogate anti-fetal cell and anti-tumor cell cytotoxicity by LNC's from primiparous hamsters. It is important to determine whether the inhibiting antigens bound to sensitized LNC are indeed embryonic antigens. Washed exudate, LNC's or spleen cells from pretumor and tumor-bearing hamsters which are cytotoxic to SV40 target cells *in vitro*, do not confer protection passively to normal hamster recipients challenged with SV40 tumor cells. This suggests, but does not prove, that a central impairment of cellular immunity may exist.

Studies aimed at distinguishing between TSTA's and embryonic antigens have involved efforts to purify soluble antigens from tumor and fetus and to monitor immune reactivity to these antigens *in vivo*. It has been concluded that (1) antibody directed against TSTA is not present in the course of virus tumor induction in the hamster model, and (2) TSTA antigen elicits CMI in the course of SV40 tumor development, but the IAT method does not detect antibody coordinate with this sensitization, nor is IAT antibody present (detectable)

in animals rendered immune to SV40 oncogenesis by immunization with SV40 or SV40 tumor cells in this system. The immunoglobulin detected by the IAT procedure is only present in hyperimmunized recipients receiving multiple (6-15) injections of x-irradiated SV40 tumor cells. One-way specificities are noted when using this assay with serum from hyperimmune donors, i.e., adenovirus target cells used for absorption of antibody pick up cross-reactive antibody induced by SV40 tumor cells, but not by the reverse combination. Hence, specificity detected and reported for this assay method in several publications from another laboratory are not confirmed in this model system when using autochthonous tumors. Multiple antibody specificities are observed by the IAT method of assay even when a single tumor cell strain is used as immunogen.

Initial studies show that both soluble and fetal antigens, but not normal adult antigens, induce high titers of IAT antibody which is not induced when the antigens are membrane bound. It is hoped that the IAT procedure will be useful for studies using "blocking" antigens and for investigating fetal antigen induced enhancement against virus tumor formation.

Significance of Biomedical Research and the Program of the Institute: Fetal antigens are a new class of antigens appearing on viral-transformed cancer cells, and these antigens are of significant potential usefulness in early cancer detection, as an index of tumor progression, and as antigens useful for the monitoring of cancer therapy. The activation of cryptic fetal antigen expression as a product of viral transformation provides a significant, exploitable "marker" of early viral transformational events and the phenotypic expression of these events. Fetal antigens appear to be auto-soluble *in vivo* and *in vitro*. This makes them highly suspect as a major contributor to tumor progression in the face of a host immune response. Under controlled conditions fetal antigens may be employed to interrupt successful virus tumor induction. Fetal antigens can be used in immunotherapy against tumors. Human and rodent fetal antigens have been demonstrated to cross-react in the systems examined to date. Rodent fetal antigens may thus prove useful in human cancer situations for detection and in monitoring therapy. Basic understanding of the analogy between cancer cell survival and fetal cell survival in the face of immunologic adversity perpetuated by the host and the mother, respectively, is leading to the development of a fundamental concept which may afford new avenues for cancer control. Human cancers may only have fetal antigens. Hence, the study of these antigens in rodents is anticipated to be invaluable in the basic characterization of human cancer antigens.

Proposed Course: The projects described above will be brought to a close and the work on the relationship between fetal and viral antigens will be readvertised.

Date Contract Initiated: January 1, 1965

UNIVERSITY OF CALIFORNIA (NO1-CP4-3211)

Title: Studies of Interrelationship of Viruses, Genetics and Immunity in the Etiology of Human Cancer

Contractor's Project Director: Dr. Paul I. Terasaki

Project Officer (NCI): Dr. Paul H. Levine

Objectives: (1) To detect cellular and humoral immunity in cancer patients and to determine the specificity of these reactions; (2) to understand the cellular component of the immune response to cancer and its interaction with antibodies in terms of resistance or susceptibility to cancer; (3) to study the strength of the immune response to different viral and viral-induced antigens in cancer patients for its relationship to various cancers; and (4) to examine the relationship of genetic markers (HLA and others) for linkage to the incidence of cancer in families.

Major Findings: Cell-Mediated Immunity. The specificity of cell-mediated immunity tested against cultured human tumor cells is not confined to "histologic" types of tumors. Extensive data from testing over 5,000 effector-target combinations against 45 targets from a variety of cancers showed that patients with other types of tumors and normal persons reacted as well as the patient with the same cancer as the target. The reactivity of persons tested appears to reflect the ability to respond by these persons with a strong correlation in the reactivity against all targets. Normal healthy persons were generally strong reactors, while cancer patients showed a decline in reactivity with progressing disease. To resolve the controversy over the specificities in cell-mediated immunity to human cancer, the effect of enriched and depleted cell fractions on target cells was studied. Granulocytes were strongly toxic to target cells, and the nonspecific effect which was related to release of enzymes was inhibited by heparin. Monocytes in the numbers present in the effector cell suspension had very little effect. For antibody-activated CMI or LDA activity, B cells were shown to be more reactive. Because of the difficulty in determining what is a specific reaction, the active cell in the direct CMI test is still under investigation. Depression in the ability to respond by cancer patients was further supported by the development of a system specifically to immunize and assay for cell-mediated immunity against allogeneic target cells in vitro. The test using release of ^{51}Cr from target cells after mixed lymphocyte-target cell interaction in culture showed that lymphocytes from normal persons were more readily sensitized and caused greater ^{51}Cr release than lymphocytes from cancer patients.

Humoral Immunity to Cancer. In testing by indirect immunofluorescence against frozen tumor sections from kidneys, prostate glands, ovaries, and normal tissue from the same kidneys, 14% of the sera were found to react positively in comparison with 0.7% and 3.8% for normal persons and patients with nonmalignant diseases. This provides evidence for an antibody response by patients to a tumor-associated antigen which is unrelated to present classifications of cancer. The specificity of this reaction requires further examination.

Interaction Between Humoral and Cellular Immunity. Antibodies which enhance or activate lymphocytes to react against target cells beyond the level of lymphocytes alone were detected in 48 of 251 sera from cancer patients using 7 different target tumors and at least 2 different effector cells from normal persons. Conventional HLA reactivity has been identified and eliminated, but low levels of activity are being examined further using these sera in parallel LDA studies with lymphocyte targets. The specificity of the reaction against cultured tumor cells is under investigation. Depressed reactivity by lymphocytes from cancer patients employed as effector cells was also observed in the antibody-activated cell-mediated cytotoxicity test and the LDA assay.

A microassay system has been developed for detection of herpes type I and II viruses. The lysis of cultured cells by herpesvirus was assessed in the microtest plate by counting the surviving cells after fixation and staining by electronic image analysis. The activity of the virus preparation and titration of neutralizing antiserum is measured rapidly and efficiently.

HLA Studies. HLA types of 320 Hodgkin's disease patients were investigated. Age, sex, and stage greatly influence the distribution of HLA antigen frequency. Among 21 American patients with Burkitt's, HLA1 and HLA8 were high. The number of HLA specificities present in healthy geriatric patients over the age of 75 was significantly increased. No given specificity was significantly increased, suggesting that perhaps mere heterozygosity had led to increased survival. Division of cancer patients into young and old cancers indicated that young patients (under 35 years old) tended to have fewer antigens. Computation of the gene frequencies in the Hardy-Weinberg equilibrium showed excess in homozygosity among young cancer patients.

In collaboration with Dr. Henry Lynch in Omaha, two new large families have been typed, and to date the findings have been consistent with the idea that HLA is linked to susceptibility.

Baseline studies on population association of HLA with cancer has been continued on more of the rarer cancers.

Significance to Biomedical Research and the Program of the Institute: This study attempts to assess the role of humoral and cell-mediated immunity, including interaction between the two responses, against antigens specific for the tumor, for viruses, and for cells infected with viruses, in relation to susceptibility to cancer and to the prognosis of the disease. The ability to respond, which is related to resistance, susceptibility, and incidence of cancer, is being studied for linkage to histocompatibility and other genetic markers through the study of families with high incidence of cancer. From family studies thus far, the susceptibility to cancer appears to be linked to HLA. If this finding could be definitely confirmed, this genetic marker for susceptibility could be an extremely useful tool in the study of host resistance factors to the etiologic agents of cancer.

Proposed Course: Studies in the specificity of cell-mediated cytotoxic reactions will be continued to resolve differences observed between laboratories. The interaction between lymphocytes and antiserum for

antagonistic and synergistic activity, and the specificity of these reactions will be studied. The study of cell-mediated reactions will emphasize better-defined target systems, such as virus-infected cell cultures, for tests with lymphocytes from patients with various cancers and from normal people. Viral-related antigens will be further analyzed by serological studies. In future HLA studies emphasis will be placed on family studies, some of which have already been identified in Los Angeles. Cancer population studies on the effect of HLA homozygosity and HLA association to cancer in the young age groups will be continued.

Date Contract Initiated: July 12, 1971

CENTER FOR DISEASE CONTROL (NO1-CP4-0202)

Title: Epidemiologic Studies of Leukemia and Related Diseases

Contractor's Project Director: Dr. Clark Wright Heath, Jr.

Project Officer (NCI): Dr. Adi Gazdar

Objectives: The objectives of this contract are to conduct epidemiologic studies of leukemia and related diseases pertinent to the viral etiology of cancer: (1) ad hoc field investigations concerning community case clusters, multiple-case families, and animal tumor-associated cases, combining where possible epidemiologic and laboratory investigations; (2) systematic studies concerning case clustering, both time-space and interpersonal contact; (3) cohort study of heterophile-positive infectious mononucleosis with respect to subsequent cancer risk; and (4) case-control study of nasopharyngeal cancer, seeking both epidemiologic information and laboratory data concerning viral antibodies, EB virus, and genetic histocompatibility makeup (HLA typing).

Major Findings: Ad Hoc Field Investigations. Detailed field investigations have been undertaken in six instances: (a) a group of 3 cases of multiple myeloma occurred among employees of a single department of a corporation in Greenwich, Connecticut. No unusual epidemiologic features linking these cases were found; (b) two cases of acute leukemia occurred in schoolmates at a high school in Wantagh, New York, where 3 similar cases had occurred in a 2-year period several years ago. No particular connections between these 2 groups of cases were found; (c) three cases of Hodgkin's disease occurred in students or former students at a high school in Watertown, Connecticut. Investigation of this cluster is not yet complete; and (d) two cases of Burkitt's tumor occurred in young people living in Buford, Georgia. This investigation is also not yet finished.

Two investigations have concerned multiple-case families. One was a large kindred from northern Vermont in which two cases of macroglobulinemia and 3 other lymphomas have occurred. The other involved a large family from Tennessee containing 3 cases of Hodgkin's disease. Several other studies of

multiple-case cancer kindreds are in various preliminary stages of investigation. Work in all of these family situations includes lymphocyte HLA typing and the collection of sera for seroepidemiologic study. Eight specimens of leukemic cells have been collected in connection with ad hoc investigations, with efforts to establish cell lines. To date 3 of these 8 specimens have yielded permanent cell lines; 4 are not yet permanently established.

Case-Clustering Studies. Time-space analyses have been completed for data concerning childhood leukemia in London, England and San Francisco, and are in process for the state of Rhode Island. Results thus far show only scattered evidence of time-space clustering and no consistent patterns. A systematic study of the question of interpersonal contact among cases of leukemia and lymphoma, especially Hodgkin's disease, is being performed using Connecticut tumor registry data, involving identification of cases of leukemia and lymphoma among present and former teachers and students in Connecticut high schools. Work on this project is presently in the data-collection phase.

Infectious Mononucleosis Followup. This project involves followup of approximately 7,000 cases of infectious mononucleosis with matched controls at selected U.S. colleges. To date information has been collected on approximately 1,800 case-control sets at the University of Nebraska, Georgia Tech, and the University of California at Santa Barbara. Data are presently being collected for an additional 2,100 cases at the University of California at Berkeley and at Yale University, with negotiations in progress for followup of an equal number of cases at the University of Michigan. A special analysis of potential problems with respect to questionnaire non-response is being conducted at the University of Nebraska.

Nasopharyngeal Cancer. Case-control data are being collected at several hospitals in the metropolitan Boston area, where a total of 7 interviews have thus far been conducted. Negotiations are being conducted with the M.D. Anderson Hospital to develop a similar system for collecting case-control data there. Plans for this phase of the study have yet to be formalized.

Significance to Biomedical Research and the Program of the Institute: These various epidemiologic studies are designed to provide clues regarding the potential importance of infectious agents for the etiology of cancer. This is being done by seeking patterns within multiple-case families and community clusters, by defining features of specific tumors (NPC) or possible premalignant states (IM), and by assessing the potential importance of interpersonal contact for specific kinds of leukemia and lymphoma. Should objective epidemiologic evidence suggesting an etiologic role for infectious agents emerge in any of these settings, specific intensive longitudinal laboratory/epidemiologic studies would be indicated. Specimens collected in the course of these epidemiologic studies provide highly characterized material of particular importance to laboratory workers in the Virus Cancer Program.

Proposed Course: During the next contract year (1974-1975), it is expected that the contract will continue as in the past with ad hoc field investigations, intensifying efforts for combined seroepidemiologic studies, particularly with respect to multiple-case families. It is planned to complete the analysis of interpersonal contact clustering in Connecticut before the end of 1974, and hopefully to complete the infectious mononucleosis followup study by the end of fiscal year 1975. The NPC study will likely be a continuing project through the next contract year. More seroepidemiologic efforts with respect to the question of feline leukemia virus and its infectiousness for humans may well be added to the contract in the next year.

Date Contract Initiated: July 1, 1967

CHILDREN'S HOSPITAL OF PHILADELPHIA (NO1-CP3-3272)

Title: The Propagation and Seroepidemiology of EB Virus

Contractor's Project Director: Dr. Gertrude Henle

Project Officer (NCI): Dr. Paul H. Levine

Objectives: The major objective continues to be the demonstration of an etiologic relationship of EBV to Burkitt's lymphoma and other human malignancies. These studies entail: (1) improvement of existing and development of new techniques for detection of EBV-related antigens and titration of corresponding antibodies, as well as search for methods to measure cell-mediated immune reactions in EBV-associated diseases and, if successful, their application to detection of blocking (tumor enhancing) factors; (2) determination of frequencies and titers of antibodies to various EBV-determined antigens in EBV-associated diseases, with emphasis on longitudinal study of patients in efforts to detect disease-related differences in antibody patterns, and to ascertain whether changes in the spectra and titers of various antibodies are referable to preceding or subsequent clinical events, thereby providing prognostic information and support for a causal rather than casual relation of EBV to given human malignancies; and (3) attempts to improve yields of infectious virus from present sources and search for new sources in efforts to obtain potent EBV preparations originating from various EBV-associated diseases or healthy carriers, for comparison of their antigenic and other biologic properties.

Major Findings: Surveys for EBV neutralizing (N) antibodies were completed using the microtest developed in the contractor's laboratory which is based on the abrogation of inhibition of colony formation by lymphoblasts from non-producer lines. N antibodies appear regularly in the course of infectious mononucleosis (IM) or after inapparent primary EBV infections and persist thereafter, presumably for life. Practically all sera with, but none without, antibodies to EB viral capsid antigens (VCA) have N activity, explaining why anti-VCA serves as a dependable indicator of immunity to IM. African patients

with Burkitt's lymphoma (BL) and patients with nasopharyngeal carcinoma (NPC) have mean N titers 5-10 times higher, and patients with Hodgkin's disease (HD) show an overrepresentation of high N titers as compared to appropriate controls.

A micro-cytotoxicity test was developed based on ^{51}Cr release from pre-labeled Raji or F-265 cells after exposure to human sera and rabbit complement. Serum surveys showed some degree of relation of the cytotoxicity test to EBV; that is, (a) anti-VCA negative sera were less frequently cytotoxic ($\geq$ 2 times spontaneous release) than anti-VCA positive sera; (b) the incidence and titers of cytotoxic activity tended to increase with an increase in anti-VCA titers; and (c) sera cytotoxic for Raji or F-265 cells were, as a rule, non-toxic for cells free of EBV genomes (MOLT-4). Overall, the percentages of cytotoxic sera were, however, similar for BL, NPC, IM or HD patients. These results have shown that the cytotoxicity test is of limited value in the study of EBV-associated diseases.

Double gel diffusion precipitation tests were carried out, the initial aim being to relate the precipitates to known EBV-related antigens; i.e., VCA, EBV-induced early antigens (EA) of the D or R varieties, EBV-associated nuclear antigen (EBNA), etc. Unexpectedly, all anti-VCA negative sera produced at least one and often two lines of precipitation with concentrated extracts of cells, not only from EBV genome-carrying producer or non-producer lines of lymphoblasts, but also from EBV genome-free blastoid cells (MOLT-4, LM/DM, K-562) as well as leukocytes from cord blood or anti-VCA negative healthy donors. Furthermore, many animal sera yielded one or both of these two lines. The nature of these "non-specific" precipitinogens has not been determined, except that fetal calf serum was excluded. Sera with high titers of antibodies to VCA, D or R and EBNA produced additional lines of precipitation with extracts of cells from producer and non-producer lines (before and after superinfection with EBV), which probably were EBV-specific but have not yet clearly been identified as one or the other known antigen.

Anti-complement immunofluorescence tests for detection of EBNA were employed for two general purposes. (a) Identification of EBV genome-carrying cells in newly established continuous cultures was readily and rapidly achieved by this method, testing lymphoblast cultures derived from HD patients submitted by Dr. Alan Epstein, Stanford University Medical Center; somatic hybrid cells obtained by fusion of lymphoblasts from producer lines with cultured human epithelioid cells and submitted by Dr. Ronald Glaser, Hershey Medical School; and emerging lymphoblasts in cultures of IM leukocytes obtained in this laboratory. Work on NPC biopsy cells sent by Dr. John Ho (Hong Kong) is in progress. (b) Tests for anti-EBNA in human sera were adapted to a routine procedure. A study on the emergence of this antibody in the course of IM has been completed. Anti-EBNA appears, as a rule, late in this disease (>1 month after onset) although a few patients respond early (<3 weeks). Anti-EBNA persists thereafter, presumably for life, since no anti-VCA positive healthy donors were found who failed to show this antibody. The late appearance and the persistence of anti-EBNA give rise to intriguing speculations as to the sources of the antigen (transformed cells?) for stimulation of antibody production.

The detection of EBV-specific IgM antibodies has been hampered by considerable technical problems. The techniques reported by Schmitz and Scherer have overcome these difficulties. Dr. Schmitz spent 4 weeks in the contractors' laboratory to assist them in introducing his methods. In blind tests, early acute phase IM sera were readily identified.

Horizontal serologic followup of patients with BL, NPC, HD, renal transplants, and other conditions have been continuing in collaboration with various investigators and clinicians. (1) Burkitt's lymphoma. Antibodies to the R component of the EA complex provide prognostic information on patients brought to remission by therapy. Those without anti-EA or showing steady declines in anti-R are likely to become long-term survivors, whereas those maintaining or developing high anti-R titers are prone to have many, ultimately fatal relapses. Confirmation of these results obtained with patients seen at the Kenyatta National Hospital, Nairobi, is being sought now with patients from Uganda and Ghana in collaboration with Drs. Ziegler, Magrath and Nkrumah. (2) Nasopharyngeal carcinoma. Since antibodies to VCA and the D component of the EA complex increase in titers with the stage of the disease--that is, the total tumor burden--they were found, in turn, to decline again, especially anti-D, after eradication of the tumor. EBV-specific serology may thus serve to detect an extension of the disease and to monitor the effectiveness of therapy. Confirmation of these results is being sought in longitudinal studies of individual patients in collaboration with Dr. John Ho (Hong Kong). Similar studies of Swedish and American NPC patients are in progress with Dr. Brian Henderson and Dr. André de Schryver. (3) Hodgkin's disease. Studies on HD patients have been continuing in collaboration with Dr. Henry S. Kaplan (Stanford University Medical Center) and Dr. Bo Johansson (Radiumhemmet, Stockholm). The patients can be divided serologically into those who (a) have no antibodies to EBV; (b) continuously maintain low anti-VCA titers; (c) continuously maintain high anti-VCA titers with or without anti-EA responses; (d) converted from low to high titers; and (e) converted from high to low titers. Efforts are under way to determine whether these serologic groups can be related to clinical staging, histologic types of the disease or other parameters. These correlations are delayed by the need for reviewing clinical records and histologic preparations in the light of recent modifications of the criteria for the various classifications. (4) Renal transplant patients. These are being studied in efforts to evaluate the effects of immunosuppressive therapy on persistent EBV infections. At present it appears that therapeutic immunosuppression rarely activates latent EBV infections, in contrast to the frequent reactivation of other herpesvirus carrier states (CMV, HSV or VZ). These results suggest that, while immunosuppressive therapy reduces the resistance of the patients, it at the same time reduces the habitat of EBV and the cells to which it appears to be restricted for its replication.

Efforts to detect humoral or cell-mediated immune responses against EBV transformed cells have not as yet led to a convenient, reproducible and clearly interpretable technique. Peripheral leukocytes obtained during the acute phase of IM and seeded in soft agar were found to yield a few colonies. The number of colonies was often significantly reduced when the patient's own serum was incorporated in the medium, but not when anti-VCA negative sera were

used. Apart from the limited availability and numbers of acute phase leukocytes, such preparations may contain "effector cells" which may reduce the number of colony-forming cells. Obviously, a system must be developed free of such handicaps.

Significance to Biomedical Research and the Program of the Institute: The primary purpose is to prove the etiologic relation of EBV to certain human malignancies. Fingerprints of EBV have been found in nearly all BL and NPC biopsies. This virus transforms normal lymphoid cells *in vitro* into permanently growing lymphoblasts which have malignant properties and are indistinguishable from cultured BL cells. EBV may induce lymphoproliferative malignancies in several non-human primate species. Patients with BL or NPC generally have antibodies to EBV-related antigens, often at very high titers, and changes in the spectra and titers of various antibodies are referable to clinical events. EBV-related serology thus may serve to detect advancing disease, to provide prognostic information, and to monitor the effectiveness of therapy. Despite the mounting evidence for a causal relation of EBV to BL and NPC, present data do not rigidly exclude a passenger role of the virus in these malignancies. Furthermore, if the role of EBV were causal rather than casual, other factors must undoubtedly be operative to permit this common virus to express its evident oncogenic potential. Among these factors immunologic defects deserve special consideration. The types of antibodies studied most extensively are directed against EBV structural and EBV-induced intracellular antigens which could not affect the tumor. It is most essential, therefore, to search for humoral and cell-mediated immune responses directed against EBV-transformed cells. If successful, methods would become available for detection of blocking (or tumor enhancing) factors. Pertinent studies on IM patients may reveal how they control EBV-transformed cells. It then can be determined whether this mechanism fails to materialize on rare occasions or becomes inoperative under certain circumstances so that transformed cells escape control. Such evidence would significantly strengthen a causal role of EBV in human malignancies, and provide a firmer basis for consideration of EBV-specific preventive measures.

Proposed Course: (1) Further attempts will be made to separate EBV-specific from non-specific precipitinogens and to identify the active components with known antigens. (2) Further efforts will be made in collaboration with Dr. Ho to detect EBNA in NPC biopsy cells. (3) Serial sera from BL, NPC, and other patients will be tested for anti-EBNA to determine whether clinical events may be reflected in changes in titers and provide another source of prognostic information. (4) Anti-EBNA titers will be compared with titers of antibodies to the soluble (S) complement fixing antigen (Raji cell extracts), to determine whether the two tests measure the same or different antibodies. Conversely, efforts will be made to determine whether S antigen preparations contain EBNA. (5) The EBV-specific IgM test will be applied to serial sera of BL and NPC patients to determine whether relapses may be preceded by evidence of reactivation of the persistent EBV carrier state. (6) Efforts will continue to develop methods for the detection and quantitation of humoral or cell-mediated immune responses against EBV-transformed cells, with special emphasis on the development of such responses in the course of IM. If and when a reproducible technique becomes available, a search will be made for

blocking (tumor enhancing) factors in sera from BL and NPC patients in various stages of the malignancies. (7) Longitudinal studies of BL, NPC, HD and organ transplant patients will continue, with the addition of new serologic procedures as mentioned above, or as they become available in the future. (8) Studies have been initiated, partly with support from another source, to follow the intracellular development of EBV-related antigens with the aid of a series of sera having defined spectra of antibodies and FITC- or peroxydase-conjugated antibodies to human IgG for immunofluorescence and ultrastructural examination. Different sources of virus and various metabolic inhibitors applied at selected time intervals will be used. These efforts may provide conditions for optimal production of given antigens and possibly suggest means for enhanced virus production.

Date Contract Initiated: March 1, 1973 (This is a continuation of Contract PH43-66-477, initiated February 2, 1966).

GEORGE WASHINGTON UNIVERSITY (NO1-CP2-3251)

Title: In Vivo and In Vitro Studies of the Immune Response to Virus-Associated Antigens in Lymphoma and Breast Cancer Patients and Controls

Contractor's Project Director: Dr. T. C. Alford

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

Objectives: (1) To evaluate the cellular immune response in patients with malignant diseases by in vitro assays (leukocyte migration inhibition, lymphocyte stimulation, cytotoxicity reactions against target cells, and determination of T- and B-cell lymphocyte sub-populations) and in vivo assays (delayed hypersensitivity reactions to extracts of malignant and normal tissues); and (2) to correlate the in vivo and in vitro assays with the stage and course of the patient's malignant disease.

Major Findings: Studies on Patients with Breast Cancer. In vivo and in vitro assays are being performed in patients with breast cancer. In vitro skin tests (primarily allogeneic) for delayed hypersensitivity using 3M-KCl extracts of primary breast cancers have shown only a few positive reactions; however, individual tumor preparations of breast cancer may be quite antigenic. The majority of the breast cancer patients have been tested in the immediate postoperative period (5-10 days) when tumor is absent. The KCl breast cancer extracts appear to be specific, since no patient with other types of cancer have had a positive skin reaction. When 3M-KCl breast cancer extracts are further separated in Sephadex, three peaks are identified as measured at 230 nm. One or more of these three peaks usually gives a delayed hypersensitivity skin response in breast cancer patients and is primarily in the area of 50-75% of the bed volume of the gel; however, similar separations of normal breast tissue from these breast cancer patients also are positive. It would appear that in vitro assays using leukocyte migration inhibition are

more sensitive than the skin tests. The majority of these skin test negative patients showed leukocyte inhibition to the KCl breast cancer extracts, but not to normal breast extracts.

Studies on Patients with Lymphoma. Cellular immune responses have been studied in patients with lymphoma. Skin testing, lymphocyte transformation (LBT), and direct inhibition of leukocyte migration (MI) have been used to test responses to common infectious antigens, e.g. PPD, and to Epstein-Barr virus-associated membrane antigens (VAMA). Fifteen patients have been skin tested. Five of the fifteen were anergic. Four anergic patients had widespread, severe disease. Two patients reacted to VAMA. One of these is in remission and has had no treatment for over one year. The other reactive patient has widespread disease and has been on cyclic combination chemotherapy. Fifteen patients have been evaluated by LBT. Nine had normal responses to phytohemagglutinin (PHA), four had a suppressed response, and three did not respond. Two patients responded to VAMA. Reactions to VAMA have been studied in eleven patients with MI. Five were not affected. Three showed inhibition; two of these had widespread disease, and the third was in remission. Three of the eleven showed increased migration. The significance of this response is not known. In summary, some lymphoma patients do react to VAMA. Further serial studies are planned to see if this reaction correlates with the course of the patient's illness.

Significance to Biomedical Research and the Program of the Institute: Human tumors probably contain tumor-associated antigens. Deficiencies in cellular immunity may be responsible for the development of malignancy. Some data suggest that several malignant diseases, such as Burkitt's lymphoma, breast cancer, and squamous cell carcinoma of the head and neck, contain antigens induced by Epstein-Barr virus or herpesvirus simplex. The contractor's studies are designed to assay cellular immunity to virus-associated and tumor-associated antigens, and to correlate immune responsiveness with the clinical stage and course of the patient's malignancy. These data may help to clarify the relationship between immune response to specific viral and tumor antigen, tumor load, and clinical disease. The data may also help clinicians to detect early cancer, to monitor therapy, and to determine relapse or recurrence of malignant disease.

Proposed Course: Serial studies are to be continued on patients with breast cancer and lymphoma who are currently being studied. Additional patients will be assayed and MTV-associated antigens will be added to the group of viral antigens utilized in these studies.

Date Contract Initiated: April 13, 1972

HEBREW UNIVERSITY-HADASSAH MEDICAL SCHOOL, JERUSALEM (NO1-CP3-3342)

Title: A Multidisciplinary Study of Hodgkin's Disease in Israel

Contractor's Project Director: Dr. Natan Goldblum

Project Officer (NCI): Dr. Paul H. Levine

Objectives: To investigate the interrelationship between environmental and genetic factors in the etiology, pathogenesis and spread of Hodgkin's disease in Israel and, in particular: (1) to evaluate clustering among social contacts, in space and time, and to develop methods for the study of clustering; (2) to evaluate Hodgkin's disease in immigrant and native-born Israelis, including studies of HLA typing, and EBV and other virus antibodies; and (3) to review the histological sections of all malignant lymphomas diagnosed throughout Israel during 1964-1972 so as to provide basic information necessary for the immunoepidemiologic studies.

Major Findings: An analysis of mortality from lymphoma in Israel between 1950 and 1971 is almost complete. Over the years there has been a rise in mortality from non-Hodgkin's lymphoma and, in most population groups, from Hodgkin's disease. The rise in mortality from non-Hodgkin's lymphoma occurred from the age of 50 years, and less consistently among children and young adults. The increase in Hodgkin's disease mortality was apparent from the age of 35 years. Mortality from Hodgkin's disease and other lymphomas was related to the continent of birth of immigrants. There were no clear-cut relationships with age at immigration or period since immigration.

Interviews with cases of Hodgkin's disease or with their surviving relatives, diagnosed since 1960, have been carried out. To date, 416 (67%) of 608 patients (or relatives) have been interviewed and most of the material has already been coded. Histological information is available for most of the patients interviewed.

Histological sections of 962 cases of malignant lymphomas, during the 5-year period, 1964-1968, have been reviewed. Diagnosis of malignant lymphoma was confirmed in 828 cases (86%), and 214 of these were classified as Hodgkin's disease. It is important to note that 31 (14%) of these cases had been registered as lymphosarcoma or reticulum cell sarcoma in the Israel Cancer Registry, and so would not have been detected had they not reviewed slides of all malignant lymphoma. Mixed cellularity and nodular sclerosis each accounted for 30% of cases, lymphocytic predominance for 17%, and lymphocytic depletion for 18%. Four percent of Hodgkin's cases could not be further classified for technical reasons. Incidence rates for various population groups are at present being calculated. Preliminary results suggest that the lymphocytic predominant type may account for a higher proportion of cases of Hodgkin's disease among European-born than among Asian- or African-born Jews. Lymphomas diagnosed during the years 1969-1972 are at present under review.

To establish the association between human histocompatibility antigens and Hodgkin's disease, blood samples have been collected from patients with a

histopathologically established diagnosis. Eighty-five patients are presently undergoing HLA typing by the contractor. Bloods from all new untreated cases are shipped to Dr. Terasaki for testing. Several thousand sera were collected for EBV and other viral antibody tests, from normal individuals of various ages, different ethnic groups, and from several geographic areas.

Establishment and organization of clinical centers in the major hospitals in the country have been completed. Four such centers are operating at present, in Jerusalem, Haifa, and in the Tel Aviv area. They draw and treat over 95% of Hodgkin's disease cases. Over 250 patients have already been traced through this mechanism, and are being followed up for HLA typing, collection of sera, and other immunoepidemiologic tests and information. The majority of new cases have been entered into the study prior to treatment, and biopsy materials have been obtained from numerous patients. Cell suspensions are being routinely prepared from each biopsy. A portion of the suspension is spread on slides for immunofluorescent and other tests; also, 5 - 10×10^6 cells are suspended in RPMI 1640 containing 30% fetal calf serum, antibiotics, and 10% DMSO, and frozen at -70° C. Cells have been cultured from this biopsy material (lymph nodes, spleen, and liver), including those of "controls" from non-Hodgkin's lymphomas and normal tissues.

Significance to Biomedical Research and the Program of the Institute: A comprehensive study of all cases of Hodgkin's disease arising in a large, well-defined population, with comparable measurements of virus exposure, immunologic status, and genetic background on a control group, is needed by the Program to determine the relationship of environmental and genetic factors in the etiology of this disease. The opportunity afforded by this well-identified population offers an excellent possibility of determining the cause of Hodgkin's disease. The mortality findings are consistent with the hypothesis that environmental, probably infective, factors play a role in the etiology of both Hodgkin's disease and other lymphomas. The reviewed and "Rye-classified" cases of Hodgkin's disease will provide accurate histological information on the cases included in the interview study. They will also enable one to determine the incidence rates and the frequency of the various histological types to be determined for the various population groups in Israel.

Proposed Course: This will be a population-based study on immigrant and native-born populations in Israel. An attempt will be made to check the histological diagnoses of cases of Hodgkin's disease diagnosed in the earlier period, 1960-1963, included in the interview study, and to confirm the diagnosis and Rye classification of all newly-diagnosed cases of Hodgkin's disease included in the HLA typing, in the virus antibody studies, and in cell culturing. Laboratory studies will include HLA typing and serology, initially consisting of testing for antibodies to EBV. Sera will be stored and will be available for testing against other possible candidate oncogenic viruses. Biopsy material from Hodgkin's disease patients will be grown in culture, and a search will be made for the presence of specific antigens, EBV and others, in the original biopsy, as well as on the cultured cells.

Date Contract Initiated: June 29, 1973

INTERNATIONAL AGENCY FOR RESEARCH ON CANCER (NO1-CP4-3296)

Title: Sero-Epidemiologic and Laboratory Studies on Nasopharyngeal Carcinoma and Burkitt's Lymphoma

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

Project Officer (NCI): Dr. Paul H. Levine

Objectives: The overall objective of the contract is to evaluate the role of the Epstein-Barr virus (EBV) in the etiology of Burkitt's lymphoma (BL) and nasopharyngeal carcinoma (NPC). The first specific objective was to carry out a prospective study in a child population of 35,000 in Uganda; the children were to be bled once and then to be followed for at least five years in order to obtain pre- and post-BL sera. The serological study of these paired sera should test the validity of four hypotheses which have been formulated regarding the etiological relationship between EBV and BL. Two sub-objectives were: (1) to study the role of heavy malaria burden in the development of BL; and (2) to investigate the epidemiological behavior of EBV infection in the study population by the follow-up of randomly selected groups. The second objective involved the investigation of the sero-epidemiological behavior of EBV in populations at different risk for EBV-associated diseases, carried out in Hong Kong, Singapore, and Uganda. The third objective is the investigation of the role of EBV in the development of NPC through the integration of field and laboratory studies.

Major Findings: With regard to the prospective study by December 31, 1973, 27,448 sera had been collected in the 0-5 year old population. In addition, about 3,000 sera have been collected from 6-8 year old children and mothers of babies under one year. Case detection has been given high priority, and by January 1, 1974, four cases of BL had been detected in the prebled population. This is in line with expectations. In the first three cases the time interval between pre- and post-BL sera was between seven and nineteen months; details of the fourth case are not yet available. Testing of these paired sera has not yet been started. As suggested by both the NCI Review Committee and the IARC Tenth Scientific Council (January 1974), it is intended to wait until at least five or six prebled BL cases are on hand before the testing is commenced.

Analysis of the initial phase of the case control study, which is built into the case detection, showed that BL patients more often than controls have experienced blisters in or around the mouth. Antibody studies directed towards all human herpesviruses showed that BL patients have higher titers than controls, not only to EBV but also to cytomegalovirus (CMV) and varicella/zoster and, to a lesser extent, to Herpes simplex virus (HSV). These titers were obtained by the indirect immunofluorescent assay, and not by complement-fixation from which no differences between BL patients and controls were observed. The epidemiological study built into the BL case detection

indicates that BL cases tend to cluster along rivers and smaller water systems; furthermore, a number of BL cases were observed among cousins.

The malaria study, integrated into the survey, has progressed satisfactorily: every child had a malaria smear taken, and 4,391 were read both quantitatively and qualitatively. The analysis of the available results indicates a significant variation in the intensity of malaria infection from place to place in the survey area. Sickle trait studies carried out in October 1973 in the groups which were rebled showed that frequency of the sickle trait varies from between 15-30% within the study area, which is not more than could be expected by chance. Results from previous case control studies by Kafuko in 1969 revealed 15% sicklers in controls but none in BL cases. This study is being continued in view of conflicting results obtained by other investigators. The measles study, aimed at evaluating the immunological competence of children who have experienced a heavy malaria burden, is carried out in collaboration with Dr. Munube of the East African Virus Research Institute. Initial observations indicate that measles antibody response in children is not at all related to measles history reported by the mothers. The continuing BL study in the North Mara District of Tanzania reported a number of BL cases in siblings. The validity of this finding and its possible genetic versus environmental significance is being evaluated.

The major findings with regard to the 2nd objective (sero-epidemiological study of EBV infection in populations at different risk for EBV-associated diseases) were as follows: By December, 1973, the serum collection in representative populations in Singapore and Hong Kong had been terminated, with a coverage of 2,071 sera from the Toa Payoh housing estate in Singapore (Chinese 1,041, Indians 721, and Malays 309, covering all ages), and 949 sera from 216 families in Sau Mau Ping housing estate in Hong Kong. In Uganda the EBV sero-epidemiological study covered eight population clusters chosen at random in the survey area, each comprising about 120 individuals. Four such clusters have now been covered. Between June and December 1973, viral capsid antigen (VCA) antibody tests were performed on the following sera: 1,010 from Singapore, 892 from Hong Kong, and 420 from Uganda. Part of them were also tested for complement-fixing (CF) antibodies. The pattern emerging indicates that the Chinese have a lower VCA antibody response than Indians but much higher CF titers (using S antigen). The Ugandans tend to be closer to the Chinese for VCA and CF titers. In addition, the VCA testing covered 347 sera from babies bled sequentially in Hong Kong (Plan A), plus older children aged 2-4. Preliminary analysis of the results indicates that, in comparison with Ugandans (where the sample size was not of the same magnitude), the prevalence of infection at one year of age is lower in Hong Kong. The comparison will only be valid when further sera from the same age groups in Uganda have been tested.

With regard to the 3rd objective, the major findings were as follows: The human leukocyte antigen (HLA) pattern observed in Chinese NPC patients (deficiency of HLA on the second sublocus) is also being investigated in Tunisian NPC patients by Dr. Betuel in Lyon, and in Professor Dausset's laboratory in Paris. In order to know if the deficiency follows Mendelian laws, family studies have been initiated in Singapore. Preliminary results

indicate that this is the case, and that the deficiency is not merely a consequence of the disease. By the end of 1973, when the study was terminated, the NPC questionnaires from Singapore totalled 2,654 covering 517 families. Included was a section on socio-economic conditions, which is now being related to the finding of serological differences. In Hong Kong the questionnaire study covered 2,255 individuals among 385 families. A case control study is being carried out in Hong Kong in NPC patients, with other cancer patients as controls. Their respective families are also included in the comparison. By the end of 1973, 120 NPC cases and their families and 96 controls plus families had been interviewed.

In Lyon and collaborating laboratories three major findings were achieved: (a) The finding of EB nuclear antigen (EBNA) in NPC biopsies from Tunisia was confirmed. The most positive cells appeared to be the lymphocytes closely associated with epithelial tumor cells, and which appear to belong to the 'B' sub-group. (b) The relevance to disease development and the cross correlation of the various EBV serological reactivities has been investigated in NPC patients at different stages of the disease and in various controls, all of Chinese origin. Most of the EBV reactivities [VCA, early antigen (EA), CF] seem to be independent of each other. The NA reactivity, however, may be related to CF reactivity. The two most relevant antibody reactivities to disease development which emerged were the EA and CF/S. (c) Sera from BL cases and controls were tested for immunofluorescent antibodies (IFA) against human herpesviruses. EBV, CMV, and varicella/zoster IFA antibody titers were simultaneously elevated in BL patients, as compared with controls. Antibodies against HSV were elevated to a lesser extent.

Significance to Biomedical Research and the Program of the Institute: The objectives of the contract should elucidate the etiological role of EBV in two human tumors, BL and NPC. The prospective BL project is the only study in progress aimed at finding direct proof that a human tumor is caused by a virus. The comparative study of the behavior of EBV in populations at varying risk for EBV-associated diseases has shown that only a comparison based on representative samples of these populations is valid. Initial testing has shown that indeed there are significant differences among populations having different risks of NPC. The results obtained in blood genetic typing, together with the sero-epidemiological results, may provide the means for detecting high risk groups among a normal population for one human tumor, namely NPC.

Proposed Course: The main BL survey will continue until 35,000 children between 2 and 5 years of age have been bled. This target is expected to be reached by July/August 1974, at which time the number of personnel in the field will be reduced to a minimum. In addition, children from 6 to 8 years, and mothers of babies under one year of age, will be bled. The BL case detection now has top priority in the project, and this will continue so until 30 to 35 cases have been detected. This is expected to take another three years. By July 1974 it is hoped to have the five to six cases required to carry out the first serological testing of the paired sera (pre- and post-BL). This will be done with approximately 10 control sera for each patient, all the sera being coded and tested in two or three laboratories. A proposal to this

effect was accepted by the Tenth Scientific Council of IARC in January 1974. A case control study, involving examination of matched controls and their families, will be continued simultaneously with the case detection. The detailed mapping of all new cases in the area and the registration of environmental data will provide the basis for the continuing general epidemiological study of BL. The familial occurrence of BL is being further investigated both in Uganda and in Tanzania. The collection of malaria slides will continue in the main cohort study as well as in the substudy, in BL cases and their families, and in controls and their families. In the main study only one-third of the slides will be examined for species and number of parasites, but in the substudy and BL case control study all smears will be examined. Malaria antibody studies will be restricted to BL cases and controls, and to their families. The frequency of the sickle cell trait (AS) will be compared in BL patients and controls and their families in the case control study. Measles vaccinations and followup measles antibody and malaria parasite counts will be continued. With regard to the sero-epidemiology of EBV in different populations, the proposed course is to finish the testing and analysis of results, and this should be completed during 1974. The NPC questionnaires will be analyzed, with the immediate aim of comparing the socio-economic status of the various ethnic groups in Singapore with their corresponding EBV serological reactivities. The collaborative laboratory study of NPC will include the investigation of the presence of EBV markers in epithelial tumor cells and subsequent cultures, as well as the establishment of the degree of relevance of the serological reactivities with regard to disease development. A particular objective in 1974 is to see if, from the serological results, one can determine some high risk group among the Chinese population using both HLA and serological markers. It is intended to try to relate the HLA pattern and EBV serological reactivities in the framework of the HLA family studies undertaken by Dr. Simons in Singapore. A special anthropological study will also be undertaken in Hong Kong among the high-risk boat-people.

Date Contract Initiated: June 11, 1970

JOHNS HOPKINS UNIVERSITY (NO1-CP3-3337)

Title: Immunologic Reactivity Against Tumor and Possible Virus-Associated Antigens in Families of Leukemic Patients and Controls

Contractor's Project Director: Dr. Paul Anderson

Project Officer (NCI): Dr. Paul H. Levine

Objectives: (1) To perform assays for cellular and humoral immunity against tumor-associated antigens in patients with acute leukemia and lymphoma; (2) to search for common antigens in these tumors, and to look for immunological reactivity of family members and unrelated individuals to the tumor antigens; and (3) to correlate the results of these assays with each other, with the

clinical state of the patients, and with viral evidence and epidemiological data in order to obtain further information relevant to the cause and control of these tumors.

Major Findings: Studies have extended the numbers of patients who demonstrate relative anergy on first presentation with acute leukemia, as demonstrated by skin testing. Patients in remission after chemotherapy tend to regain normal skin reactivity to conventional established delayed hypersensitivity antigens.

Family members of patients with acute adult leukemia have a high degree of response to leukemic blasts in the macrophage migration inhibition assay (MIF) and the ^{51}chromium release cytotoxicity cell-mediated immunity assay (CMI). Over 80% of HLA identical siblings were shown to react to leukemic blasts in the mixed leukocyte culture (MLC) test, presumably in response to tumor-specific antigens. Patients in remission, and patients after bone marrow transplantation for leukemia, show a high incidence of response in MLC, MIF, and CMI assays to autologous leukemic blasts.

The question of cross-reactivity or common antigenicity of these anti-leukemia reactions has been studied in a preliminary way. Using the CMI assay and the MIF test, patients in remission have shown broad cross-reactivity of positive responses to leukemic blasts of the same cell type from other patients, and to leukemic blasts of different morphological types. Remission patients with acute myelogenous leukemia (AML), for instance, have also shown responses to other AML cells, to chronic myelogenous leukemia blast crisis cells (CML/BC), to acute myelomonocytic leukemia cells (AMMOL), acute monocytic leukemia cells (AMOL), and less frequently to acute lymphoblastic leukemia cells (ALL). Two of 4 remission patients tested showed positive MIF and CMI responses to a tissue culture line (Raji) of Burkitt's lymphoma cells.

In preliminary studies of a number of oncology ward personnel, physicians, and oncology laboratory technicians, extensive reactivity was found against leukemic blasts, using CMI and MIF testing. These reactions were very cross-reactive, as described above, with positivity demonstrable to a broad panel of leukemic cell types. Some normal individuals have been studied who have had no known exposure to patients with neoplasia, or to tumor cells. These individuals usually are negative when tested with MIF or CMI assays against a battery of leukemic blasts.

The numbers of leukemic patients and their family members tested for serum antibody to leukemic cells were extended. Several human sera have been shown to have highly specific complement-dependent cytotoxicity to leukemic blasts, particularly those of ALL. One patient (a pediatric ALL patient) demonstrated antibody to his own tumor cells, as well as having cytotoxicity to the tumor cells of 3 of 6 other ALL, and 1 of 2 CLL patients, but no reactivity to cells from 3 AML and 4 normal controls. In the screening of patient leukemic cells against the panel of cytotoxic sera, 5 of 12 with ALL and 1 of 3 with CLL were positive with one or more sera, while cells of 11 patients with AML, 2 with CML, 3 with aplastic anemia, 2 with lymphoma, 7 with infectious mononucleosis, and 2 normal patients were negative with all test sera. The pattern of serum reactivity suggests at least 2 partially overlapping specificities

serologically detected on acute lymphoblastic leukemia cells. Seven established lymphoid cell lines from normal and leukemia patients, as well as from patients with Burkitt's lymphoma, were also tested, and by direct assay Raji showed reactivity with two antileukemia sera. Three ALL derived lines were negative.

Further studies using 3 molar KCl extracts of mouse lymphoid and sarcoma tumor lines have been performed. Using these extracts, anti-tumor reactivity could be correlated using skin testing and the MIF and CMI assays in these rodent models.

Collaboration with the Laboratory of Tumor Cell Biology at the NIH (Dr. R.E. Gallagher, Dr. R.C. Gallo) has been initiated. Cells from leukemic patients are being sent for assays of RNA virus-like RNA and RNA-dependent DNA polymerase (RDDP). Leukemic blasts from several of the patients have been shown to have virus-like RNA and RDDP. In the 3 cases studied thus far, remission cells have not shown such viral evidence.

Significance to Biomedical Research and the Program of the Institute: Clinical, epidemiological and laboratory studies suggest that acute leukemia in man may be a virus-induced disease. Study of immune reactions to tumor-associated antigens, and the cross-reactivity of such reactions, in patients with leukemia, family members, and in exposed and non-exposed unrelated controls, should be useful in evaluating the role of possible oncogenic viruses in human tumors, and in providing assays for monitoring possible antiviral therapy and immunotherapy.

Proposed Course: In the coming year, it is proposed to continue to use the same basic technical approaches to define and expand observations regarding antileukemia immune responses. The study of serological reactions to leukemia will be extended by absorption experiments to further define the number and relationship of the specificities detected by the serum panel on both tumor and cultured cells. Screening for active sera and susceptible cells will continue, and clinical correlates will be studied where possible. CMI and MIF reactions to leukemic cells by remission patients, family members, and controls will be extended in an effort to describe more fully the epidemiology of such reactions. Using MLC and MIF reactions, KCl extracts of human leukemic cells will be evaluated for their ability to stimulate results comparable to those seen with whole cells, and as possible standardized testing reagents. Similarly, viral antigen preparations from known animal tumor viruses may be examined for reactivity comparable to that seen using human tumor KCl antigen extracts. Efforts will be expanded to provide leukemic blasts and normal remission cells for biochemical assays for evidence of viral nucleic acids and enzymes, and to correlate viral evidence with immune reactivity to leukemic cells.

Date Contract Initiated: May 1, 1971

LITTON BIONETICS, INC. (NO1-CP-43252)

Title: Application of Immunologic Techniques to Studies on the Viral Etiology of Human Cancer

Contractor's Project Director: Dr. Maneth Gravell

Project Officer (NCI): Dr. Paul H. Levine

Objectives: The major objectives of this contract are: (1) to provide virological, immunological and data management support for other I-E Segment contracts, (2) to maintain the American Burkitt's lymphoma registry, and (3) to study the relationship of Epstein-Barr virus (EBV) to human lymphoma.

Major Findings: The major effort of this contract has been devoted to providing supportive services for other I-E Segment contracts, including those of Dr. Evan Hersh and Dr. Joseph Sinkovics of the M.D. Anderson Hospital, Dr. T. Crandall Alford of George Washington University, Dr. George Santos of Johns Hopkins University, and Dr. George Bekesi of Mt. Sinai Hospital. Supportive services rendered include assays for type C virus infectivity or neutralizing antibody, EBV serology, *in vitro* cell-mediated immunity assays, preparation of skin test antigens, propagation of lymphoid cell lines, immunofluorescent tests to distinguish T and B lymphocytes, storage and distribution of samples, and data management.

A primary support function has been to coordinate the safety and potency testing of formalin-inactivated Rauscher leukemia virus (RLV) to be used in vaccine studies with human cancer patients by Dr. Evan Hersh. In support of Dr. Hersh's contract, the contractor has maintained the central serum bank for the 20 patients thus far immunized, has distributed over 300 sera to 7 collaborating laboratories, and has maintained the central data file. In addition to coordinating the RLV testing efforts of other investigators, they have performed tests for RLV neutralizing antibody on sera from rhesus monkeys immunized with RLV. Although these sera have been negative for neutralizing antibody, they contain non-neutralizing RLV antibodies. Sera have been distributed to collaborating laboratories to test for such antibodies by radioimmunoprecipitation.

The ^{51}Cr release cytotoxicity assay was used to monitor the cellular immune response of cancer patients and rhesus monkeys receiving RLV. ^{51}Cr release assays employed peripheral blood lymphocytes from either cancer patients or monkeys and target cells that contained RLV or RLV-associated antigens [i.e. Raji cells infected with RLV, cells from an RLV-induced mouse lymphoma (RBL-5), or uninfected control cells]. As measured by this test, no specific cellular immunity was developed by cancer patients or monkeys injected with formalin-inactivated RLV. Evidence of specific cell-mediated immunity to RLV antigens, however, has been obtained by Dr. Hersh using skin testing and the *in vitro* lymphocyte blastogenic assay.

Fifteen (15) new cases of Burkitt's lymphoma were reported to the American Burkitt's Lymphoma Registry, bringing the total to 161 cases, 112 of which

have been confirmed. A lymphoid cell line, designated NAB, was established from an autopsy tumor specimen of one of these cases (K.N.). Cells of the NAB line have typical blastoid appearance by histochemical staining, and have been shown by immunofluorescence to have surface receptors predominantly of the IgM type with "patch and cap" mobility. These cells contain a complement receptor and form EAC rosettes; thus, like lymphoid cell lines established from African Burkitt's tumors, the NAB cells resemble type B lymphocytes. Although the NAB cells show no evidence of Epstein-Barr viral capsid antigen (VCA) or early antigen (EA) when tested by indirect immunofluorescence, EA can be induced in these cells by treatment with 5-iododeoxyuridine. Dr. Gary Pearson of the National Cancer Institute has shown the NAB cells to contain Epstein-Barr nuclear antigen (EBNA) by the anticomplement immunofluorescence technique. These results suggest that at least some American Burkitt's tumor cells contain the EBV genome.

Patients with human lymphomas other than Burkitt's type have frequently been found to have elevated EBV antibody titers. Using the lymphocyte stimulation assay, a study was made on the capacity of peripheral blood lymphocytes from lymphoma patients to respond to soluble antigen from a lymphoid cell line (Raji) established from a Burkitt's tumor. Preliminary data from 28 lymphoma patients studied reveal a high correlation between EBV VCA antibody titers and the capacity of patient's lymphocytes to respond to Raji soluble antigen.

Significance to Biomedical Research and the Program of the Institute: The major objective of this contract is to provide supportive services for other I-E Segment contracts; therefore the significance of this work varies from contract to contract. As a general theme, the aim of the contract is to obtain information regarding the role of viruses in human cancer, and to develop methods for the early diagnosis and control of cancer. The relationship of EBV to human lymphoma is not well understood, even though elevated antibody levels have been found in a number of human neoplasms. Studying cases of Burkitt's lymphoma in North America and Africa affords an opportunity to determine whether the African and American diseases are the same, and to compare and identify specific host and environmental factors which might contribute to the disease.

Proposed Course: "Back-up" support will be provided for other I-E Segment contracts, as well as presently-supported ones. Support functions will include viral and immunological testing, collection, storage and distribution of clinical or laboratory samples and data management. The research effort will continue to determine the role of EBV and other viral, host or environmental factors which might contribute to human lymphoma.

Date Contract Initiated: November 1, 1973

MAKERERE UNIVERSITY, UGANDA (NO1-CP7-0047)

Title: Epidemiological Study of Burkitt's Lymphoma in Uganda

Contractor's Project Director: Dr. George Kafuko

Project Officer (NCI): Dr. Paul H. Levine

Objectives: Since malaria has been considered to be a contributing factor in the etiology of Burkitt's lymphoma, the major objective has been to study the relationship between malaria and Burkitt's lymphoma in the susceptible childhood population of the West Nile district. Specific objectives are: (1) to determine the degree of malaria parasitemia and species of parasite in an adequate subsample of the 35,000 cohort, so that the status of these factors in children can be elucidated before and after they develop BL; (2) to measure serially IgG immunoglobulins, malaria antibodies, and parasites by species and counts in a 200-300 subsample, in order to determine the immune response to malaria typical of children in the study area; (3) to investigate G6PD and hemoglobin type of BL patients and controls to determine factors possibly preventing BL; (4) to determine the measles antibody response in a sample of young children or infants to be immunized by the West Nile seroepidemiology teams in order to ascertain whether the response to measles vaccination is impaired in children with heavy malaria infection.

Major Findings: The malaria survey is paced with the prospective seroepidemiologic survey (IARC contract number NO1-CP4-3273); this contract was terminated on September 25, 1973, but the work was continued under the IARC contract. Up to November 1973, there were 28,358 malaria slides collected since the survey started, including those from controls and from mothers in the main study, the substudy, and case control study. Just over half of these have been read, including counting of parasites. The four counties of the West Nile district of Uganda in which surveys were done were Maracha, Aringa, Terego, and Madi. Initial blood smears were examined from 4,785 children under 6 years of age, and 3,819 (80%) were positive for malarial parasites. Among 9 parishes initially surveyed the parasite rates varied from 64% in Maracha to 80% in Madi, the overall average being 72.7%. Only *Plasmodium falciparum* and *P. malariae* were seen, varying from 78.5% to 82.0% among the parishes, and *P. malariae* from 15.0% to 21.4%. Among 624 children resurveyed, there were 374 (60.0%) positive smears, the parasite rates varying from 54% in Madi to 71% in Terego; *P. falciparum* varied from 86.1% to 92.4%, and *P. malariae* from 7.6% to 13.9%. The parasite density index varied from 2.5 to 3.0. The highest infections and highest parasite density index in the resurveys were found in Terego. There were 5,515 spleens examined initially. The rates varied from 11.0% to 92.0%, the highest being in Terego. In resurveys, 623 spleens were reexamined, the rates varying from 5.0% in Madi to 33.0% in Terego.

Sickle cell trait, AS type hemoglobin, and G6PD are known to confer relative immunity to malaria. A second substudy in 1973 on sickle cell (AS) hemoglobin yielded 30.2% in Aniya, 16.9% in Terego, and 17.8% in Maracha. As in the previous 1972 study, these percentages are not more than could be expected by

chance. [In 1972 Kafuko had found no AS hemoglobin in 32 BL cases, as compared with 9 (15%) in controls; however, this does not agree with findings of other investigators, since Pike found about half of BL cases had AS hemoglobin as compared with controls.] No conclusion can be reached until AS hemoglobin is measured in the BL cases which occur in the West Nile district, with carefully selected controls for comparative analysis. Comparisons between BL and controls have yielded no differences in deficiency of this enzyme between BL cases and controls; therefore, this investigation will be discontinued in the West Nile district.

Malaria antibody studies will be continued in 1974 only on BL cases and controls and their families by Dr. C.C. Draper at the London School of Hygiene and Tropical Medicine. Blood is collected on blotting paper, and antibody is determined by immunofluorescence. In 1971 antibody tests, all children were found infected with malaria below two years of age, primarily with *P. falciparum*. Little difference was found in the antibodies of 46 BL patients, 39 controls, and 50 bloods from the general population. Dr. Munube of the East African Virus Research Institute found that 25% of the measles "negatives" had antibodies, indicating unreliability of the mother's information; therefore, all children aged 9 months to 5 years are now being given measles vaccine. In the substudy the postvaccination antibody levels are being followed from 6 months to 2 years after vaccination. Simultaneous malaria parasite counts are being done, in order to determine whether or not malaria inhibits the response to measles vaccination.

Significance to Biomedical Research and the Program of the Institute: This contract attempts to ascertain the relationship between malaria and Burkitt's lymphoma, and between sickle cell trait and Burkitt's lymphoma. In Africa, Burkitt's lymphoma appears to be restricted to the malaria belt. The disappearance of BL in areas where malaria is well-controlled emphasizes the importance of malaria studies in any program in Africa involving the etiology of BL. The major effort in Uganda of IARC contract number NO1-CP4-3273 involves the determination of the possible role of EBV as an etiologic factor in this disease. The present malaria studies may lead to verification of another potential oncogenic factor of critical importance, since endemic malaria is suspect as a prerequisite for the development of BL in Africa.

Proposed Course: The contract with Makerere University was terminated on September 25, 1973. The proposed work is described under the IARC contract.

Date Contract Initiated: September 26, 1966

UNIVERSITY OF MIAMI (NO1-CP3-3218)

Title: Cellular Immunity in Breast Cancer Using Mouse Mammary Tumor as a Model

Contractor's Project Director: Dr. Diana M. Lopez

Project Officer (NCI): Dr. Paul H. Levine

Objectives: (a) To determine, define, and integrate the relevant reactants and factors in the interaction of mammary tumors and the host, (b) to analyze cellular immunity in relation to clinical state and interventive procedures, and (c) to compare lymphocyte responses to MTV-free and MTV-containing tumors.

Major Findings: Using BALB/cCrgl MTV-free mice and two transplantable MTV-free tumors, the *in vivo* immunogenic D1-DMBA-3 and the non-immunogenic D1-DMBA-2, it was demonstrated that extracts of the former could evoke an increased thymidine incorporation (blastogenic response) in lymphocytes of mice bearing these tumors, whereas extracts of the latter did not evoke such a response in the lymphocytes of mice bearing the corresponding tumors. Mice with D1-DMBA-2 tumors did not have sensitivity to the antigen from immunogenic tumors, and lymphocytes from animals bearing the immunogenic tumor did not respond to the D1-DMBA-2 tumor extracts. Two inferences are possible: (1) D1-DMBA-2 is incapable of sensitizing lymphocytes, or is deficient in its capacity to stimulate sensitized lymphocytes, and (2) the two tumors do not share a common antigen. With tumor progression there was a loss of the lymphocyte response to extracts of the tumor antigen in the immunogenic tumor series and to a mitogen, PHA, in both the immunogenic and non-immunogenic series. This anergy could be reversed by tumor removal and by sham surgery where the tumor mass was not disturbed, suggesting that non-immunological factors might be at play. In preliminary experiments it was found that lymphocytes of mice which remain tumor-free after surgery retain reactivity to tumor antigen, whereas lymphocytes of mice whose tumors return lose this reactivity.

Blastogenic transformation reactions were studied in lymphocytes of BALB/cfC_3H mice bearing spontaneous tumors containing MTV. No stimulation of thymidine uptake was observed in cultures containing mixtures of lymphocytes from the virus-free BALB/cCrgl mice and the BALB/cfC_3H, indicating that there was no major difference in the histocompatibility antigens of two separate lines of BALB/c mice. The antigen extract from the spontaneous tumor stimulated the lymphocytes of a mouse bearing a small MTV-positive spontaneous tumor, but not the lymphocytes of a BALB/cCrgl MTV-free mouse with the transplantable D1-DMBA-3 tumor. The reciprocal combination also failed to elicit a blastogenic response under conditions where the autologous combination caused a significant reaction. These preliminary findings strengthen the contention that the D1-DMBA-3 tumor is free of MTV, for if virus were present in this tumor, one would have expected that the lymphocytes of the animal with the spontaneous tumor would have reacted to the viral antigen; however, definitive proof of this point will require experiments employing purified MTV and lymphocytes from mice of the two lines.

Preliminary indications were obtained that the separation of mouse spleen cells in Ficoll-Isopaque yielded a population of lymphocytes with distinctive specificity of reponse to tumor antigen as opposed to PHA. In preparation for cytotoxicity studies a tissue culture of the D1-DMBA-3 tumor has been developed. The cells in passage 6 were shown to be tumorigenic by

transplantation to BALB/cCrgl mice. For the cytotoxicity study experiments are being undertaken with procedures utilizing ^{51}Cr and ^{3}H-thymidine-labeled target cells.

Significance to Biomedical Research and the Program of the Institute: The genesis, evolution and fate of mammary tumors appear to be determined by multiple factors including viruses. Immunological reactions can be clearly demonstrated both in the host and through *in vitro* procedures. The present work is geared toward the collection of more information about antigens and immunologic responses in the experimental host, with reference to clinical stage of disease and interventive procedures. The use of MTV and its antigens against the background of previous and new information with virus-free antigens is expected to provide new information about the role of the virus, viral and virus-induced antigens in immunity and in non-responsiveness at the organismic and cellular levels. The work may help to elucidate the impact of virus infection (with or without associated tolerance) on specialized cellular populations of the lymphoreticular system. The correlative approach is of direct significance to human breast cancer, since it involves an assessment of certain immunological parameters with the clinical course and with therapeutic interventions. The study with the non-immunogenic tumor is of special interest, inasmuch as human breast tumors may also be characterized by different degrees of antigenic potency.

Proposed Course: A new contract has been proposed to extend the present studies to permit a comparison of tumors and hosts representing an MTV-free system, a system contaminated by the virus, and a system where tumors are induced by the virus. The effect of the virus on host resistance will be assessed. Relevant antigens (TSTA, viral and viral-induced) will be separated, and their contributions to host immunity as well as their evocation of lymphocyte response will be determined. In addition to blastogenic reaction the tests will include cytotoxicity and migration inhibition. Attempts will be made to separate and characterize the cells involved in the various functions of immunity in these systems.

Date Contract Initiated: October 13, 1972

MOUNT SINAI SCHOOL OF MEDICINE AND HOSPITAL (NO1-CP4-3225)

Title: Stimulation of Immunity to Virus and Tumor Antigens by Enzymatically Treated Autologous Tumor Cells

Contractor's Project Directors: Dr. James F. Holland
Dr. Julius G. Bekesi

Project Officer (NCI): Dr. Paul H. Levine

Objectives: (a) To pursue a program of chemoimmunotherapy in lymphoma bearing AKR mice using neuraminidase-treated cells; (b) to study the

stimulation of immunity to virus- and tumor-specific antigens in successfully treated and control mice; (c) to examine the efficacy of allogeneic Gross leukemia virus-induced E_2G leukemia as an immunogen; (d) to study the effectiveness of maintenance therapy using compounds which show antiviral activity, e.g., adenine arabinoside, Palm O-araC, Virazole, and other drug combinations which may suppress the induction of clonogenic lymphoma cells in the treated host; (e) to study the effect of Statolon and synthetic polyanions-interferon inducers on the relapse of lymphoma in AKR mice; (f) to determine the function of the spleen in the reinduction of secondary lymphoma in AKR mice: (g) in collaboration with Dr. Oldstone of the Scripps Clinic and Research Foundation, to determine the antibody-antigen complexing in the sera and kidney of animals receiving chemotherapy alone and AKR mice treated with chemotherapy and immunotherapy; (h) to determine the viremia of AKR mice in the experimental protocol (in collaboration with Dr. Gravell at Litton Bionetics, Inc.), in order to elucidate the mechanism of action of neuraminidase-treated leukemic cells in AKR mice infected _in utero_ with GMLV and carrying large amounts of the virus throughout their life; and (i) in relation to the Acute Leukemia Group B, to identify and study families of particular interest to investigators in the Virus Cancer Program (identical twins, siblings with cancer, etc.).

Major Findings: AKR mice acquire Gross virus prior to their birth, but the virus remains dormant in the animals until they reach the age of 6 months, when they develop spontaneous lymphoma. By 12 months of age, about 90% to 95% of the mice die from leukemia. Studies were made on the efficacy of various carcinostatic agents with and without neuraminidase-modified syngeneic or allogeneic Gross virus-induced E_2G leukemic cells in this animal model system because of the similarity of the disease to human acute lymphocytic leukemia. Chemotherapy followed by active immunotherapy using neuraminidase-treated AKR lymphoma cells delayed the reappearance of viable clonogenic lymphoma cells (from day 5 to 13 for spleen, and from day 12 to 21 for thymus). Chemotherapy alone sustained an increase in life span of 150-300%, but only 5% of the treated AKR mice survived beyond 100 days. Drug therapy plus immunization with neuraminidase-treated spontaneous leukemic AKR cells or with Gross virus-induced allogeneic E_2G leukemic cells (administered s.c. or i.d.) resulted in 20% to 40% of the animals surviving beyond 120 days without evidence of disease. Longer observation of such treated mice beyond 150 days showed, however, that in spite of "cell cure" (between 150 to 260 days after the initial diagnosis), a relapse of leukemia followed. Using the ^{51}Cr cytotoxicity assay for the _in vitro_ monitoring of immunity, the immunized mice yielded an antibody titer of 128 about 21 to 28 days after initiation of active immunotherapy.

Splenectomy, particularly if performed after chemotherapy and followed by immunotherapy, significantly increased the survival of leukemic AKR mice as compared to those animals which received the same therapeutic regiment but were only sham splenectomized. Experiments performed in clinically diagnosed leukemic AKR mice with Virazole showed an apparent anti-tumor effect; multiple injections of Virazole resulted in about an 85% increase in survival time of leukemic AKR mice. In other experiments clinically diagnosed AKR mice first received combination chemotherapy followed by anti-viral therapy. Virazole

resulted in marked delay in the reappearance of viable clonogenic lymphoma cells in treated AKR mice measured by the explantation-splenomegaly assays, as well as increased survival time of these animals compared to animals receiving cytoreductive therapy alone. A study on AKR mice having Gross virus-induced lymphoma with or without cytoreductive therapy and/or active immunotherapy indicated 9-β-arabino-furanosyl-adenine (ara-A) was ineffective.

Statolon, which has strong effects against Friend leukemia virus (FLV), Moloney sarcoma virus (MSV), and Rauscher leukemia virus, resulted in an increased survival time of 200 to 260% when combined with chemotherapy, depending on the route and concentration of Statolon (chemotherapy alone gave an increased life span of 130%). The combination of Vincristine + Palm O-araC + BCNU or MCCNU followed by injection of Statolon significantly inhibited the appearance of secondary lymphoma (as measured by the explantation-splenomegaly assay) and increased the survival time of leukemic AKR mice from 170% to 380% in another series of experimental groups. (Vincristine + Dexamethasone with Statolon was ineffectual in leukemic AKR mice.) Statolon not only significantly prolonged the appearance of lymphoma in AKR mice, but also substantially increased the immunological responsiveness of the host when using neuraminidase-treated leukemic cells. An excellent working arrangement was developed with Dr. Oldstone at the Scripps Clinic and Research Foundation where sera and kidneys from protocol mice will be evaluated for specific viral antibody-antigen complex. Similarly, Dr. Gravell at Bionetics and Drs. George and Eva Klein at the Karolinska Institute, are measuring the amount of viremia in the protocol animals, as well as testing the sera from AKR mice in the immunotherapy protocol by cytotoxicity, immunofluorescence, mixed hemadsorption, virus neutralization, and immune adherence tests. These assays are also being used to monitor immune reactions in leukemia patients on Phase I studies of chemoimmunotherapy.

Significance to Biomedical Research and the Program of the Institute: The contractor has successfully explored the parameters of neuraminidase incubation of spontaneous and experimental tumor cells and established treatment protocol which lead to their increased immunogenecity. They have had success with combined chemotherapy and immunotherapy using neuraminidase-treated cells in the cure of animals with leukemia L1210 in $DBA/_2$ hosts, 316 melanomas in BALB/c mice, and in the cure of a substantial number of AKR mice having Gross virus-induced autochthonous leukemia. Similar results were obtained with Gross virus-induced allogeneic E_2G leukemia in AKR (leukemic) mice. No other data exist concerning the successful use of chemoimmunotherapy with neuraminidase-treated cells in an autochthonous system. Their data indicate substantially greater resistance to challenge than in any other reported experimental leukemic system using other types of immunization (such as chemically-modified leukemic cells, irradiated leukemic cells, MER or BCG). The knowledge gained from the treatment of transplantable and spontaneous leukemia and mammary tumor with *Vibrio cholerae* neuraminidase, with and without chemotherapy, is highly relevant to man suffering from neoplastic disease. Information obtained during this contract is now beginning to be applied in a clinical study of persons suffering from neoplastic disease.

Proposed Course: It is intended to continue and further extend these studies in AKR mice harboring spontaneous leukemia. A study will be initiated with C_3H mammary tumor in C_3H mice which are free of MTV or neonatally infected with MTV. A major attempt will be made to ascertain the host's humoral and cellular immunity which is directed to a viral transplantation, and possible "private" antigens in vivo and in vitro. Administration of various carcinostatic agents will be continued. Special attention will be paid to the reversible and possibly irreversible immunosuppressive action of these therapeutic agents. The efficacy of Statolon and polyanions-interferon inducers on the relapse (reinduction) of lymphoma in AKR mice and mammary tumor in C3H mice will be studied. The immunogenic dose, site, and frequency of immunization with autochthonous, syngeneic or allogeneic tumor cells, and the optimal conditions for the enzymatic treatment of target cells with neuraminidase will be established. It is intended to quantitate the immunological response of the host by mixed lymphocyte culture, ^{51}Cr cytotoxicity assay, blastogenic transformation, MIF, and other cytocidal bioassay techniques which may influence the schedules of the clinical therapy employed. Collaborative studies with Dr. Oldstone will be continued in order to determine the antibody-antigen complexing in the sera and kidneys of AKR mice receiving chemotherapy or chemotherapy plus immunotherapy. Collaborative studies will be continued with Drs. Eva and George Klein at the Karolinska Institute to test sera obtained from AKR mice in the immunotherapy protocol by various immunological assays, e.g., cytotoxicity, immunofluorescence, virus neutralization, and immunoadherence tests. These tests are expected to elucidate the mechanism of action and specificity of neuraminidase-treated leukemic cells in AKR mice infected in utero with GMLV and carrying large amounts of Gross virus throughout their life. With the approval of the NCI Clinical Research Committee, monitoring of procedures for immune stimulation of cancer patients will be pursued in the areas of acute myelocytic leukemia and breast cancer.

Date Contract Initiated: August 6, 1973

NEW YORK MEDICAL COLLEGE (NO1-CP3-3398)

Title: Immunologic Measurements as a Guide to the Behavior and Viral Etiology of Breast Cancer

Contractor's Project Director: Dr. Maurice M. Black

Project Officer (NCI): Dr. Clarice Gaylord

Objectives: (1) To look for a correlation between biological behavior of breast cancer and immunological responses to autologous breast cancer tissue; (2) to evaluate the antigenicity of various types of breast lesions, viz. normotypic, precancerous atypia, in situ carcinoma, and invasive carcinoma; (3) to search for evidence of the role of a mammary tumor virus in human mammary carcinogenesis by immunologic procedures and molecular hybridization

procedures using murine MTV (MuMTV) as a probe (in collaboration with Dr. Dan Moore); and (4) to determine immunological responses of "control" women to MuMTV and *in situ* breast cancer according to "risk" factors, viz. parity, exogenous estrogens, family history of breast cancer, etc.

Major Findings: Immunological measurements were performed on 64 breast cancer patients and 39 control women (with and without benign breast lesions). The cancer patients were tested as follows: skin window tests vs. autologous breast cancer (35); leukocyte migration inhibition (LMI) tests vs. autologous breast cancer (39), vs. homologous *in situ* breast cancer (173), vs. homologous invasive breast cancer (53), vs. benign breast tissues (44), vs. KCl extracts of cancerous and benign breast lesions (24), vs. common environmental antigens (44), vs. RIII mouse milk containing mammary tumor virus (MTV) (61), vs. RIIIf mouse milk without MTV (22), and vs. C57BL mouse milk without MTV (18). The control women were tested with the LMI procedure against the following targets: vs. invasive breast cancer (19), vs. *in situ* breast cancer (87), vs. benign breast lesions (25), vs. KCl extracts of cancerous and benign breast lesions (13), vs. common environmental antigens (34), vs. RIII mouse milk containing MTV (35), vs. RIIIf mouse milk without MTV (8), and vs. C57BL mouse milk without MTV (8).

The skin window procedure was used to test cellular hypersensitivity responses of breast cancer patients against cryostat sections of autologous breast cancer tissues. The immunological responses to autologous breast cancer were also evaluated in terms of the cellular responses seen microscopically in sections of the primary tumor (diffuse lymphoid cell infiltration, perivenous lymphoid cell infiltrations) and in regional lymph nodes (sinus histiocytosis, follicular hyperplasia). Moreover, each breast cancer was classified as to nuclear grade, histologic type, and stage. All patients (cancer and control) were identified as to age, family history of breast cancer and the use of exogenous estrogens. Cellular hypersensitivity responses to skin window tests correlated with L-RE responses in the primary tumor and regional lymph nodes, and both were prognostically favorable.

The leukocyte migration procedure was used as a measure of cellular hypersensitivity responses of human leukocytes against human breast tissues. The migration of leukocytes from control women, with and without benign breast lesions, was commonly inhibited by Varidase, but rarely inhibited by any of the mouse milk samples or by any of the benign or malignant breast lesions. The migration of leukocytes from breast cancer patients was commonly inhibited by Varidase, but was not inhibited by virus-free mouse milk samples or by benign breast tissues. However, migration inhibition (≥25%) was found in 31 percent of breast cancer patients tested against RIII milk, in 33 percent of tests against homologous *in situ* breast cancer, 29 percent of tests against autologous invasive breast cancer, and 16 percent of tests against homologous invasive breast cancer tissues. Responsiveness to breast cancer tissues was correlated with a high degree of cross reactivity against RIII mouse milk. Conversely, leukocytes which responded to RIII milk cross reacted in the majority of tests against homologous *in situ* breast cancer in approximately one-third of tests against invasive breast cancer, but were nonresponsive to RIIIf milk, C57BL milk, and benign breast lesions. It appears that the

antigenicity of human breast cancer tissue is largely a reflection of a component which is similar to that found in MuMTV-infected lactating mammary parenchyma. Such antigenicity is more regularly found among in situ breast cancer tissues than among invasive breast cancer tissues.

The studies of the response of human leukocytes to mouse milk samples were performed in collaboration with Dr. Dan Moore and his associates, Institute for Medical Research. Dr. Moore's group provided precisely defined milk samples for immunological studies in Dr. Black's laboratory, while Dr. Black supplied precisely classified (pathologically and antigenically) breast tissue samples to Dr. Moore's laboratory for hybridization studies aimed at the detection of viral genomes. Although the data are still limited (18 breast cancers) it appears that the hybridization of RNA derived from human breast cancer with DNA probes prepared from MTV RNA may be correlated with histopathologic and antigenic characteristics of the human donor tissue.

Significance to Biomedical Research and the Program of the Institute: The data provide additional support for Black's contention that immunogenicity appears and is maximal during the in situ phase of mammary carcinogenesis. Moreover, there appears to be a high degree of similarity between murine MTV-associated antigenicity and the antigenicity of human breast cancer tissues. Further support for the participation of murine MTV in human mammary carcinogenesis is provided by the hybridization studies. These data are pertinent to the phenomenon of human mammary carcinogenesis in general and the potential for immunoprophylaxis in particular.

Proposed Course: During the coming year the contractor plans to exploit the leads developed to date with particular reference to correlations between clinicopathologic, immunologic, and biochemical measurements: (1) more precise definition of the antigenically similar components of MTV-containing RIII milk and human breast cancer tissue; (2) immunologic responsiveness of "control" and breast cancer patients to MTV-containing RIII milk and in situ breast cancer tissues by age, family history of breast cancer, and prior use of exogenous estrogens; and (3) correlation between the structural (stage, nuclear grade) and antigenic characteristics of breast cancers and the presence of viral genomes (hybridization with DNA probes prepared from mouse MTV). In addition to providing data highly relevant to the viral etiology of human breast cancer, such studies are prerequisites for defining the most appropriate "antigens" and the optimal conditions for the development of immunoprophylaxis of human breast cancer and approaches to tumor immunotherapy.

Date Contract Initiated: June 26, 1972

ROBERT B. BRIGHAM HOSPITAL (N01-CP1-2172)

Title: Cell-Mediated Tumor Antigens as Measured by Macrophage Migration Inhibition

Contractor's Project Directors: Dr. John R. David
Dr. W. Hallowell Churchill

Project Officer (NCI): Dr. Gary Pearson

Objectives: (1) To develop assays capable of monitoring the cellular immune response to human tumor; (2) to explore specifically the usefulness of inhibition of macrophage migration or inhibition of leukocyte migration as a measure of cell-mediated immunity to human tumors; and (3) to study in animals mechanisms of cellular cytotoxicity in vitro because better understanding of the mechanism in animals should in turn lead to more effective assays in man.

Major Findings: The direct leukocyte migration assay, initially described by Bendixen and Soborg, and modified by Rosenberg and David, has been used to search for cellular immunity to two soluble antigens, SK-SD and PPD, and to breast cancer antigens solubilized by the 3MKCl antigen extraction technique. For the soluble antigen, a positive assay was considered to be 82% or less. When the result of the in vitro assays were compared to skin tests against SK-SD and PPD in a large series of normals, there were essentially no false positives and 26% false negatives (i.e., negative in vitro assay with a positive skin test). Normals who yielded a false negative on one test tended to do so on repeat tests. Breast cancer patients have been tested on the day of operation with an allogeneic 3MKCl extract, and post-operatively with autologous or allogeneic extracts.

The results of the patients' in vitro assays were always compared to those of normal individuals which were tested simultaneously with the same antigens. The initial 12 patients were tested without a preincubation step. In this group, one had been tested 5 times, 3 tested 3 times, 2 tested twice and the remaining tested only once. Using 82% or less as positive, 4 of these patients had at least one positive reaction to a breast cancer antigen; however, 2 false positives to one particular alloantigen were observed. In these two instances, significant inhibition of the normal cells but not the patient's cells was observed. Dose response curves with breast cancer antigens did not reveal any consistent pattern, but all assays were done with antigen concentration of 200 μg or greater. After the first 12 patients, technical modifications were introduced with the hope of obtaining greater sensitivity; the cells were packed in 20 lambda capillaries at lower centrifuge force than previously. Finally, a preincubation step with antigen was introduced prior to placing the cells in capillaries.

Early in the current reporting period, the series of 7 patients tested with the modifications noted above were completed. Four of these patients were from a group of patients followed by Monaco and McDonough, who have also tested these patients for skin reactivity to breast cancer antigens and assayed for cellular immunity by the colony inhibition technique. The results did not suggest that the technical modifications described above effected a substantial improvement in the assay. Reactivity against the control antigen in 43% of normals was negative despite known skin reactivity against the same antigen. There was only one definite positive reaction against an allo-breast

cancer antigen, and this was counterbalanced by a false positive reaction in a normal against another allo-breast cancer antigen.

Mechanisms of the leukocyte migration assay were studied by Dr. Ross Rocklin. His findings suggest that there are at least 2 distinct migration inhibition systems that are related to delayed hypersensitivity, and that polymorphonuclear indicator cells from a third party may be used to detect production of this mediator. Some of the variability of the direct leukocyte migration assay may be the result of variations in indicator buffy coat cells from donor to donor. It is now possible to use an indirect leukocyte migration technique in which all supernatants, in a manner similar to the system for detecting MIF, are tested on indicator polymorphs from one donor. This modification could improve leukocyte migration assay sensitivity and reproducibility.

Significance to Biomedical Research and the Program of the Institute: The leukocyte migration assay might be used for study of groups of patients with vigorous cellular immunity against certain soluble antigens, such as SK-SD and PPD. Polymorphonuclear leukocytes, separated from peripheral white blood cells by sedimentation on Ficoll, may be better indicators of production of LIF (leukocyte inhibitory factor) than whole buffy coat cells; however, contrary to other reports, the contractor was not able to obtain evidence that this assay is useful in detecting cellular immunity to tumor antigens.

Proposed Course: This contract was terminated on October 26, 1973.

Date Contract Initiated: June 28, 1971

SCRIPPS CLINIC AND RESEARCH FOUNDATION (N01-CP3-3204)

Title: Immunopathologic Study of Leukemia

Contractor's Project Director: Dr. Michael B.A. Oldstone

Project Officer (NCI): Dr. Tadao Aoki

Objectives: (1) To identify and quantitate the viral antigens of Gross leukemia virus and antibodies to these antigens that are found in the virus antiviral antibody complex of AKR mice; (2) to apply the knowledge learned through the elution, recovery, identification and quantitation of viral antigens in the AKR model to the study of tissues from patients with leukemia and cancer; and (3) to develop quantitative, immunospecific, and sensitive assays for the detection of antiviral antibodies in the presence of antigen excess.

Major Findings: The incidence of immune complex disease in AKR/J mice has been studied. These mice spontaneously develop leukemia as a result of their natural infection with Gross leukemia virus and have an 84% incidence of

immune complex disease. Among over 300 leukemic and non-leukemic 6-10 month old individual AKR/J mice studied, 84% had significant deposits of host immunoglobulin G and complement along the renal glomerular basement membrane and in the mesangia in a granular, lumpy-bumpy pattern. Pools of kidneys from AKR/J mice showing IgG and complement deposits have been homogenized, the antigen-antibody complexes eluted off by low acid, low molar buffer treatments, and the various antigens and antibodies identified. Evidence has been found for the presence of gs-1 and gs-3 antigens, and antibodies to GSA, other envelope viral antigens, and murine reverse transcriptase. In other experiments, antibodies toward tumor-specific antigens have been found complexed in the kidney. In this case, mice inoculated with neuroblastoma cancer cells have been followed for development of immune complex disease. Such mice develop immune complex disease. Elution of the antigen-antibody complex and its subsequent recovery identifies the antibody as being specific for the tumor membrane antigens. Studies of renal tissues from patients with African Burkitt's showed evidence of IgG and complement deposits in a granular pattern along the glomerular basement membrane and mesangia. Elution of the immunoglobulin and its recovery from glomeruli has identified the antibodies as having specificity towards several EBV antigens.

Significance to Biomedical Research and the Program of the Institute: Over the past several years, there has been intensive investigation of the immunopathogenesis of persistent viral infections and virus-induced immune complex disease. Of particular importance and application to the cancer field and to the program of the institute are the contractor's observations that in persistent viral infections in which a low antibody response is made, viral-antiviral antibody complexes may be found in the circulation and subsequently become trapped in the renal glomeruli. Because of antigen (virus excess), free antibody is not found in the circulation, but can be recovered, identified and quantitated in the renal glomeruli. The techniques used in the Principal Investigator's laboratory for analyzing the viral antigen-antiviral antibody complex have led to a convenient marker for identifying the persisting agent in the absence of detectable free antibody. This suggests that in looking for markers to identify the cause of human cancer, the techniques used in animal models of persistent infection should be applicable. Of prime importance in this regard is the observation that in spontaneous leukemia of AKR mice, antibody to Gross virus or Gross virus cell surface antigen can be found and recovered from the renal glomerulus where they have been deposited in the form of antigen-antibody immune complexes. Hence, direct immunologic evidence has been found for identifying the etiologic agent in spontaneous murine leukemia by immunochemical techniques, and possibly these procedures will be of importance in the study of spontaneous leukemia and cancer of man.

Proposed Course: The proposed course of this study is to identify all the antigen-antibody complexes present in the immune complex of AKR mice, to establish specific immunologic sensitive assays for identifying the antigen and/or antibody, and to apply this technology to the study of human cancer. In addition, the role of viral antigens on the plasma membrane, and the role of antibodies in inducing antigenic change and formation of antigen-antibody complexes, are also being evaluated.

Date Contract Initiated: September 1, 1972

UNIVERSITY OF TEXAS (NO1-CP3-3292)

Title: Immunity to Sarcoma-Related Antigens in Patients and Controls

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

Project Officer (NCI): Dr. Gary Pearson

Objectives: The overall major objective of this contract is the collection of evidence for human sarcoma-specific antigens as a means of identifying a human sarcoma virus. This is being approached through the following projects: (1) the establishment of human sarcoma cell cultures for immunological and virological investigation largely in collaboration with other contractors in the VCP; (2) long-term evaluation of the correlation between the clinical course of patients with sarcomas and immunological parameters measured *in vitro* (by lymphocyte-mediated cytotoxicity assay, by serum factors antagonistic to or synergistic with the lymphocytes, and by specific serological studies directed against relevant animal sarcoma viruses) and *in vivo* (by skin tests utilizing sarcoma cell membrane preparations as antigens); (3) determination of the possible role of an infectious agent in the sensitization of lymphocytes from clinically normal, healthy individuals to long-term cultured sarcoma cell lines in the *in vitro* cytotoxicity assay; and (4) determination of the nature of the infectious agent released by some of the sarcoma cell lines.

Major Findings: A LabTek chamber/slide assay involving testing leukocytes and sera of patients against established cultures of tumors revealed that 54 patients with sarcomas yielded leukocytes cytotoxic to cultured sarcoma cells in 105 of 126 tests (83%), and to cultured carcinoma cells in only 13 of 126 tests (10%). The leukocytes of 13 patients with carcinomas of the type against which they were tested were cytotoxic to cultured carcinoma cells in 20 of 21 tests (95%), and to cultured sarcoma cells in 12 of 21 tests (57%). The leukocytes of patients with sarcomas thus clearly distinguished between sarcoma and carcinoma cells grown in the same medium.

In the cytotoxicity assay, 2 major populations of lymphocytes could be distinguished: (2) the population of "committed" lymphocytes interacting with tumor cells promptly after cocultivation (early phase cytotoxicity): these lymphocytes are considered to have undergone pre-sensitization to tumor antigens *in vivo*; (b) the population of "noncommitted" lymphocytes interacting in a protracted fashion with allogeneic cells, disregarding whether these were neoplastic or non-neoplastic (late phase cytotoxicity); these lymphocytes were considered to have acquired cytotoxic potency *de novo in vitro* to either normal HL-A antigens or to tumor antigens or to both.

Correlation of the *in vitro* assay with clinical course has also been investigated. A good correlation of the assay with clinical course constituted either: (1) tumor regression with cytotoxic lymphocytes and/or synergistic serum factors (Group A); or (2) continuing tumor growth without reactive lymphocytes and/or with blocking serum factors (Group B). Lack of correlation occurred when tumor growth continued despite cytotoxic lymphocytes and/or synergistic serum factors, or in the absence of blocking serum factors (Group C). Unclassifiable patients awaiting further evaluation are those with contradictory results upon repeated *in vitro* testing (Group D). Twenty-six from Group A, 12 from Group B, 6 from Group C, and 9 from Group D, of 38 (Group A plus Group B) out of the 53 patients with sarcomas (71.6%) thus correlated well with the *in vitro* assay.

Six patients with sarcomas were studied for correlation of blocking serum factors and tumor growth. Disappearance of blocking serum factors coincided with temporary tumor regression in 4 cases; tumor growth continued until death despite absence of blocking serum factors in 2 cases. The occurrence of blocking or unblocking serum factors correlated well with the clinical course of a pair of identical twins: one member of the pair had rhabdomyosarcoma and blocking serum factors, the healthy twin circulated unblocking serum factors. Treatment of the sarcoma-bearing twin, with plasma and leukocytes of the healthy twin, however, failed to maintain a chemotherapy induced remission.

Immune reactions of patients with sarcomas and normal individuals were compared in a microplate assay, using as target cells established sarcoma and carcinoma cell lines. Purified lymphocytes of 20 patients with sarcomas were cytotoxic to cultured sarcoma cells in 21 of 44 tests (48%), and to cultured carcinoma cells in 6 of 21 tests (28%). Purified lymphocytes of normal donors were cytotoxic to cultured carcinoma cells in 8 of 17 tests (47%), and to cultured sarcoma cells in 11 of 29 tests (38%). The lymphocytes of patients with sarcomas thus clearly distinguished between sarcoma and carcinoma cells; the cytotoxic capacity of normal lymphocytes, however, is high. Some lymphocyte preparations augmented the growth of target cells: carcinoma cell growth was more frequently enhanced by normal lymphocytes (24% of the tests) than by lymphocytes of patients (14% of the tests); sarcoma cells were stimulated to grow only occasionally by lymphocytes. Serum factors antagonistic to or synergistic with cytotoxic lymphocytes occurred also in normal sera: almost half of the tests in which normal sera were tested demonstrated serum factors blocking the cytotoxicity of lymphocytes to a tumor target cell line.

Significance to Biomedical Research and the Program of the Institute: The significance of this project is multiple: (1) 7 of 13 (54%) of normal individuals tested possessed circulating lymphocytes which were cytotoxic to sarcoma cells. If this finding is confirmed in a larger number of tests, it will strongly suggest that human sarcomas are viral-induced, the virus spreading horizontally and having a low clinical pathogenicity; thus, the project proposed herein may lead to the discovery of a human sarcoma virus(es); (2) the project may provide an *in vitro* assay capable of monitoring immune reactions of patients with sarcomas to their tumors; the high incidence of immune reactions to sarcoma cells in the normal human population pre-empts the

use of the assay for diagnosis, but the correlations of the assay with the clinical course of patients with sarcomas predict usefulness of the assay in prognostication and evaluation of investigational immunotherapy protocols; (3) further characterization of the infectious agent released by human sarcoma cell lines could provide important leads to the identification of a human sarcoma virus; and (4) established human sarcoma (and other tumor) cell lines will be available for further (biochemical, etc.) studies to interested investigators.

Proposed Course: Efforts will continue to develop cell lines from primary sarcoma cultures. Cultures of normal fibroblasts will also be initiated and stored deep-frozen in the viable state. These cultures will be particularly important in studies designed to determine whether the reactivity of lymphocytes from normal individuals is directed against tumor-specific antigens. This area will receive a high priority. Particular emphasis will be placed on identifying and characterizing the filterable agent released by human sarcoma cell lines in collaboration with other investigators in the VCP. Sera from sarcoma patients and controls will be tested for antibodies cross-reactive with known animal sarcoma viruses. Immune reactions of patients with sarcomas will be tested against autologous cultures of sarcoma cells and of normal fibroblasts, and against allogeneic established sarcoma cell lines. Immune reactivity of normal individuals will be similarly tested. The immunological assays to be used will be the lymphocyte-mediated cytotoxicity assay measuring also serum factors antagonistic to or synergistic with the cytotoxic lymphocytes; a macrophage migration inhibitory assay; and serum antibodies in indirect immunofluorescence (both membrane and cyto-plasmic) and in complement fixation tested against sarcoma cells. The immune reactions of patients with sarcomas will be correlated with responses to skin tests with sarcoma-cell-membrane preparations, and with the clinical course of the patients. Special attention will be given to those patients whose clinical course fails to correlate with the *in vitro* assay. The anti-tumor reactions of normal individuals will be investigated for tumor-specificity. The viral etiology of sarcomas will be investigated by attempting to induce oncornaviruses in established sarcoma cell lines with IUdR and DMSO; virion synthesis will be visually evaluated by electron microscopy. Human cells will also be infected with the woolly monkey sarcoma virus to determine if immune reactions to this virus, or to cells harboring this virus, are detectable in the human population.

Date Contract Initiated: April 1, 1971

UNIVERSITY OF TEXAS, M.D. ANDERSON HOSPITAL AND TUMOR INSTITUTE
(N01-CP3-3301)

Title: Oncogenic Virus-Associated Immunity and Immune Responses in Human Cancer

Contractor's Project Director: Dr. Evan M. Hersh

Project Officer (NCI): Dr. Paul H. Levine

Objectives: (1) To study the human immune response to the Rauscher leukemia virus, including studies of both cell-mediated and humoral immunity; (2) to generate reagents useful in studies of immunity to RLV antigens and the possible viral etiology of human neoplasms; and (3) to develop methodology for the possible viral immunotherapy of human cancer. This will ultimately involve immunization with oncogenic viruses or preparations derived from oncogenic virus transformed cells for the treatment or prevention of recurrence of cancer.

Major Findings: Testing and evaluation were completed on the first 20 patients selected for this study. Thirteen had solid tumors and seven had adult acute leukemia. All patients were considered to have widely disseminated disease beyond cure and to have a known limited life span. In order to qualify for the study and before informed consent was obtained, the patients were required to be ambulatory, to be in good general condition, to have an estimated survival of at least three months, and to be immunocompetent according to standard in vivo and in vitro clinical tests. Sixteen of the patients were on various types of chemotherapy and four were on BCG immunotherapy during the study period. Prior to entrance into the study, the patients underwent delayed hypersensitivity skin testing with a battery of five skin test antigens including dermatophytin, dermatophytin-O, candida, varidase and mumps. To enter the study, they were required to be skin test positive, that is, to show delayed hypersensitivity to at least two of these antigens. In addition, prior to entrance into the study, the patient's peripheral blood lymphocytes were cultured and stimulated with phytohemagglutinin (PHA). The PHA response was required to be at least 20,000 counts per minute for acceptance into the study.

The Rauscher leukemia virus preparation used in this study was grown in a BALB/c mouse cell line. The supernatant from these cultures was collected and the virus was separated from the culture fluid by first centrifuging the debris at 7000 x G for 10 minutes and then double sucrose density gradient centrifugation, first in a model K zonal centrifuge and then in a B-29 rotor. The density zone from 1.13 to 1.17 was collected. Among the 20 immunized patients, two-thirds mounted a cell-associated response as measured by lymphocyte blastogenesis to antigen extracted from RLV-infected cells, half of the patients mounted a cell-mediated response as measured by delayed hypersensitivity, and half of them mounted a humoral immune response measured by radioimmunoprecipitation. The twenty patients had been immunized with four doses of the Rauscher leukemia virus every two weeks and were followed according to protocol. There were no untoward side effects, and no unusual changes in the patient's course of disease. There were no changes in the PHA or SLO responses, but there was a significant increase in the lymphocyte response to both the RLV preparation and to the JLV-V9r antigen. This was both in terms of counts per minute and stimulation index. The response to the lymphocyte RLV vehicle did not increase very much, but the response to the JLS-V9c antigen (the control antigen) did increase significantly. Thus,

while anti-viral immunity and anti-tumor-associated immunity developed, there was also some development of what is presumed to be anti-mouse immunity.

The bulk of the response occurred during the first three to four weeks after immunization, after which the response fluctuated around a given level. The lymphocyte response to the RLV preparation was greater than that to the JLS-V9r antigen. The median responses were low because five or six of the patients failed to develop immunity to the immunization. The lymphocytes of 14 patients showed an increase in their response to the virus as determined by counts per minute, and 15 showed such an increase in terms of the stimulation index. A true response to immunization with the formalin-fixed virus was defined as an increase in both the counts per minute and the stimulation index. Fourteen patients showed a true response by this criterion in terms of their *in vitro* lymphocyte reactivity to the virus preparation, and nine patients showed it in terms of their *in vitro* lymphocyte response to the JLS-V9r antigen.

The immunizations were intradermal, and immunization sites were examined at 24 and 48 hours. Five patients showed some degree of delayed hypersensitivity to the virus on their first test, suggesting previous exposure to an antigen in the RLV preparation. Thirteen patients showed a response post-immunization, and ten patients showed a true response to immunization by changing from negative to positive, or showing a greater than 100 percent increase in diameter of induration. Cell-mediated immunity in terms of delayed hypersensitivity thus developed in one-half of the patients. There was no significant difference between the response of patients with leukemia, melanoma or other solid tumors, or between patients receiving chemotherapy, chemotherapy plus BCG or BCG alone. This was determined by both the chi-square test and the Wilcoxon signed rank test. The patients with leukemia had the highest pre-immunization values, while patients receiving BCG alone had the highest post-immunization values.

Antibody responses were measured by radioimmunoprecipitation by Dr. M.G. Hanna, Jr. of the Oak Ridge National Laboratory. Approximately one-half of the patients developed antibody to the RLV as measured by radioimmunoprecipitation. No patients had antibody prior to immunization. The maximum responses usually occurred toward the end of the immunization period during week 6-8. This suggests that further immunizations might further raise the antibody titer, and this may be necessary to produce neutralizing antibody. The latter would be important in the development of viral immunotherapy. In most, if not all, of these patients, both pre- and post-immunization, there was a significant anti-mouse antibody titer demonstrable prior to absorption. Virus precipitating antibody was present which could be absorbed out by exposure of the serum *in vitro* with 3T3 cells, or with JLSV-6 cells, or more effectively by passage of the serum *in vivo* in the mouse. Several of the sera containing antibodies to the Rauscher leukemia virus were tested against the RD114 virus, and no precipitation was seen. These sera were also examined for complement fixing activity by Dr. R. Huebner, and for neutralizing activity both by Dr. R. Huebner and Dr. M.G. Hanna, Jr. Some complement fixing activity was found; however, the high anticomplementary

activity of the sera precluded their full evaluation. Neutralizing activity was not found; also, treatment of patients with BCG without immunization did not produce an anti-RLV antibody titer in them. There was a highly significant correlation between the development of antibody and the development of delayed hypersensitivity to the virus, and between development of an antibody and the development of *in vitro* lymphocyte reactivity in terms of counts per minute to the JLV-V9r antigen. The delayed hypersensitivity responses did not correlate significantly with the blastogenic responses. Since leukemia patients had a higher pre-immunization lymphocyte response to the RLV preparation than non-leukemia patients, studies have been initiated to follow serially the lymphocyte responses of a group of non-immunized leukemia patients. Both new patients and patients with long-standing disease (including remission and relapse cases) have been selected for this study, the objective being to determine whether there is indeed an increased level of spontaneous immunity to RLV in human leukemia. Ten (10) patients have been placed in this study, and a total of 20 is anticipated.

Significance to Biomedical Research and the Program of the Institute: These studies may lead to the possible development of a vaccine with potential utility in acute leukemia. They will contribute materially to our understanding of the immune response to oncogenic RNA viruses and will have considerable applicability if it is determined that an RNA virus causes any form of human cancer.

Proposed Course: The next phase of the study will be to utilize the human reagents generated in the first study and study the specificity of the human leukemia antigens that have been cross-reacting with animal oncogenic viruses.

Date Contract Initiated: May 25, 1972

TRW SYSTEMS GROUP, TRW INC. (NO1-CP3-3252)

Title: Preparation and Purification of Viral Antigens and Antiviral Antibodies

Contractor's Project Director: Dr. Norman Weliky

Project Officer (NCI): Dr. Vincent Hollis

Objectives: The objectives were to isolate those antigens from EBV-infected or transformed cells which react with human sera found to be positive for Epstein-Barr virus by the fluorescent antibody test. Because of the low titer of antisera and antigens currently available, complement-fixing positive antigens were examined first, with the intention of isolating immunodiffusion-positive antigens and fluorescent-positive (or

radioimmuno-positive) antigens, comparing their identity with respect to source and reactivity with different human sera. The purified antigens were to be used to prepare animal antisera specific for those antigens, for standardized detection, assay and other purposes for which the antisera could be used. Further purification of such animal antisera would be undertaken as necessary.

Major Findings: Starting materials for the isolation and characterization of complement fixing (CF) antigens were P3HR-1, HK-Ly-28 and Raji cell line lysates, with pellets removed at 2,000 x g. These were again fractionated by centrifugation at 44,000 x g yielding (a) a supernatant fraction, N-44-S and (b) a pellet designated P-44-7, after further washing with neutral buffer. Inactivation of CF antigens during separation procedures led to investigations of antigen stability under the chemical conditions encountered. This approach was extended to characterize further the CF antigens. Some of the current significant results are as follows:

(1) Soluble CF antigens were partially purified by the use of immunoadsorbents (with conjugated human serum), by ammonium sulfate precipitation and by precipitation in acid. Most bands which were stained after electrophoresis of crude soluble antigen (N-44-S) in 7% polyacrylamide gels, were no longer present, particularly in gels with immunoadsorbent purified antigen. (2) The purified soluble P3HR-1 CF antigens lost activity upon storage for four days at 4° or -80°. (3) By comparing chemical and physical conditions for stability and inactivation, and by selective absorption of antisera with immunoadsorbents, the following CF antigens could be distinguished: (a) P3HR-1 soluble antigen in the N-44-S fraction is different in stability from P3HR-1 particulate antigen in the P-44-7 fraction. (b) P3HR-1 N-44-S soluble antigen is different in stability from Raji N-44-S soluble antigen. The relationships between these antigens and VCA, EA and ACIF antigens remain to be investigated. (c) HK-Ly-28 N-44-S soluble antigen is different in stability from P3HR-1 soluble antigen. Little if any CF antigen is present in Raji P-44-7 particulate antigen, verifying reports in the literature. (4) The soluble CF antigen of the Raji cell N-44-S fraction is not present, by stability tests, in the P3HR-1 N-44-S fraction. Any proposed relationship between the antigens, with respect to the steps in the synthesis of EBV or cell transformation would have to be sequential. (5) The P3HR-1 N-44-S soluble CF antigen seems to be composed of subunits which appear to be labile and/or dissociable under specific physical and chemical conditions.

Significance to Biomedical Research and the Program of the Institute: Epstein-Barr virus has been associated with Burkitt's lymphoma, nasopharyngeal cancer and infectious mononucleosis. More tenuous relationships have been proposed between EBV and other diseases. Results of clinical tests, such as fluorescent-labeled antibody tests on cultured BL and NPC cells using human serum correlate with tumor development. The test sera used are from human patients and normal individuals. Questions arise as to the identity of the antibodies and antigens detected from one individual to another as used by different investigators. It is necessary to identify, distinguish between, and correlate antibodies and antigens detected by different sera using

different cells and detection techniques, in order (a) to be sure that different investigators are describing the same or different antigens, (b) to determine which antigens correlate best with the progress of disease at its different stages, (c) to understand the part played by these antigens in the progress of viral infection, cell transformation and production, and (d) to distinguish viral- from cell-associated antigens. It is expected that the purified antigens can be used for vaccines, and also used to prepare animal antisera specific for those antigens, for standardized detection, assay and other purposes. The association of EBV with tumor development suggests the possibility of the development of further clinical tests for early diagnosis, susceptibility and regression. This also might serve as a model for other DNA virus-associated diseases which may be discovered.

Proposed Course: This contract was terminated in February, 1974.

Date Contract Initiated: June 15, 1970

TULANE UNIVERSITY, DELTA REGIONAL PRIMATE RESEARCH CENTER (N01-CP3-3396)

Title: Role of Humoral and Cellular Immunity in Determining the Outcome of *Herpesvirus saimiri* Infections in Squirrel Monkeys

Contractor's Project Director: Dr. William P. Allen

Project Officer (NCI): Dr. Dharam V. Ablashi

Objectives: The overall purpose is to investigate the role that immunological factors may play in infections of *Herpesvirus saimiri* (HVS) in the squirrel monkey (*Saimiri sciureus*), its natural host. Elucidation of these factors may provide a basis for understanding resistance to the oncogenic potential of this and other similar viruses in their natural hosts. Specifically the aims are: (1) to determine what immunological factors are elicited by squirrel monkeys infected with HVS; (2) to characterize the humoral and cellular aspects of the immune response, and determine which play a role in the outcome of HVS in this natural host; (3) to establish breeding colonies of squirrel monkeys which will provide virus and antibody negative animals for these studies; and (4) to make controlled observations on the temporal relationships of natural transmission of HVS and the antibody responses to natural infection in developing infant squirrel monkeys.

Major Findings: Breeding of squirrel monkeys was initiated at this primate center in late 1972. Five breeding groups with a male to female ratio of 1:6 yielded a total of 29 viable infants the first year, which was a breeding efficiency of 35 percent. The breeding program has been expanded so that it now includes approximately 70 first-year females, 90 second-year females, and 26 males. Three subspecies are included in this program (Peruvian, Ecuadorian, and Bolivian). Hand-rearing from within 3 hours of natural delivery was successful with 13 of 15 babies. Eleven of these infants are now

approximately 6 months old and have been held isolated from virus-infected adults. The seronegative status of these infants has been maintained since birth. Four infants born in July and August, 1973, have been maintained with their mothers: 2 within a breeding colony and 2 in separate cages. These animals are being incorporated into studies of natural transmission of HVS. Another four infants that were handreared in isolation will be reserved to initiate a breeding colony of seronegative squirrel monkeys.

All adult feral monkeys in the breeding program have antibodies to HVS. Attempts to isolate virus from lymphocytes from these adults by co-cultivation with VERO cell monolayers have been successful for approximately 30 percent of the animals tested. HVS was isolated from throat swabs from 2 of 30 animals tested. Pooled squirrel monkey serum has been fractionated to obtain preparations rich in IgM, IgG, or IgA. Monospecific antisera capable of differentiating these immunoglobulins have been prepared by immunization of rabbits or goats. These antisera are not commercially available and are required in order to determine what classes of antibody are made in response to HVS infection.

In order to determine if altering the immune capability of squirrel monkeys will affect the outcome of HVS infection, immunosuppression experiments have been initiated in seropositive animals using two drug regimens useful in human organ transplantation: (1) cyclophosphamide and (2) azathioprine and prednisolone in combination. Experiments were designed to determine the maximum tolerated dosages of these drugs, and animals were monitored for changes in hematocrit, and total white blood cell and differential counts. During the course of treatment the animals were immunized with various antigens, and evidence of humoral immunosuppression in the two groups has been obtained, particularly as regards the development of mercaptoethanol insensitive precipitating antibody. As a terminal phase of this experiment the untreated controls and cyclophosphamide-treated groups have been re-infected with HVS and will be examined serologically and histopathologically for differences in response to the infection. Two monkeys were immunized with Freund's complete adjuvant to utilize the classical cellular response to tuberculin as a model to develop procedures to be used in testing cellular immunity to viral and tumor antigens. The response to phytohemagglutinin is also being used in this work. Attempts have been made to detect the release of macrophage-migration-inhibitory-factor (MIF) from peripheral blood lymphocytes cultured with antigen. To date MIF has not been demonstrated using rabbit or guinea pig macrophages as indicator cells; therefore, peritoneal exudates are being induced in squirrel monkeys in efforts to demonstrate MIF with a completely homologous system.

Significance to Biomedical Research and the Program of the Institute: The suggestion that viruses may induce neoplastic diseases in man as well as in animals has loomed as one of the foremost biomedical research challenges in recent years. A major component of the evidence supporting this suggestion is represented by the association of Epstein-Barr virus (EBV) to Burkitt's lymphoma and other human lymphoproliferative diseases. There are several well-documented similarities between the natural history of EBV in man and HVS

in squirrel monkeys. A most pressing question on the relationship of EBV and human disease is the role played by the immune response. The squirrel monkey infected with HVS promises to provide an excellent model system to investigate many parameters of the immune response at both the humoral and cellular levels. The inability of EBV to elicit oncogenesis in most human beings, and a similar lack of tumorigenesis in squirrel monkeys infected with HVS, strongly suggests the need for a detailed investigation of immune mechanisms. Many of these studies can only be done in a laboratory host system. The results could lead to greater insight on the interaction of viruses and human neoplastic disease.

Proposed Course: Within the next six months it is anticipated that an effective regimen of chemical immunosuppression in the squirrel monkey will have been established. It is anticipated also that approximately 53 viable infants will be born to the breeding colonies. Most of the infants will be hand-reared in isolation from the colony. Some will be used to determine their response to infection with HVS, both with and without immunosuppressive treatments. By this time it is anticipated that satisfactory techniques will be available to measure parameters of cellular immunity, as well as humoral antibodies in these animals. Determination of the classes of immunoglobulins involved in the humoral antibody response to HVS will be completed during the next contract year. Observations will be continued on the natural transmission of HVS in the breeding colony. Horizontal transmission will be studied in infants raised with their mothers in the colony; with their mothers in isolation; and hand-reared in isolation. The possibility of vertical transmission will be investigated also by attempting to isolate HVS from Caesarian-delivered infants.

Date Contract Initiated: June 29, 1973

UNIVERSITY OF WASHINGTON (NO1-CP3-3236

Title: Immunological and Transplantation Studies on Dogs with Cancer for the Detection of an Oncogenic Virus Carrier State

Contractor's Project Director: Dr. Rainer Storb

Project Officer (NCI): Dr. Gary Pearson

Objectives: This contract involves studies in dogs with lymphosarcoma and solid tumors, with the following objectives: (1) to set up and assess the usefulness of immunologic assays for investigation of tumor-specific antigens of canine tumors; (2) to study the relationship between genetics and susceptibility to cancer in dogs as a model for human studies; (3) to carry out allogeneic marrow grafts for purposes of developing a model to study the suspected viral induction of malignancy in human donor transplant cells; (4) to study the immune status of dogs with tumors before, during, and after treatment; (5) to attempt to identify oncogenic viruses in canine lymphosarcomas and breast cancer; and (6) to supply dog tumors to other investigators

approximately 6 months old and have been held isolated from virus-infected adults. The seronegative status of these infants has been maintained since birth. Four infants born in July and August, 1973, have been maintained with their mothers: 2 within a breeding colony and 2 in separate cages. These animals are being incorporated into studies of natural transmission of HVS. Another four infants that were handreared in isolation will be reserved to initiate a breeding colony of seronegative squirrel monkeys.

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Proposed Course: Within the next six months it is anticipated that an effective regimen of chemical immunosuppression in the squirrel monkey will have been established. It is anticipated also that approximately 53 viable infants will be born to the breeding colonies. Most of the infants will be hand-reared in isolation from the colony. Some will be used to determine their response to infection with HVS, both with and without immunosuppressive treatments. By this time it is anticipated that satisfactory techniques will be available to measure parameters of cellular immunity, as well as humoral antibodies in these animals. Determination of the classes of immunoglobulins involved in the humoral antibody response to HVS will be completed during the next contract year. Observations will be continued on the natural transmission of HVS in the breeding colony. Horizontal transmission will be studied in infants raised with their mothers in the colony; with their mothers in isolation; and hand-reared in isolation. The possibility of vertical transmission will be investigated also by attempting to isolate HVS from Caesarian-delivered infants.

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in the VCP for virological investigation.

Major Findings: Immunologic Function in Dogs with Spontaneously-Occurring Malignancies. The contractor had previously demonstrated that dogs with lymphosarcoma (LSD) have uniform histological and clinical features and are a suitable model of spontaneous malignancy in a randomly bred species. The immune status of 62 untreated LSD and the clinical features and immune status of 35 dogs with spontaneously occurring solid tumors (STD) have been investigated. Studies of humoral immunity revealed low IgG levels in LSD but not in STD. Hemagglutinin titers following sheep RBC immunization were suppressed in LSD and normal in STD. Similar findings were obtained following primary and secondary immunization with bacteriophage. Cellular immunity, assessed by first and second set allogeneic skin graft survival, was impaired in LSD but not in STD. Response to PPD challenge following sensitization with BCG and in vitro lymphocyte blastogenesis (LB) following stimulation by phytohemagglutinin or allogeneic lymphocytes were deficient in both LSD and STD. These results demonstrated that LSD have a marked deficiency of immunologic function, while STD are relatively intact immunologically.

Lymphocyte Reactivity to Autochthonous Tumor Cells in Dogs with Spontaneous Malignancies. Lymphocyte reactivity to autochthonous tumor cells was determined using fresh, incubated, cryopreserved, or trypsinized tumor cells from 29 dogs with malignant lymphoma and 24 dogs with solid tumors. Lymphocytes were stimulated in vitro by autochthonous irradiated tumor cells, and after 6 days in culture, were incubated with ^{3}H thymidine. In 18 of 29 lymphoma dogs and 15 of 24 solid tumor dogs, significant reactivity of lymphocytes to autochthonous tumor cells was seen. No consistent effect of autologous serum on lymphocyte reactivity was found. It was concluded that tumor cells from most dogs with spontaneous malignancies have tumor-associated antigens capable of stimulating autochthonous lymphocytes in culture.

Macrophage Migration Inhibition Assay (MIF). The leukocyte migration inhibition assay has been utilized to demonstrate tumor-specific immunity in dogs with spontaneously arising neoplasms. Several dogs sensitized to BCG and many others sensitized to allogeneic histocompatibility antigen were tested. Consistent, reproducible and specific migration inhibition was found. Inhibition was demonstrated with the direct test, i.e. inhibition of migration of peripheral lymphocytes from experimental animals in the presence of antigen in the form of disrupted lymphocytes from sensitized animals (autologous and non-related controls were included). Inhibition was also found with the indirect test where concentrated supernates from mixed cultures of sensitized cells and antigenic cells were found inhibitory to the migration of lymphocytes from unrelated dogs. To date lymphocytes from 22 dogs bearing spontaneous neoplasms have been tested for ability to produce migration inhibition factor (MIF) when exposed to thoroughly washed autologous tumor cells. Of 19 dogs with lymphomas, 6 produced MIF. In addition, 2 of 3 solid tumor-bearing dogs also had positive tests. All clearly positive findings were made utilizing the indirect test, though both tests were employed routinely. It is not clear why immunity was not

demonstrated in more of these dogs. It seems likely, however, that many of these dogs lack tumor specific immunity.

Cytotoxic Cellular Immunity Test (^{51}Cr Release). Attempts are being made to establish the ^{51}Cr release assay in allogeneic histocompatibility combinations in which pre-existing states of cell-mediated immunity are clearly defined by other *in vitro* and *in vivo* tests. Evidence for high level killing was obtained, indicating that this test may be applicable to tumor specific immunity in dogs bearing spontaneous neoplasms.

Lymphocyte Reactivity to Soluble Tumor Extracts. Soluble tumor extracts prepared by the KCl solubilization method of Meltzer *et al*. have also been used as stimulants for inducing blastogenesis of autologous lymphocytes in the tumor dogs. Seven of eight dogs tested showed positive blastogenic response to autochthonous tumor extract with stimulation index $\geq$ 2.0.

Delayed Hypersensitivity Cutaneous Reactions to Autologous Tumor Extracts. Tumor extracts were prepared by the same method used for the blastogenesis test prepared from autologous buffy coat cells used as controls. Positive reactions were confirmed by histology. Three of 12 tumor dogs tested exhibited positive delayed hypersensitivity cutaneous reactions to autologous tumor extract, while none responded to autologous buffy coat cell extract. These data may be significant in view of the fact that six of these twelve dogs were anergic to PPD challenge.

Microcytotoxicity Assay. Dogs were sensitized with tissues from unrelated DL-A incompatible donors and also from DL-A compatible donors. All 3 dogs that were immunized with whole blood from histoincompatible donors showed strong and reproducible lymphocyte cytotoxicity to the donor fibroblasts. The cytotoxicity became weaker but was still significant after 3 months. With the exception of one, all 4 dogs grafted with skin from histoincompatible donors showed weak but significant cytotoxicity to target fibroblasts after the first graft and stronger cytotoxicity after the second graft. Of 8 dogs that were grafted with skin from histocompatible littermates, six showed significant cytotoxicity to donor skin fibroblasts. Cultured tumor cell lines will be used as target cells for the detection of tumor specific immunity in dogs and man by this test.

Irradiation-Induced Plasma Particles. Previously, a plasma particle was discovered having a density of 1.16 gm/ml containing a DNA polymerase which utilized exogenous DNA primers. This particle appeared in the plasma of dogs with malignant lymphosarcoma following total body irradiation, and also in the plasma of normal dogs infused with lymphosarcoma cells prior to total body irradiation. The particle was not present in normal dogs subjected to total body irradiation, nor was it present in normal dogs into which lymphosarcoma cells had been infused without irradiation therapy, nor in normal dogs which received lymphosarcoma cells following total body irradiation. It therefore appeared to be tumor-specific and induced by irradiation. Subsequent studies, however, have failed to provide further information on the identity of these particulates. Electron microscopy of both plasma pellets containing particles and of lymphosarcoma tissue biopsied following irradiation has been negative with regard to presence of C type

particles. Furthermore, very large quantities of plasma particles collected by multiple unit plasmaphoresis of the irradiated lymphosarcoma-bearing dogs appear to have either very low or absent endogenous DNA polymerase activity. Differential template specificities were not determinable due to low enzyme activity. It has therefore not been possible to demonstrate the presence of a true reverse transcriptase in these canine particles. In collaboration with Dr. Werner Schaefer at the Max Planck Institute for Virology in Tuebingen, Germany, studies were conducted on the plasma particle material for the presence of interspecific (gs-3) mammalian C type virus antigen. These studies were also negative. The negative results may have partially been due to inability to obtain the concentration of virus particles which Dr. Schaefer needed for these tests. Further consideration is presently being given to more sensitive assays, such as different electron microscopic techniques and the radioimmunoassay for gs antigen. At the present time, there is no evidence that the irradiation-induced plasma particle is a C type virus. Attempts are now being made to detect viral RNA in the plasma particles by hybridization with labeled poly u, which is reported to be a sensitive test for RNA tumor viruses.

Attempts to Rescue Virus from Cultured Canine Lymphosarcoma Cells. Attempts to observe cultured canine lymphosarcoma cells for induction of virus particles after exposure to bromodeoxyuridine have not yet been successful. Established canine lymphosarcoma cells have been cultivated with RD cells following exposure of the lymphosarcoma cells to inducing agents like bromodeoxyuridine. No type C virus was seen. Attempts were made to modify the ability of canine lymphosarcoma cells in tissue culture to express any associated viruses by passage of these cells through immunosuppressed mice. Ascites tumors were produced in some instances by injecting adult cyclophosphamide-treated BALB/c mice intraperitoneally with up to 10^7 cultured canine lymphosarcoma cells.

Attempts to Rescue an Endogenous Canine C Type Virus. Because of the apparent efficiency with which RD cells rescue endogenous viruses from a number of species, attempts were made to rescue a putative endogenous canine type C virus by passage of RD cells in fetal pups which were injected in utero between the 48 and 62 day of pregnancy. The first attempt failed. Dog placentas are being scanned by electron microscopy for the presence of an endogenous virus.

Production of Antisera Against Lymphoma Cell Lines. Rabbits and goats are being immunized with cells derived from individual lymphoma cell lines in an attempt to produce xenogeneic antisera against canine lymphoma-associated antigens. After absorption on canine non-malignant cells, the antisera will be tested with lymphoma cells initially using the complement fixation assay which is in use in the laboratory for histocompatibility typing purposes. Later, immunofluorescent studies are contemplated.

Treatment of Canine Malignancies by 1200 R Whole Body Irradiation (TBI) and Autologous Marrow Grafts. The randomly bred dog with spontaneously-occurring malignancy is a model suitable for therapeutic studies potentially applicable

to man. Twenty-five dogs with malignant lymphoma (L) and 18 dogs with solid tumors (ST) were treated with supralethal exposure to TBI followed by infusion of previously aspirated autologous marrow. In all 25 L dogs, marked decrease in peripheral lymphadenopathy was seen within 48 hours of TBI. Seventeen L dogs died within 14 days of grafting, generally of infection related to radiation-induced granulocytopenia. Seven of 8 L dogs had partial or complete remissions and survived 28, 48, 65, 67, 77, 144, and 215 days. One dog is still alive. Survival and clinical response were not dependent on clinical status before TBI, marrow cell number infused, or marrow involvement with lymphoma. Three of the 8 dogs with remissions were leukemic at the time of marrow aspiration. Of the 18 ST dogs (ca palate, 3 melanoma, 3 mastocytoma, 3 osteogenic sarcoma, 3 ca breast, 3 miscellaneous tumors), 8 survived more than 14 days after TBI and autologous marrow grafting. Two of the 8 showed no tumor response, 3 showed minimal response and 2 (1 mastocytoma, 1 ca breast) showed significant partial tumor responses. One dog was given TBI and autologous marrow after excisional surgery of recurrent ca of the breast and is now alive without evidence of disease > 14 months. Clearly additional therapy is needed to sustain remissions achieved with TBI in dogs with L and to achieve useful clinical responses in dogs with ST. Nevertheless, the remissions achieved in L dogs, even in the face of malignant cells in peripheral blood, are encouraging. These studies represent baseline data necessary to assess the therapeutic effectiveness of contemplated immunotherapeutic approaches involving TBI and marrow grafting.

Allogeneic Marrow Grafts. Allogeneic grafts have been performed following 1200 R total body irradiation (TBI) in 14 lymphoma (L) dogs and 7 solid tumor (ST) dogs. Of the L dogs, 8 died within the first 10 days while 6 have survived > 11, 17, 28, 50 and 65 days. The dog surviving 50 days died of unknown cause without evidence of lymphoma, while the other deaths are attributable to graft-versus-host disease (GVHD). So far there has been no recurrent tumor in a long term survivor of an allogeneic graft. Among the ST dogs, 5 died in the immediate post-grafting period, and 2 died on days 27 and 78 with progressive tumor. Any survivors which will have had allogeneic grafts from donors of opposite sex are potential candidates for demonstrating "induction" of tumor in donor cells.

Significance to Biomedical Research and the Program of the Institute: The dog with malignant disease is of particular interest as an experimental model for the continued study of spontaneous tumors in a randomly bred species. It is reasonable to suppose that principles derived from studies in such dogs may be more readily translated to human neoplastic disease than would be possible in studies of inbred animals or with induced or transplanted tumors. Evidence is being sought for the presence of an oncogenic virus in dogs with lymphosarcoma and breast carcinoma. Such evidence would be of special importance in understanding the relationship of tumors and virus--one of the major aspects of the Virus Cancer Program. Similarly, the nature and importance of tumor-specific antigens in malignant disease is a major focus of current investigations supported by the National Cancer Institute. It is planned to continue to explore a number of different asays for tumor-specific antigens, in an attempt to establish the incidence and importance of such

antigens in naturally occurring dog tumors. Finally, marrow transplantation is a current therapeutic approach to the treatment of human leukemia. One problem enoucntered in these clinical studies has been recurrence of leukemia in donor-type cells, which has been attributed to viral transformation. Similar studies are being conducted in tumor dogs in an attempt to observe this phenomenon in an animal model as an approach to the identification of an infectious agent in the etiology of canine malignancies.

Proposed Course: (1) Efforts will continue toward applying immunological assays for the detection of tumor-specific antigens on canine tumors. Particular emphasis will be given to the specificity of the immune reactions being detected with different types of cancer; (2) allogeneic marrow grafting will be continued to study the question of recurrence of malignancy in donor cells; (3) efforts will continue to identify and characterize the polymerase-containing particulate released into the plasma of lymphosarcomatous dogs following whole body x-irradiation using more sensitive biochemical methods; (4) attempts to induce the production of a canine endogenous virus will continue. The major approaches will be co-cultivation of canine tumors with cells from other species and activation procedures; (5) DL-A typing will continue on tumor-bearing dogs to determine the possible relationship of genetics to the susceptibility to different types of cancers; (6) studies will continue on the immune status of dogs with tumors before, during, and after treatment; and (7) canine tumors, particularly breast cancers, will be supplied to other investigators in the VCP for virological investigation.

Date Contract Initiated: November 1, 1971

5. PROGRAM MANAGEMENT SEGMENT

Dr. J. B. Moloney, ADVO, DCCP, NCI, Chairman
Dr. Louis R. Sibal, OADVO, DCCP, NCI, Executive Secretary

UNIVERSITY OF NORTH DAKOTA (NO1-CP6-0008), GRAND FORKS, NORTH DAKOTA

Title: Quantitative Studies on the Transmission of Selected Herpesviruses and Type C RNA Viruses by Arthropods.

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

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

Objectives: The major objective is to determine whether the following diseases, induced by DNA or RNA viruses, can be transmitted from infected animals to healthy susceptible animals by arthropods, with special reference to virus quantitation: Marek's disease, cytomegalovirus inclusion disease (Henson), and Friend leukemia. Mechanical and biological transmission studies will be undertaken. Specific objectives are (a) to determine oncogenic virus levels in arthropods which have fed on experimentally infected donor animals of known viral blood titer (extrinsic incubation studies), and (b) to determine infection and transmission rates in those arthropods which demonstrate potential biological vectorial ability.

Major Findings: Experiments were conducted to determine how long cytomegalovirus (Henson) could be maintained in an active state in certain species of insects. Viability of CMV in these insects following a single blood meal from CMV-infected mice was as follows: *Aedes aegypti*, 4 days; *Anopheles quadrimaculatus*, 4 days; *Culex pipiens pipiens*, 1 day; and *Culex pipiens quinquefasciatus*, 1 day. *Stomoxys calcitrans* flies fed on infected mice maintained virus for 23-26 days, and those flies fed on infected salivary gland-blood mixture maintained virus for 10 days. The virus was detected again in the latter group of flies at between 22 and 28 days.

Studies were initiated with stable flies (*Stomoxys calcitrans*) and Friend leukemia virus to determine viability in flies fed a single blood meal containing FLV. Maintenance of these flies was then carried out by feeding them on normal mice rather than on human blood as was done previously. Viability of FLV was determined at intervals of 1, 3, 7, 10, 14 and 18 days postfeeding. The flies were randomly selected and individually ground in a TenBroeck homogenizer in 0.5 ml P.S.S. solution, and injected into a single recipient mouse per experiment. FLV in the flies was demonstrated at 1, 3, and 7 days following a single infected blood meal, using *in vivo* disease parameters only. Biological transmission attempts were made at 1, 2, 3, 4, 8, 9, and 11 days intervals following the initial FLV-infected blood meal. One- to 14-day old suckling mice were exposed to the infected flies, and then returned to the mothers. They will be observed for 3 months, and then examined for FLV infection. Collaborative experiments with Life Sciences Inc., St. Petersburg, Florida, are in progress on insect transmission of Marek's disease.

Significance to Biomedical Research and the Program of the Institute: Viruses may be transmitted biologically or mechanically by arthropod vectors. Mosquitoes, biting flies, fleas, ticks, and mites may suck blood from both man and domestic animals (including birds) closely associated with man. It is, therefore, important to investigate whether oncogenic viruses present in infected animals are potentially transferable to man. If such a vector is found, measures can be taken to control or prevent contact between the vector and human hosts. These basic studies will permit one to determine which arthropods should be further investigated in this regard.

Proposed Course: Insect transmission experiments will be continued with Marek's disease virus, murine cytomegalovirus (Henson strain), and Friend leukemia virus, using mosquitoes, flies, fleas, and other arthropods. Additional species will be used as test insects in transmission experiments when available in sufficient numbers.

Date Contract Initiated: October 27, 1965

LITTON BIONETICS, INC. (NO1-CN-23294), BETHESDA, MARYLAND

Title: Operation and Maintenance of the Frederick Cancer Research Center at Frederick, Maryland

Contractor's Project Director: Dr. Robert E. Stevenson

Project Officer (NCI): Dr. William W. Payne

Objectives:

A. To develop and establish large-scale virus production and purification capabilities to meet NCI program requirements.

B. To conduct developmental research relative to virus production of those oncogenic or suspected oncogenic viruses for which no established protocols exist or for which existing protocols have failed to consistently provide a suitable product.

C. To prepare special viral diagnostic and test reagents including developmental research to either improve established techniques or improvise new ones as necessary.

D. To perform basic research in support of existing NCI programs pertaining to the role of virus in the etiology of human neoplasms; pursue research objectives, determined by NCI staff, reflecting those areas of scientific investigation that require special emphasis and/or expanded activities and that can maximally utilize FCRC facilities.

E. To evaluate potential hazards associated with research activities in viral oncology and chemical carcinogenesis and to develop adequate protective measures in support of the NCI Office of Biohazards and Environmental Health.

F. To develop and implement a safety and environmental control program for the Frederick Cancer Research Center (FCRC) and to support applied and basic studies that will be performed by the Office of Biohazards and Environmental Control.

G. To establish, maintain and operate an Advanced Systems Laboratory for research in viral oncology, which shall contain the most modern equipment and safety features available for special programs conducted by NCI and visiting scientists.

H. To operate an animal farm for the breeding of rodents to meet the needs of research programs at FCRC and for shipment to other NCI operations as production permits.

Major Findings:

Those Projects that are completely funded by Viral Oncology are the following:

Project 1, Virus Production. During the period 5 February 1973 to 27 January 1974, 8,840 liters of viruses were produced and 8,622 liters were concentrated in 122 separate product lots, yielding a total of 11,031 milliliters of concentrated and purified virus. Of this amount, 7,608 milliliters were released to investigators within The Virus Cancer Program, thereby fulfilling 100 percent of that amount requested by the Office of Program Resources and Logistics for investigators, and 842 milliliters were released within the FCRC for product characterization. Approximately 96 percent of the material was Rauscher murine leukemia virus (RLV). A second agent, woolly monkey virus (SSV-1), was phased into production, with current production levels at 50 liters per week. Five small lots of other viruses were produced for Program investigators. In addition, a system for suspension culture production of RLV was developed, and a new high-producer RLV seed lot developed.

Project 2, Developmental Research. High titered EBV was produced by optimizing growth parameters of the P_3HR-1 cell line. The values studied included cell growth curves, virus harvest time, purification methodology and storage conditions. Liter volumes were produced under these conditions maintaining high virus titer.

Mouse mammary tumor virus was studied *in vitro*; significant progress was achieved in increased levels of MMTV antigens, DNA polymerase, and type B particles in the absence of type C particles by hormonal treatment of the cultures. Biochemical and biological characterization of the *in vitro* product was initiated.

The Gibbon ape virus project was terminated during the year to concentrate on the above projects.

The quality control activity formerly associated with this project was transferred to a new product evaluation group; this project continues to provide EM services, however, required for this work.

Project 3, Viral Diagnostic and Test Reagents. The RLV reverse transcriptase enzyme has been purified 30-fold with 50 percent recovery by column chromatography. The enzyme is free of cellular polymerases, RNase, DNase, terminal transferase, and RNA polymerase activity. Its molecular weight and activity with various synthetic primer-templates indicate that it is the viral enzyme. It contains RNase H but this activity can be removed by further chromatography on affinity columns. The purified enzyme has been injected into rabbits and guinea pigs, and the resultant IgG has been isolated and shown to possess potent inhibitory activity.

The RLV p30 was initially purified by isofocusing but can now be purified simultaneously with the RDDP. This latter material shows a single protein band on SDS gels with a molecular weight of 30,000. Antiserum has been prepared against the isofocused material and is currently being prepared against the column-purified material.

All of the product characterization of the virus produced within Project 1 was being performed within this project but was transferred to a separate section in November, 1973.

The FCRC Viral Oncology Quality Control testing program was reorganized on November 27 with the creation of a Product Evaluation Section to provide product characterization tests, and a Product Evaluation Committee to guide the testing program. The reorganization was coupled with the physical relocation of the Product Evaluation Section so that all technical personnel are grouped in adjacent laboratories. The net effect of these actions is expected to provide an expanded, responsive testing program which provides for better biological standardization.

Project 5, NCI Office of Biohazards and Environmental Control. A number of applied research programs were undertaken during the year. A laminar flow system for handling experimental animals was designed, constructed and installed in an animal room in the safety building. The system has been extensively tested with a variety of animals and infectious diseases. The system will prevent animal cross-infection and will protect animal handlers. Disinfection studies of oncogenic viruses by various chemicals is being carried out in order to list suitable disinfectants that may be used to inactivate oncogenic viruses.

Project 13a, Herpes Simplex Virus. The Herpesvirus (HSV) project supported the work of Dr. Albert B. Sabin in testing 600 human sera from patients with numerous cancer types. It was found that at least nine varieties of advanced cancer sera specifically react in complement fixation (CF) tests against HSV-induced nonvirion antigen(s). Sera from persons free of cancer, or with recurrent HSV infections, do not react in the CF test.

Reagents produced to support the CF work were HEp2 cultures infected for production of stock virus and virion antigen, primary guinea pig kidney cultures grown in guinea pig serum for nonvirion antigen and stock virus for immunization of guinea pigs, and guinea pig kidney cultures in fetal calf serum for nonvirion antigen for CF testing.

Project 13b, Virus Disease Modification. Seven compounds were tested for antiviral activity against Rauscher Leukemia Virus. Compounds VDS 29, VDS 31 (Levamisole), and VDS 24 reduced splenomegaly and/or viremia, but only at an early stage of the disease.

In the LSTRA Leukemia tumor system a greater number of compounds have been evaluated as supplements to BCNU chemotherapy. Those compounds which significantly increased both the median survival time and incidence of tumor regression included interferon, VDS 31, analogs of VDS 31 (VDS 31A, 31C, 31D) VDS 52 (tilorone hydrochloride), and analogs of VDS 52 (VDS 60, 63, and 65).

Two in vitro assays have been adopted for use in the study of host immune response to chemical and biological stimuli. These are the Jerne Plaque technique (a method for detecting humoral immune response) and the Lymphocyte Stimulation Assay (a method for quantitating cell-mediated immunity). The latter assay can be utilized for immunostimulation either in mice or in splenic lymphocytes cultured in vitro. Stimulation of splenic lymphocytes in vitro and the humoral response in vivo was noted with VDS 31 using these procedures. Analogs of both VDS 31 (VDS 31A and 31C) and VDS 52 (VDS 56, 62 and 63) were also positive in the Lymphocyte Stimulation Assay. An additional substance, a Staphlococcus aureus extract, was also positive in the Jerne Plaque Assay.

Both the in vivo and in vitro chemotherapy sections are continuing to work on the improvement of existing laboratory procedures as well as the development of additional techniques.

AMV virus was partially purified and subsequently utilized for purification of the RNA-dependent DNA polymerase (reverse transcriptase). By the modification of previously reported procedures, a method was obtained for purification of RLV reverse transcriptase. Both of the purified transcriptases were utilized in drug inhibition studies and found to differ in sensitivity to streptovaricin U, the AMV reverse transcriptase being more sensitive. Preliminary studies indicate that the site of inhibition by streptovaricin U is located on the polymerase molecule and not on the template. Tilorone hydrochloride and seven of its analogs (VDS 57, 58, 59, 60, 62, 63 and 64) were all found to be potent inhibitors of both RLV and AMV transcriptase with the RLV enzyme appearing to be more sensitive. The analogs containing either dimethylaminoethosy-or piperidinobutyryl-substituents were excellent inhibitors.

Project 13c, Purification and Characterization of Virus Associated Tumor Antigens. Soluble antigens have been extracted from syngeneic SV40-transformed BALB 3T3 tumor cells with 3M KCl. These antigens elicit a specific delayed hypersensitivity reaction in syngeneic tumor immune BALB/c mice. Subfractions of the extracted antigens have been tested for tumor protection. Results have shown that several fractions are necessary to provide this protection. A method has been devised to determine picogram quantities of anime sugars. This method will be employed in antigen characterization.

Project 13d, Primate Virus Section. The NCI Primate Virus Section has been established at FCRC. Laboratories have been completely renovated and

facilities for the study of human and non-human primate oncogenic viruses are now in operation. Containment facilities for the use of moderate risk viruses have been established and satisfy EISH-FCRC Biohazard Standards; a radioisotope program has been approved and facilities for the monitoring and disposal of radioactive waste materials have been provided.

Various human and non-human primate cell lines are now being propagated and experiments focusing on the role of Herpesvirus saimiri; EBV and human cell adapted RLV and other primate viruses in oncogenesis are presently being conducted. The use of the facility as a center for the evaluation and testing of reagents and sera and as a training facility for visiting scientists, both from the U.S. and abroad, is being contemplated.

Those Projects that are partially funded by Viral Oncology are the following:

Project 4, Environmental Control. Several training programs were conducted during the year, and support was given the NCI Office of Biohazards and Environmental Control, who sponsored a seminar at FCRC on Centrifuge Biohazards. The various control, inspection training programs and applied research in the areas of biological, chemical and radiological safety were carried out without significant difficulties. Special emphasis and effort have been directed towards safety in the use, handling, storage and disposal of chemical carcinogens. This will remain an area of intense effort in the future.

Project 6, Advanced Systems Laboratory. O'Connor SA-4 Project - Studies were initiated in March, 1973, for the purpose of evaluating this human oncogenic candidate virus and are now in the final stage. The cells, under two different culture systems have been evaluated for optimal induction permitting large-scale production of the viral agent as demonstrated by reproducible polymerase and simultaneous detection assays. In addition, a new enzyme, an RNA-directed DNA polymerase (RDDP), has been isolated and characterized. Polymerase is not neutralized by anti-mouse RDDP serum. The large-scale probe material necessary for species identification is in the initial stage of production. This material in sufficient quantity will be used for hybridization work by Dr. E. Chan at Columbia University, New York.

Studies in Biochemistry - A serum protein, common to mice bearing over 15 tumor lines, has been identified. This protein is being purified and characterized. A monospecific antiserum, prepared with the use of acrylamide gel slices, is being utilized in this analysis.

Developmental Electronmicroscopy - The present objectives and research projects of the Developmental Electronmicroscopy section are: (1) to develop more efficient and useful methods of utilizing the electron beam for quantitative and qualitative measurements of biological specimens with the transmission (TEM) and scanning electron microscopes (SEM); (2) to develop the appropriate techniques of specimen preparation for these measurements; and (3) to perform independent and collaborative research using the TEM and SEM.

Project 12, Animal Breeding. During calendar year 1973, the Animal Farm produced over 252,000 laboratory animals of 26 different species or strains for use in cancer research programs. The production of F 344 rats and $B_6C_3F_1$ hybrid mice has been increased to meet additional requirements. Rapid expansion of the breeding colony for the nude Athymic mouse has been required as the unique usefulness of these animals has become more widely recognized and the realization that they can be maintained in the laboratory when reasonable precautions are taken to preserve their health. Heavy demand for all three strains of guinea pigs has continued. Many intricate manipulations are now in progress within all production colonies, which include timed pregnancy, issue of newborn, aging and the production of large numbers of hybrid combinations. The initial shipment of mice in filtered containers was received at FCRC from the VRB barrier colony the last week in November and these animals are housed in barrier Building 1033. This shipment is the beginning of the effort to reconstitute all rodent colonies at FCRC free of all demonstrable evidence of viral contamination. Alterations to Building 1021 have been completed. A gnotobiotic capability is now possible with the completion of the renovations to Building 1030.

Significance to Biomedical Research and the Program of the Institute:

The Viral Oncology projects that have been initiated at FCRC include an expanded program to make available a wide variety of high quality biological resources that are necessary for NCI programs in virology, immunology, chemotherapy, molecular biology, genetics and electron microscopy. They will also provide important information and safety design criteria in the area of biological hazards and environmental control that will be made available to a large number of laboratories engaged in cancer research. Plans will also include the establishment of high caliber laboratory facilities to be employed for special investigations and advanced workshops involving workers from both this country and abroad. Finally, the basic research effort will augment the Cancer Program by providing information on problems related to the elucidation of human neoplastic disease relative to detection, prevention and control.

Proposed Course:

Although programs will be carried out in a manner similar to those mentioned above, effort will be directed toward an increased emphasis upon project flexibility to utilize FCRC staff and facilities as advantageously as possible to reflect Program needs, and to elevate the level of integration of all Viral Oncology FCRC activities not only with each other in-house but also with other outside laboratories engaged in related work.

Date Contract Initiated: June 26, 1972

SUMMARY REPORT

6. SOLID TUMOR VIRUS SEGMENT CONTRACTS

July 1, 1973 through June 30, 1974

Introduction

Emphasis in VCB-STV programs has continuously been on those problems in cancer which appeared to be most amenable to attack. In trying to summarize the 1974 achievements of these programs, it became obvious that, with some exceptions, the work of most of the STV contract and inhouse research projects were so frequently intermeshed with those of others in efforts to achieve major objectives (cited below) that it was frequently impossible to discuss individual project accomplishments of one contract without at the same time discussing the contributions of two or more other groups. This is reflected in the foregoing VCB summary. It is also apparent in the publications made by VCB-STV scientists during the 1974 fiscal year.

The major targets of the STV programs can be listed as follows:

A. Determination of the specific roles of RNA and DNA viruses in the etiology of cancer, including those transmitted endogenously by genetic inheritance and those transmitted exogenously, either vertically or horizontally.

B. Identification at the cellular level of specific host gene control factors such as regulating mechanisms which either predispose to increased or decreased RNA virus and tumor expressions.

C. Studies of the specific host immune response factors which either predispose and/or increase virus and cancer expressions including immune responses that may eventually be used to prevent cancer or control its growth.

D. Studies of exogenous carcinogenic factors present in the human ecology of agents which precipitate virus expressions as well as cancerous responses. In this area special emphasis is placed on interactions between viral and chemical carcinogens.

Dr. John Riggs (California State Department of Public Health), in collaboration with Dr. Paul Arnstein (NCI Project Officer), has successfully transplanted a number of human malignant human tumors into anti-thymocyte serum treated (ATST) mice. Two of 13 attempts resulted in growth of the tumor material. Among 36 previously transplanted human tumor lines, 10 "picked up" an NIH Swiss mouse type C virus; and of seven transplanted into highly "switched off" C57/L mice, one was positive for murine type virus. Thus, this method adopted to recover the putative human type C virus was successful in recovery of the murine xenotropic virus. Two animal tumor lines are currently being co-cultivated with human tumors in the ATST mice in order to favor emergence of the putative xenotropic human oncorna virus.

Studies on vaccine-induced resistance of ATST mice to transplantation of human tumor cells indicate that resistance is easily established and demonstrable. These studies are providing important methodology in the search for the human tumor virus, and the feasibility of vaccine and other approaches to cancer prevention and control. Since the neoplasms produced in the ATST mice remain morphologically "human," experiments can be designed to test chemical, physical or biologic influences on human solid tumors without the involvement of human tumors.

Dr. Thomas Kawakami and his associates (University of California, Davis) have isolated two type C RNA viruses from subhuman primates (woolly monkey and gibbon ape viruses) which appear to be associated with neoplasias in the respective species, but do not appear to be the xenotropic viruses in either case. These viruses are being studied intensively by a number of parameters (biochemically, immunologically, by electron microscope, etc.) and compared with isolates of other species. Immune response studies have revealed that these animals develop antibody to both the envelope and group specific viral antigens. The methodology used to recover the above isolates is now being applied to the human system. The finding of two type C viruses associated with tumors of different primate species is evidence that the higher animals, including man, are likely to be among the growing number of species harboring type C viruses associated etiologically with cancer.

Drs. Bishop and Varmus (University of California School of Medicine, San Francisco) have assembled a relatively complete array of techniques for the study of RNA tumor viruses by molecular hybridization. In studies on the genetic relatedness of type C viruses, they have the genomes of type C viruses from different host species were generally not related; i.e., an endogenous virus of chickens shared no nucleotide sequences with DNA from avian species other than chicken. This group is also working on methods to develop cultured sources of all major variants of the mouse mammary tumor virus (MMTV) and to describe the chemical and humoral factors which facilitate growth of the virus. In molecular hybridization studies with nucleic acids from normal and malignant human breast tissue, no detectable hybridization between MMTV DNA and RNA from human tissues was obtained. In addition, reactions between MMTV DNA and human DNA were observed only if the conditions for hybridization were non-stringent. In the latter instance, the observed reactions were not species-specific and were therefore of uncertain significance. These studies are providing an important insight into the mechanism by which RNA tumor viruses bring about malignant transformation.

Dr. Frank Lilly (Einstein Medical College), working on the genetic and immunologic factors in viral leukemogenesis, has defined two new genes governing response to the B-tropic Friend virus. He has also demonstrated a very strong correlation between leukemia and virus titer and a much weaker correlation between the disease and both H-2 type and sex, showing that Fv-1, which is the major factor influencing virus titer in the cross used, was a major factor governing leukemia occurrence in these mice. He has also shown that either Fv-1 or a closely linked gene governs the level of expression of the gs-1 antigen in the (BALB/c X AKR) X AKR backcross. A very interesting finding was the demonstration of genetic control of chemical carcinogenesis governing tumor response. These studies are relevant to the human cancer

problem, since one of the basic facts about tumor biology is that genetic mechanisms of the host are known to exert major control over expression of oncogenicity. By defining the loci and markers associated with leukemogenesis, it should be possible to undertake systematic studies of the precise immunochemical mechanism governed by individual loci, with the objective of encouraging or altering their immunogenetic effects to provide maximum resistance against cancer.

Studies or RNA tumor viruses at Flow Laboratories, Inc. This contract serves as the major source in the VCP of reagents used for typing species specific type C viruses of a host of animal categories. Dr. Raymond Gilden and his associates have done much of the characterization of many of the subunits of the mouse, rat, hamster, cat and primate type C viruses using highly sophisticated methodology, much of which was developed at Flow. The expertise of the laboratory is particularly useful in characterizing new isolates and determining their relationship to each other and their probable species of origin. This contract also carries major responsibility for producing and safety testing formalin-killed vaccines from concentrated banded mouse viruses and hamster, rat and primate viruses.

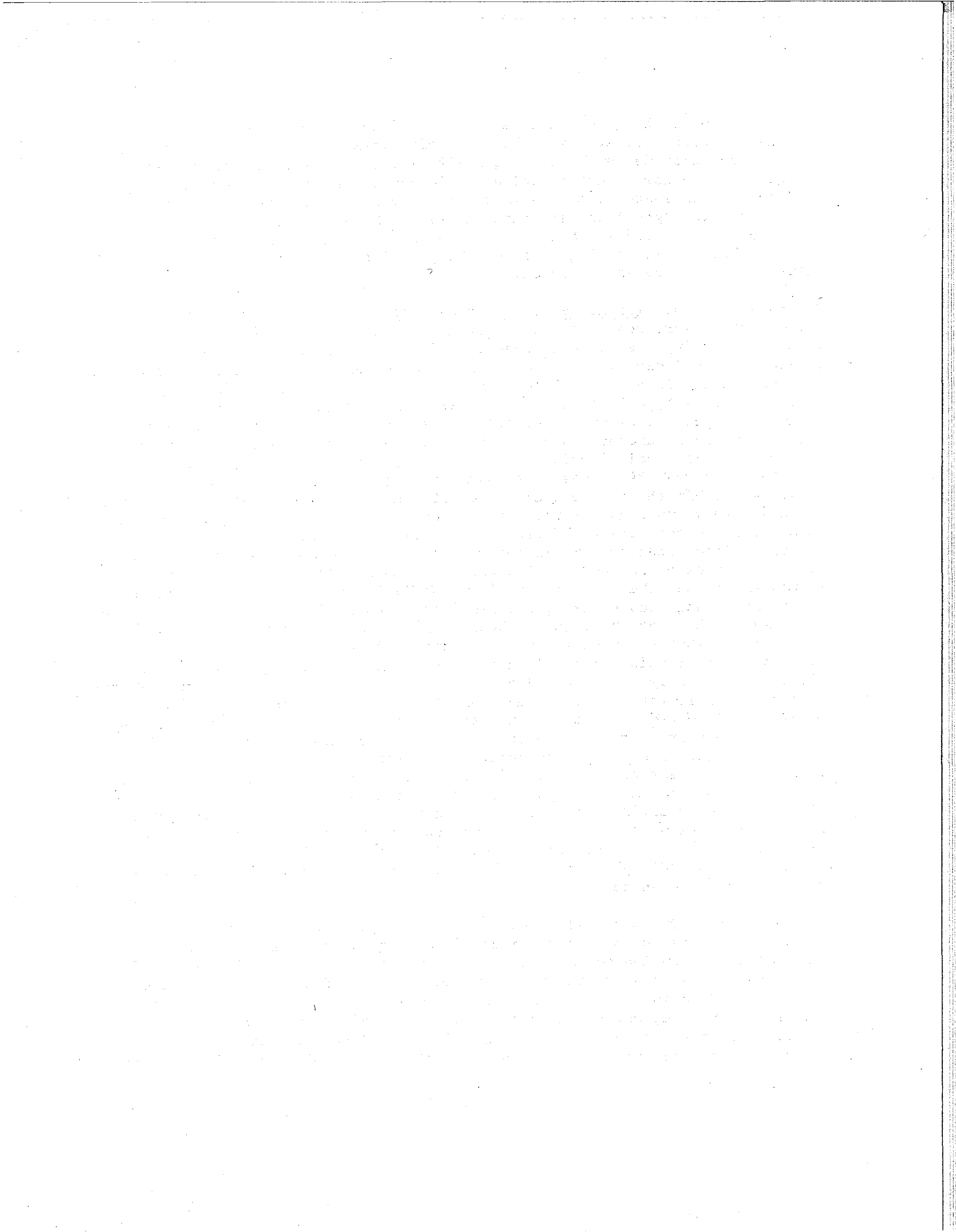
In collaboration with Dr. Berge Hampar (NCI Project Officer), this group has been working on the DNA herpesviruses as well as the type C RNA viruses. Studies with the Epstein-Barr virus have concentrated on the mechanism of latency and virus activation. Findings suggest that some as yet undetermined priming event may be required before cells become susceptible to drug-induced virus activation. Further, the findings suggested that the DNA synthesized during the cells' S-1 period contained unique sequences which controlled activation of the repressed Epstein-Barr viral genome. Additional studies (in collaboration with Dr. M. Nonoyama) indicated that the repressed Epstein-Barr viral genome replicated during the S-1 period, at a time which corresponded temporally to the critical period for activation induced by IdU. This was consistent with the proposition that viral activation occurred at or near the site of the repressed viral genome, and that the repressed genome might be physically associated with early replicating cell DNA. In addition to this contract being the major source of reagents for typing type C tumor viruses, the contractor's expertise for purifying viruses and subviral components and for preparing antisera of known specificity has allowed rapid and reliable identification of viruses isolated from various species. This is particularly important in instances where viruses isolated have been obtained from human tissues. The contractor is also involved in biological and biomedical studies relating to the mechanism of type C virus persistence in cells and the differentiation of events leading to cell transformation, and herpesvirus studies directed towards delineating the mechanism of their latency in human cells and determining the relevance of herpesviruses associated with human tumors. The vaccine studies will have direct relevance to modifying the human cancer experience.

Dr. David Allen (Harvard University) is nearing completion of determining the amino acid sequence of gs-b, an avian leukosis group specific antigen from avian myeloblastosis virus (AMV). These were sequences with substantial new information concerning the C-terminal portion of the molecule. In addition, micropeptide mapping was utilized to isolate peptides which may solve the

small portion of the amino acid sequence remaining to be determined. He has also made progress in studying the antigenic sites of gs-a by fractionating the cyanogen bromide peptides, by gel diffusion, and in determining their antigenicity by complement fixation and immunodiffusion. The AMV is being used as a model system prior to initiating similar studies with the interspecies antigen (gs-3) of mammalian viruses. The availability of a highly specific and purified antibody against the interspecies antigen would considerably aid in finding antigenic fingerprints of a potentially oncogenic virus in human neoplastic tissue.

Dr. Robert C. Good (Hazleton Laboratories, Inc.), in collaboration with Dr. Stuart A. Aaronson, NCI Project Officer, and several of his associates in NCI, including Drs. John Stephenson and Steven Tronick), have had several major findings. They have demonstrated multiple endogenous type C viruses present in mouse cells of different strains; and that biologically distinguishable type C viruses of one cell segregate in Mendelian fashion in appropriate genetic crosses, indicating that the loci detected represent virus structural information rather than regulatory genes affecting virus expression. Biochemical evidence, obtained by hybridization of cellular DNA with type C virus DNA probes, has shown that the virus-specific DNA is localized within the high molecular weight DNA of the mouse cell. One virus, induced from virus-negative mouse cells, caused lymphatic leukemia in a low leukemia incidence strain. These findings establish the oncogenicity of naturally-integrated type C viruses. In efforts to understand the regulatory processes involved in control of potentially malignant endogenous viral genes, it was shown that the Fv-1 genetic locus markedly influenced the expression of certain endogenous viruses, but had little or no effect on others. Further, a new and highly potent class of chemical inducers of type C virus, protein synthesis inhibitors, was discovered. These inducers differed from halogenated pyrimidines in their ability to activate distinguishable endogenous viruses of the same cell, providing further evidence that cellular regulatory factors specific for each virus must exist. In attempts to define the mechanisms involved in type C virus replication, conditional lethal mutants of MuLV were characterized into three physiological classes, defective in replication functions at the nonpermissive temperature. This group has also developed very sensitive and specific immunological assays for type C viral proteins. Immunoassays for type-specific determinants on the 12,000 and 30,000 M.W. polypeptides, in particular, have proven very useful as markers for different virus strains for genetic studies and in the identification of new virus isolates. These tests, along with biochemical methods involving DNA-DNA and RNA-DNA hybridization, have been used to search for type C virus information in human cells.

Dr. Hans Meier, Dr. Ben Taylor and their associates (Jackson Laboratory), have been working on the genetic mechanisms underlying regulating cancer expression, in studies complementary to those of Dr. Lilly at Einstein. They have found a highly significant and predictable association between endogenous viral expression in early life and leukemias and reticulum cell sarcomas with advancing age, establishing that the type C RNA virus was the major determinant of these tumors. They also found evidence of genetic control of the group-specific antigen of murine leukemia virus. Alleles permissive to



gs antigen expression were found to be dominant to their nonpermissive alleles. This group has developed a highly effective model system for evaluating the effects of an anti-viral cancer vaccine, utilizing 1-ethyl-1-nitrosourea (ENU) in defined inbred strains of mice. It was observed that ENU, administered transplacentally, caused teratogenic and carcinogenic effects in offspring of ENU-treated mothers. The outcome of ENU-induced effects was found to be primarily dependent upon maternal hereditary traits rather than the fetal genotype, and a significant association was found between gs antigen and in some cases complete viral expression and chemically-induced tumors, but the rate of tumor induction and activation of infectious or subinfectious viral expression was influenced by the mouse strain. In strains with complete virus expression the induction of tumor was accelerated; whereas, in strains with a largely unexpressed viral oncogenic mechanism, ENU induced tumors through a combined viral-chemical action. A consistently high incidence and development of tumors within less than 10 weeks occurred in certain F_1 mice, representing an ideal model for the simultaneous evaluation of anti-type C viral vaccines against tumors. Another interesting model strain was the HRS/J "hairless" mouse for studying the relationship between immunodeficiency and leukemogenesis. In studies done to date in the HRS/J, there appeared to be a deficient "collaboration" among different lymphoid cell types or a deficiency in the proliferative capacity of immunocompetent cells in the homozygous mutant resulting in an ineffective immunosurveillance against leukemogenesis. This group also developed a promising micromethod for culturing mouse peripheral blood lymphocytes that requires only 50 ul of blood, avoids the need for tedious cell separations, and obviates the use of heterologous sera. This method should be useful in investigations of the function of peripheral lymphocytes and allow for study of the genetic control governing responses to phytohemagglutinin or other antigens. In addition to providing basic information on the genetic mechanisms underlying oncogenesis and its regulation, this contractor has developed a number of highly relevant systems for studying the effects of viral inhibitors and vaccines.

The Microbiological Associates, Inc. (Bethesda) contract, which includes a subcontract with the Children's Hospital of Akron, embraces four major tasks. Major findings were as follows:

Task A: Studies related to a search for human type C virus. Dr. Lee Vernon has seen type C particles in 17/20 normal human placentas examined by electron microscopy to date. Extensive efforts are in process in the laboratories of Dr. Johng Rhim, Drs. Paul Price and Ilan Shif and Dr. Aaron Freeman (Children's Hospital of Akron) to isolate type C particles from human placentas and/or human tissue using co-cultivation, various culture techniques and chemical treatment. Type C particles were also readily found in the placenta of a rhesus monkey, and studies in other subhuman primates are planned, the latter purely for purposes of comparative morphology and implications for human studies. Dr. Vernon has also engaged in electron microscopic studies of the human paralytic disease, amyotrophic lateral sclerosis (ALS). This is of particular interest because of the discovery of a wild mouse type C virus which produces an analogous disease in mice. The latter pantropic virus conceivably could be implicated in human ALS, in view of the human exposure to mouse viruses through grain and

grain products. Replication of Ki-MSV in human cell lines of normal and neoplastic origin are in process to attempt to isolate human nonproducer (NP) cells. Such isolates may be useful in turning on a human type C virus.

Task B: Studies related to the prevention and treatment of tumors. Drs. Price and Shif have shown *in vitro* that the antibiotic streptonigrin at nontoxic levels protected Fischer rat embryo cells from transformation by 3-methylcholanthrene, 7, 12-dimethylbenzanthracene or benzypyrene, and that the same dose which inhibited chemical transformation also inhibited IdU induction of the endogenous rat type C virus. Drs. Whitmire and Kouri completed studies to determine the effect of tissue culture-derived interferon on MCA tumor induction in three strains of mice. It was found that at 4 months interferon significantly reduced tumor incidence in all groups except CF-1 neonates; however, at 10 months the reduction in tumor incidence was less significant. Since earlier studies had demonstrated that interferon reduced MCA tumor induction in CF-1 neonates using mouse sera interferon, one more series of studies will be undertaken to compare the two sources (sera and tissue culture) under like conditions. Studies on the effects of rifampicin against MCA carcinogenesis did not reveal any significant reduction in tumors with rifampicin treatment.

Task C: Carcinogen assays and cell repository. Utilizing the well-tested Fischer rat embryo cell transformation system to test the carcinogenic potential of coded fractions of smog condensates collected at different locations in the Los Angeles Area, Drs. Rhim, Price and Shif found four of the five unknowns were carcinogenic; three known controls done blindly behaved as expected. Additional systems for assaying effects of suspected environmental carcinogens are being developed *in vitro* and *in vivo* to provide greater efficacy and optimal accuracy. This task also embodies a cell culture repository. Ninety-six cultures were characterized and shipped to 16 different groups during the last 6 months. In addition, three type C viruses were isolated from human tumor cells passaged through immunosuppressed rats and mice and characterized as genetically transmitted xenotropic rat and mouse viruses. Drs. Kouri and Whitmire are also studying the effect of expression of the endogenous RNA viral genome on chemical carcinogenesis in a number of inbred mouse strains and the backcross and intercross animals derived from the parental strains, with particular emphasis on the relationship between AHH inducibility and tumor induction. Of 251 inducible mice treated with MCA, 202 developed tumors with an average latency of 131 days; of 85 noninducible animals treated with MCA, nine developed tumors with an average latency of 195 days. These data suggest a close relationship between AHH inducibility and MCA tumorigenesis.

Task D: Diagnostic and testing functions. During the past 6 months, approximately 5,600 serodiagnostic tests were performed by Dr. Jack Parker and his group on 3,900 specimens. Over 90% of the specimens were wild or colony mouse sera tested for antibodies to Sendai, polyoma or lymphocytic choriomeningitis. The remaining specimens tested included antigens and antisera for determination of anticomplementary activity, commercial rat colony sera for Sendai and polyoma antibodies and various mouse sera for the presence of antibodies to 11 murine viruses. Dr. Lee Vernon's group has examined, by electron microscopy, a total of 429 specimens for the presence

of viral particles. Tissues examined were derived from Tasks A through D, Contract NO1-CP4-3254 (Microbiological Associates), and the Poolesville facility of the Viral Carcinogenesis Branch. Dr. Ilan Shif performed over 1,900 reverse transcriptase (RT) assays for a number of groups within the Program. Dr. Shif developed a method for optimizing the conditions for the RT tests, increasing sensitivity and eliminating false positives. Preliminary studies in the development of a sensitive neutralization test using the RT assay has shown this procedure to be possible and potentially useful in evaluating sera in vaccine studies. The test is now being correlated with other test systems such as XC and focus reduction.

Subcontract, Children's Hospital of Akron, Ohio: Drs. Freeman and Igel, have succeeded in establishing several chemically transformed human cell lines, a placental line and several lines derived from human warts in culture. Experiments are now underway utilizing these lines in co-cultivation and induction experiments in attempts to isolate a human type C virus.

This contract program is a core part of a coordinated, comprehensive, targeted research program to define the role of the type C RNA genome as a determinant of oncogenesis, to develop diagnostic systems for screening carcinogens and candidate materials for cancer prevention and control in vitro and in vivo. The in vitro tissue culture systems for assaying environmental carcinogens make possible large scale, rapid screening of environmental pollutants, food additives, drugs, etc., at a fraction of the cost of equivalent tests in vivo. In view of the screening burden posed by literally thousands of new compounds each year, the tissue culture approach represents the only feasible hope of recognizing possibly dangerous carcinogens quickly. The systems developed under this contract are also important for attempts to rescue human cancer viruses, and for testing anti-cancer compounds.

Drs. Damodar Deshmukh and Nirmal Mishra, Microbiological Associates, Inc., and Dr. Padman Sarma, NCI, have initiated a collaborative effort on the isolation and characterization of type C RNA tumor viruses from various avian and mammalian species, including man. Evidence was obtained to suggest that the RD 114 viral genome is widespread in apparently normal cats. A survey of cats and humans for prevalence of feline leukemia-sarcoma virus neutralizing serum antibodies showed that virus neutralizing antibodies were found in the sera of cats without neoplasia, as well as in cats with neoplastic disease, but not in the sera of 36 veterinarians or in 33 laboratory personnel working in two laboratories engaged in feline leukemia research. Additional studies established the importance of viral envelope antigens of feline leukemia and sarcoma viruses in permitting or restricting infection across species barriers. Possible implications of these findings in natural spread of virus to other mammalian species remains to be verified.

Drs. Robert Nims and Robert Peters, Microbiological Associates, Inc. (Walkersville), and Dr. Gary Kelloff, NCI, continued in their collaborative study on the immunoprevention of spontaneously occurring neoplasms. Immunoprevention studies to date have included a determination of the optimal means of virus production and characterization and the optimal fixation and

storage of viruses. These studies led to a standardization of the vaccine preparation so that each vaccine lot can be adequately evaluated. The optimal dose of virus, route and schedule of immunization of mice with banded virus has been evaluated as measured by the footpad assay, the lymphoblast transformation assay, by neutralization tests and ultimately by the hosts ability to resist challenge by live murine leukemia viruses. Also, the importance of the immunogenicity of type C virus in cellular vaccines is being examined.

There is substantial data linking DNA viruses with certain animal and human malignancies. The biochemical methodology for detecting DNA tumor virus sequences with highly radioactive viral DNA is an extremely sensitive probe for viral DNA sequences in human tumors. Because these tests can yield decisive answers, it is important at this time to prove or exclude the presence of adenovirus and herpesvirus DNA sequences in certain human cancers. Dr. Maurice Green and co-workers at the Saint Louis University School of Medicine have begun a systematic analysis of human tumors for adenovirus 2, 7 and 12 information by utilization of highly radioactive L and H strands utilizing reagents and procedures developed previously. In addition, they are developing the procedure to detect single herpesvirus gene copies in certain human tumors by use of herpesvirus DNA preparations made highly radioactive by the nick-translation technique of Berg and co-workers. Data on the analysis of human cancers for nucleic acid sequences specific for RNA tumor viruses remains inconclusive. The future direction of this effort will be to screen human tumors for DNA polymerase activities capable of responding to a template developed by Dr. Gerard--poly 2'O-methyl cytidylate:oligo dG which appears at this time to be a more sensitive template than the widely used poly rC:oligo dG. Screening tumors with a sensitive assay will permit selection of those which can then be tested for activities templated by endogenous RNA.

Dr. Walter Eckhart, Salk Institute, is attempting to determine whether infection with a DNA virus induced the appearance of RNA tumor virus RNA in infected cells. If it is possible to understand the factors that govern expression of RNA virus genes in the mouse system, much of that information should be applicable to human cells. Dr. Eckhart reported that polyoma-infected BALB/3T3 cells do not produce RNA tumor viruses assayed by reverse transcriptase in the medium of infected cells. An endogenous type C virus from JLS-V9 cells (derived from a BALB mouse) was induced and a radioactive DNA probe to use in searching for RNA synthesis in polyoma-infected cells was prepared. Of two lines of rat cells infected with polyoma, one of the cultures has undergone morphological transformation. The size classes of RNA present in JLS-V9 cells was examined by assaying for protection of the R-MLV probe. The 35S and 20S species were found but no 70S was observed.

The Scripps Clinic and Research Foundation, under the direction of Drs. Frank Dixon, Richard Lerner and Ralph Reisfeld, is concentrating its efforts on the immunologic aspects of virus-caused cancer and autoimmune disease using the New Zealand mouse as a model. The Scripps Leukemia virus (SLV), derived from lymphoblast cell lines of the NZ mouse, proved highly oncogenic in all murine strains except NZBxW, NZWxB and C57BL/6. However, the way in which the NZ hybrids and the C57BL/6 handled SLV appeared to be

quite different, since in the former there was little elevation of serum, while in the latter there was a marked elevation. While all three strains developed anti-nuclear antibodies (ANA) when infected with SLV, only the NZ hybrids also developed glomerulonephritis within the first 6 months of life. Thus, it seems possible that in developing a means of controlling the oncogenic effect of SLV, the NZ hybrids develop immunologic disease. The SLV, although apparently endogenous to the NZ mouse, is not the xenotropic NZ virus. This group has also successfully isolated and purified the major glycoprotein which appears on lymphocytes transformed by oncorna viruses. These results, similar to those recently reported by Dr. T. August, indicate that this protein is one of the major targets of neutralizing antibody and as such is ideal to illustrate the relationship between vertically inherited viral genes and naturally occurring late life diseases. This protein was found to be available not only on the surface of the cell, but also on virion surfaces, a fact which is important for an understanding of the relationship between the host immune response and oncogenesis. This group is continuing to refine methods for quantitation of the number of plasma membrane associated immunoglobulin molecules per cell and has succeeded in isolating and characterizing the structure of these molecules. The results, since confirmed in other laboratories, suggest that plasma membrane associated immunoglobulin may have a unique structure which may be important for its role as a receptor molecule, and it should be possible to determine whether the amount or structure of plasma membrane associated immunoglobulin is altered in the neoplastic state. Because of the association between immunodeficiency and the neoplastic state, and the finding of type C particles in human placentas, this group has acquired a number of placentas from patients with lupus and have found these to be particularly rich in particles. They have succeeded in establishing several placenta lines, from which they are attempting to isolate and grow the virus. Present evidence indicates a universality of the type C virus or viral genome with apparent oncogenic potential in all species. Expression of this potential, however, is dependent on the immunologic responses of the host. Characterization of the viruses, viral antigens and host cell responses should provide important insights into the etiological role of the type C viruses under varying immunologic conditions, and the means of detecting and interfering with the oncogenic properties through the use of viral vaccines or manipulation of the host immune system.

Drs. Murray Gardner, Robert McAllister, Brian Henderson and associates at the University of Southern California School of Medicine have continued their comprehensive field and laboratory research program on the etiology and epidemiology of human cancer. The Cancer Surveillance Program which is a mechanism for rapid reporting of all cancer cases in Los Angeles now has the cooperation of all major hospitals (165 in number). Approximately 22,000 incident cases annually are made available for epidemiologic, immunologic and virologic studies. Epidemiologic surveys designed to obtain evidence of a milk transmitted human breast cancer virus showed no evidence of an increased risk of breast cancer associated with being breast fed, regardless of the age at diagnosis, and excess familial risk of breast cancer occurred equally in both maternal and paternal lines. Virus particles were not found by EM in milk specimens, and there was no correlation between detection of reverse transcriptase activity in milk

samples and family history of breast cancer. These results fail to support the possibility of a milk agent as an etiologic factor in human breast cancer.

A group of young wild mice trapped in Southern California were found to be remarkably prone to spontaneous lymphoma and/or lower limb paralysis. Transmission and neutralization studies have established unequivocally the indigenous type C viruses as etiologic agents of both diseases. The type C virus is spread epigenetically via milk and transplacentally. Several of the viral isolates can be grown in vitro in human cell cultures. Thus, type C RNA viruses must be considered as potential etiologic factors in humans, as well as mice, not only for cancer and possibly immunologic disorders, but also for unexplained neurologic diseases.

The isolation of temperature-sensitive mutants of avian sarcoma viruses was continued by Dr. Peter Vogt, University of Southern California School of Medicine. These class T mutants (viruses which cannot induce nor maintain transformation at 41°) are being used primarily in complementation studies to see how many viral genes are involved in the transformation process. It was found that two early coordinate mutants, ts335 and ts337, have a temperature-sensitive reverse transcriptase. Genetic revertants of ts335 and ts337 to wild type and to recombinants with wild type virus showed a correlation between virion heat lability and temperature-sensitivity of replication and transformation, indicating that a virion component, presumably the reverse transcriptase was responsible for the temperature-sensitivity of infection.

Dr. Leonard Hayflick, Stanford University, developed a unique method for enucleating mass populations of cultured and malignant cells derived from humans and animals. Of the several important questions that can be asked of this system are those involving the control of malignant properties and the cellular sites responsible for these properties. Until now the hopes that hybridization of fusion studies would unequivocally answer these questions has not been forthcoming. The interpretation of such studies has been confounded by the presence of all or a part of the genome of one of the two parental cells. The ability to enucleate mass cultures of cells now circumvent this serious limitation or interpretation of hybridization studies.

Drs. Karl and Ingegerd Hellström, University of Washington, demonstrated that lymphocyte mediated cytotoxic reactions to Rous sarcoma cells in Japanese quails can be blocked by the tumor-bearers' serum. Evidence was obtained indicating that antigen (in the absence of detectable antibody) can also block cytotoxicity and that antibodies can facilitate the formation of blocking complexes by releasing antigen from the tumor cells. The data are compatible with the hypothesis that the most efficient blockers are antigen-antibody complexes. Other studies showed that sera from certain tumor patients can increase the specific cytotoxic effect on tumor cells of lymphocytes from the respective patients (and from other patients with the same tumor) and can often bestow a tumor cytotoxic effect on lymphocytes from control donors.

Dr. Sachs, Weizmann Institute of Science, in continuing studies of the mobility of surface membrane lectin sites on normal and transformed cells, reported that the carbohydrate-containing structures on the cells that bind Con A are mobile. These experiments have provided direct evidence that in cells that are in suspension *in vivo*, malignant transformation is associated with reduction in mobility of these sites and that in cells that form a solid tissue, malignant transformation is associated with an increase in mobility of these sites. Additional studies have shown that the amount of cholesterol present in the cell surface membrane may play a significant role in malignancy, that there is a reversed cyclic change in the mobility of specific surface membrane sites in normal and transformed cells, and that there is a correlation between surface membrane glycopeptides and tumor formation.

Dr. Girardi, The Wistar Institute of Anatomy and Biology, in further studies on the mechanisms of virus-induced transformation found that the SV40 genome appears to be specifically integrated on the human chromosome C7 as indicated by expression of T antigen, TSTA antigen and rescuable infectious SV40 virions. Loss of the C7 chromosome is accompanied by loss of the above mentioned activities. These studies are being expanded in attempts to localize the EBV genome, the Rous sarcoma genome and the murine sarcoma genome in human cells which were transformed by these agents, but which are now producer cells.

Two new contracts were initiated this year. Dr. Norman Davidson of the California Institute of Technology, will apply physical methods, especially electron microscopy, to investigate the sequences and structure present in the nucleic acid molecules of RNA tumor viruses and in the integrated DNA viral genomes of transformed cells. These studies could provide important information on the mechanism of viral transformation of cells resulting in cancer. In addition, it could lead to a new means of detecting the presence of the cancer virus genome in cells and could influence approaches to cancer prevention and/or therapy at the cellular level.

A second new contract was initiated with Dr. Henry Kaplan at Stanford University on Hodgkin's disease and other human malignant lymphomas. Preliminary studies had been conducted by Dr. Kaplan the previous year under Contract NO1-CP4-3244. Cell lines have been established *in vitro* from the tumor cells of two patients with histiocytic lymphomas. Certain distinctive markers clearly identify the cells growing *in vitro* as identical with the tumor cells from the original patients. In addition, it has been established that the proportion of T-lymphocytes in the peripheral blood of patients with Hodgkin's disease is normal. Thus, the immune deficit in these patients is not attributable to a quantitative deficiency in numbers of T cells, and some more subtle form of functional impairment must be involved. It is anticipated that this contract will provide a source of Hodgkin's and other human tumor materials for cultivation and for virus isolations. If antigens specific for the Hodgkin's cells (Reed-Sternberg) can be detected, this would have major implication not only for etiology but also for diagnosis, and perhaps even for the immunotherapy of Hodgkin's disease.

The Segment has under consideration a proposed study on human xenotropic viruses by Dr. Jay Levy , the University of California School of Medicine. This group has been actively engaged in type C virus research, particularly in the murine system. Using methods similar to those employed for the recognition of the murine xenotropic viruses, they plan to begin to test human tissues for the possible presence of a xenotropic-type virus. It has become apparent that type C viruses do exist which can be produced actively by the host but cannot be propagated in other host cells. This observation may explain the failure, thus far, at isolating a human type C virus in pure culture. Once accomplished, this human virus will be most important in looking not only at its pathology in other hosts, but also as an immunologic tool for detecting other potential human type C viruses in human tumors and tissues. Moreover, only after cultivation *in vitro* can a virus be available for studying its possible role in normal cell physiology and human malignancy itself.

At present, two models of the genetic control of RNA tumor virus expression are being explored in mice: (1) murine mammary tumor virus expression, and (2) murine leukemia virus expression. It is important to use another system to broaden the base of observations to determine if the mechanisms observed are of a general nature or peculiar to those systems in which they have been described. The avian system is particularly appropriate, since it represents an entirely different class and species, and because of its intrinsic importance to public nutrition and the economy. In further support of its work on the inheritance and oncogenicity of endogenous RNA tumor viruses, the Segment is considering an Interagency Agreement with Dr. Lyman Crittenden, U.S. Department of Agriculture, aimed at defining the genetic control of resistance to and expression of endogenous avian type C viruses.

SOLID TUMOR VIRUS PROGRAM SEGMENT

Dr. Robert J. Huebner, VCB, Division of Cancer Cause and Prevention, Chairman
Dr. James T. Duff, VCB, Division of Cancer Cause and Prevention, Vice-Chairman

CALIFORNIA STATE DEPARTMENT OF PUBLIC HEALTH (NO1-CP4-3209)

Title: Studies on the Possible Role of Oncogenic Viruses in the Causation of Cancer in Man

Contractor's Project Directors: Dr. Edwin H. Lennette
Dr. John Riggs

Project Officer (NCI): Dr. Paul Arnstein

Objectives: To apply the newer knowledge of the nature of RNA viruses to the study of neoplasms of animals and man.

Major Findings: Malignant human tumors which had been surgically removed were implanted into anti-thymocyte serum treated (ATST) mice. Two of 13 attempts resulted in growth of the tumor material. Among newly acquired established cell cultures transplanted to the ATST mice, two were rated "benign" monolayers and failed to induce tumors; five were rated malignant and produced progressive tumors; two were rated borderline and produced regressive tumor nodules.

Among 36 previously transplanted human tumor lines, 10 have "picked up" an NIH Swiss type C virus by transplant into ATST mice.

Seven human tumor lines have been transplanted to highly "switched off" C57/Leaden mice, and one transplant tumor pool is positive for murine type C virus. This is the first type C viral isolate from "Leaden" mice.

Two animal tumor lines are being co-cultivated with human tumors in the ATST mice in order to favor emergence of theoretically xenotropic human oncornavirus. Mixed karyotypes are observed in the tumors produced; virus detection is in progress.

Studies on vaccine-induced resistance of ATST mice to transplantation of human tumor cells indicate that resistance is easily established and demonstrable. The phenomenon seems to be non-specific; all antigenic inoculations, whether viral or non-viral, seem to protect.

Tumor material from eight laryngeal papillomas and four genital warts were processed in the laboratory between September 1, 1973, and February 1, 1974. Cell cultures of papillomas at various passage levels are being carried in the laboratory. Immunofluorescent staining of cells of these cultures, using patients serum and also antibody against SV40T antigen, has failed to show any reaction to date.

The D 17 dog cell line was utilized in a study to determine its susceptibility to different type C viruses. Antisera to the gs-1 component of many different type C viruses was tested by indirect immunofluorescence once infection with the different oncorna viruses had been established in this cell system.

A study of ATST C57/L mice revealed that in addition to the presence of virus in newborn mice, type C virus was also present in the bone marrow of 37 day old ATST mice bearing RD cell transplant tumors.

Three of 56 laryngeal papillomas examined thus far contained papova-like viruses while one of 11 genital warts contained papova-like virus.

Significance to Biomedical Research and the Program of the Institute: The immunosuppression of mice with ATS and their subsequent tolerant state presents unique opportunities as an experimental system, not afforded in other methods of studying human cancers. One obvious advantage is the availability of a solid tumor, in which the human neoplastic tumor cells comprise the entire "malignant" component. These neoplasms are produced in the mice in 10-30 days and resemble architecturally well-known clinical tumor types. Thus, experiments can be designed to test chemical, physical or biologic influences on human solid tumors without the involvement of human subjects. The solid tumors might respond in a manner more closely resembling spontaneous human neoplasms than tumor cells in the more restrictive *in vitro* culture environment.

Proposed Course: (1) Growth of human tumor cells in ATS-treated mice for the purpose of (a) verifying that they are tumor cell lines instead of the usual fibroblasts and (b) trying to "switch-on" human type C virus by *in vivo* passage similar to the switch-on which occurred in RD cells on passage through a cat; (2) utilization of the ATS-treated mouse system for the study of viral vaccines; and (3) utilization of the fluorescent antibody (FA) procedure to look for gs-1 antigens in human tumors and tumor cell cultures and also utilization of the indirect FA procedure to look for antibodies in human cancer patients or laboratory personnel working with candidate human type C viruses.

Date Contract Initiated: June 24, 1968

CALIFORNIA, UNIVERSITY OF (NO1-CP3-3242)

Title: Comparative Leukemia and Sarcoma Viral Studies

Contractor's Project Director: Dr. Thomas Kawakami

Project Officer (NCI): Dr. Wade P. Parks

Objectives: To continue studies of the *in vitro* characterization of the woolly, gibbon and other simian type C viruses, especially by antigenic

and biochemical characterization; to continue studies of the pathogenicity of the simian viruses; to evaluate the natural history of the gibbon type C virus in specimens from Thailand and San Francisco Zoo populations; to employ serologic studies to detect shared antigenic components between the simian viruses and putative human type C viruses; to isolate or identify viruses or viral antigens in spontaneous human tumors as well as in nonhuman primate tumors using a wide variety of _in vitro_ techniques.

Major Findings: Virus detection, isolation and characterization from nonhuman primates and human tissue continued to be emphasized utilizing established _in vitro_ techniques. Since September two nonhuman primate and 24 human tumors were or are being evaluated for type C virus by electron microscopy, DNA polymerase assay, ^{3}H-uridine incorporation and serologic tests. Additional rhesus monkey tissues examined for type C viruses proved to be negative; however, seven additional gibbon type C viruses were isolated from peripheral blood cells of clinically normal gibbons. The viruses isolated from the gibbons were found to share antigenic determinants on the envelope, 30,000 dalton virus protein, and viral-DNA polymerase with known primate type C virus. The relatedness of the virus was established by IFA, neutralization, immunodiffusion and polymerase immunoinhibition tests. Biochemical studies on the primate type C viruses were continued to study the conditions for optimal activity and differentiation of the viral and host cell enzymes. Viral polymerases from different animal viruses showed similar preference for synthetic templates under identical conditions with the exception of the avian myeloblastosis virus. Avian virus differed in showing a much higher utilization of oligo dG·poly rC compared to simian and feline viruses. Examination of human tumors and placentas by electron microscopy, culture initiation and co-cultivation, and other viral activation and detection methods has yielded presumptive evidence of RNA virus in only a few instances. Human tumor with evidence of virus-like particles lacked DNA polymerase and showed no evidence of being infectious to other cell cultures. Human placenta with evidence of type C virus by electron microscopy showed low DNA polymerase activity in tissue culture fluid, but the cells could not be propagated. Placenta cells co-cultivated with heterospecies cell cultures showed increased polymerase activity but the infection could not be maintained.

Studies on gibbon and woolly monkey immune response to type C virus after infection with the homologous primate virus showed that these animals developed antibody to both the envelope and group specific viral antigen. Antibodies to the envelope antigen of the primate type C virus in gibbons and woolly monkey sera were detected by indirect immunofluorescence and radioimmune assay, while antibodies to the group specific antigen were detected by complement fixation and immunodiffusion tests. Gibbons viremic with type C virus were found to lack antibody reactive to the virus. All gibbons are being monitored for clinical changes and for type C virus antigen and antibody.

Significance to Biomedical Research and the Program of the Institute: The finding at this laboratory of two type C viruses associated with tumors of different primate species is evidence that the higher animals, including

man, are likely to be among the growing number of species harboring type C viruses. Since monkeys and man are closely related phylogenetically, the proposed studies, which are oriented toward characterization of the primate viruses and seeking possible relation with human tumors, are of direct relevance in establishing the etiology of human cancer.

Proposed Course: (1) Efforts will continue to detect, isolate and characterize viral agents from spontaneous nonhuman primate and human tumors, utilizing various rescue or viral activation methods; (2) characterization of recently isolated type C primate viruses and human virus-like particles in tumors and placentas will be continued. Comparison studies with known type C viruses will be included; (3) epidemiologic studies on primate viruses for evidence of infection in human and animal species associated with gibbons will be continued; and (4) further pathogenesis studies will be continued to establish the oncogenicity of SLV, SMV, and other type C primate viruses. These studies will involve monitoring the animals for clinical changes, including immune response to type C viruses.

Date Contract Initiated: November 1, 1969

CALIFORNIA, UNIVERSITY OF (NO1-CP3-3293)

Title: Studies on the Role of Virion-Associated DNA Polymerases in Malignant Transformation by Tumor Viruses

Contractor's Project Directors: Dr. J. Michael Bishop
Dr. Warren Levinson
Dr. Leon Levintow

Project Officer (NCI): Dr. Edward M. Scolnick

Objectives: Conduct investigation on the molecular biology of the avian and other RNA tumor viruses, particularly (a) the virion-associated DNA polymerase and (b) the virus-specific DNA and RNA in normal and transformed cells.

Major Findings: (1) Initiation of DNA synthesis *in vitro* with 70S RNA as template for RNA-directed DNA polymerase. A series of avian viruses has been surveyed. In every instance, nascent DNA initiates with pdA covalently joined to 3'terminal rA of a 4S RNA. In visna virus, initiation joins pdG to rA. The 4S RNA primer on the 70S RNA of RSV has been purified. It can be aminoacylated with methionine (and only methionine) and has many structural hallmarks of tRNA. However, rT is absent. This feature suggests that primer may be an initiator met-tRNA, but other aspects of the nucleotide sequence and the results of column chromatography do not conform to this view.

(2) The nucleotide sequence at the 5'-terminus of nascent DNA. All (>90%)

DNA transcribed directly from the 70S RNA of SR-RSV, B77-RSV, Prague-RSV and RAV-2 initiates with the octadeoxynucleotide d(A-A-T-G-A-A-G-C). A homogeneous 5'-terminal oligodeoxynucleotide (>7 residues) has also been found with visna virus, but its nucleotide sequence is not known. The occurrence of sequences of these lengths cannot be explained as a random event, and the homogeneity of the sequences implies the existence of common and reiterated initiation sites on the template RNAs.

(3) Molecular hybridization and RNA tumor viruses. A relatively complete array of techniques for the study of RNA tumor viruses by molecular hybridization has been assembled. This report summarizes the contractor's experience with these techniques. Two specialized reagents are in preparation: single-stranded DNA which uniformly represents the entire genome of RSV and single-stranded DNA which specifically represents part or all of the transforming genes of RSV. The utility of potassium iodide as solute in equilibrium centrifugation for detection of RNA-DNA hybridization was documented. Use of this solute (rather than Cs_2SO_4) avoids precipitation of RNA and the consequent trapping of DNA.

(4) Genetic relatedness of type C viruses. The genomes of type C viruses from different species of host are generally not related when tested by molecular hybridization. Even single species (e.g., cat) can harbor two unrelated classes of type C viruses. An endogenous virus of chickens (RAV-0) shares no nucleotide sequences with DNA from avian species other than chicken. These observations do not conform to the view that endogenous (and exogenous) RNA tumor viruses evolved from a common progenitor and augur poorly for the use of nonhuman reagents in the analysis of human neoplasia by molecular hybridization. However, this series of analyses may not be sufficiently extensive to exclude the possibility of important exceptions to the rule.

(5) Mouse mammary tumor virus. The production of MMTV by a variety of cultured cells is being measured with a sensitive and specific assay based on molecular hybridization. The objectives are to develop cultured sources of all major variants of MMTV and to describe the chemical and humoral factors which facilitate growth of the virus.

Several inbred strains of mice which differ considerably in their incidence of spontaneous mammary carcinoma and production of MMTV, and two colonies of feral mice all harbor approximately equivalent amounts of MMTV-specific DNA in their tissues. All mouse tissues examined also contain detectable MMTV RNA, irrespective of whether the tissues are producing virus, and irrespective of the incidence of spontaneous mammary carcinoma and virus production. These observations leave open the possibility that differences in either structural or regulatory genes (or differences in both) account for variations among mouse strains with respect to the presence, transmission and oncogenic potential of MMTV.

(6) Molecular hybridization with nucleic acids from normal and malignant human breast tissue. No detectable hybridization between MMTV DNA and RNA from human tissues has been obtained. In addition, reactions between MMTV DNA and human DNA have been observed only if the conditions for hybridization

are non-strigent. The observed reactions in this latter instance are not species-specific and are therefore of uncertain significance.

Significance to Biomedical Research and the Program of the Institute: These studies are providing an important insight into the mechanism by which RNA tumor viruses bring about malignant transformation, and perhaps will lead to significant advances in the understanding of the causation and control of human neoplastic disease.

Proposed Course: (1) The nature of 4S RNA primer on 70S RNA. Studies of the nucleotide composition and sequence will be continued, along with efforts to characterize and locate the binding sites for primer on 70S RNA.

(2) Reagents for molecular hybridization. Prepare the following radioactive DNAs: a) nucleotide sequences specific for transforming genes of RSV, and b) nucleotide sequences which uniformly represent the entire RSV and MMTV genomes.

(3) The genetic relatedness of endogenous viruses. Carry out an extended series of molecular hybridizations to determine the extent of relatedness among viruses endogenous to various species including primates.

(4) Mouse mammary tumor viruses. a) Use molecular hybridization to compare the MMTV genes present in various strains of mice and to analyze the extent of relatedness among the several variants of the virus. b) Use molecular hybridization to search for biologically inapparent infection of rodents other than mice. Attempt to infect the germ line of mice and other rodents with GR virus, resulting in the establishment of genetic transmission of the viral genome. These infections may be biologically inapparent. If established, they can be used to dissect the nature of the controls which limit the expression of MMTV genes in genetically infested mice. c) Analyze the distribution of integration sites for MMTV genomes in the DNA of different mouse strains in order to ascertain whether the site of integration is a determinant of gene expression. d) Molecular hybridization with human materials. Further efforts will be made to detect hybridization between MMTV-specific nucleic acids and both RNA and DNA from various human tissues and attempt to characterize the putative type B particles found in human milk.

Date Contract Initiated: June 2, 1971

EINSTEIN MEDICAL COLLEGE (NO1-CP3-3249)

Title: Genetic and Immunological Factors in Viral Leukemogenesis

Contractor's Project Director: Dr. Frank Lilly

Project Officer (NCI): Dr. Robert J. Huebner

Objectives: To gain a better understanding of the genetic events and underlying mechanisms that play a role in the oncogenic process.

Major Findings: (1) New genes governing response to B-tropic Friend virus. Preliminary evidence was obtained from experiments in the BALB/c X DBA/2 cross for the existence of two new genes (temporarily referred to as Fv-3 and Fv-4) governing susceptibility to B-tropic FV but with no apparent influence on response to N-tropic virus. Fv-3 has a dominant gene for resistance in DBA/2; Fv-4 has a recessive gene for resistance in DBA/2.

(2) Fv-1 and spontaneous leukemogenesis. In a large group of (BALB/c X AKR) X AKR mice, observationshave been made on the occurrence of leukemia in relation to (a) the titer of MuLV in tail biopsies taken at 6 weeks of age, (b) H-2 type and (c) sex. Results to date show a very strong correlation between the disease and virus titer and a much weaker correlation between the disease and both H-2 type and sex. These data show that Fv-1, which is the major factor influencing virus titer in this cross, is a major factor governing leukemia occurrence in these mice.

(3) Fv-1 and gs antigen expression. Either Fv-1 or a gene closely linked to it governs the level of expression of the gs-1 antigen in the (BALB/c X AKR) X AKR backcross. $Fv\text{-}1^{n}$ homozygotes of this cross show a high level of antigen expression, whereas $Fv\text{-}1^{n}/Fv\text{-}1^{b}$ heterozygotes show a low level of the antigen.

(4) Studies with cultured FV tumor cells. A transplantable tumor from the spleen of a BALB/c mouse with FV leukemia was obtained, and subsequently the cells were adapted for growth in vitro. The cells produced moderate levels of infectious FV (SFFV plus helper) in early passage generations, but later they became nonproducers by all criteria examined. The cells have a transplantation antigen which causes dose-dependent rejection when inoculated in vivo, and the sera of regressor mice contain virus-neutralizing antibodies but not cytotoxic antibodies. FV can be recovered from the nonproducers cells by (a) co-cultivation with MolLV-infected fibroblasts and (b) serial passage of the tumor cells in normal BALB/c mice. FMR antigen has not been detected on the surfaces of these tumor cells.

(5) Genetic control of chemical carcinogenesis. BALB/c mice painted with MCA develop skin tumors and not leukemia; DBA/2 mice develop leukemia and no skin tumors. Also, F_1 hybrids show the DBA/2 type of response.

Significance to Biomedical Research and the Program of the Institute: One of the basic facts about tumor biology is that genetic mechanisms of the host exert major control over the expression of oncogenicity. By defining the loci and markers associated with leukemogenesis, it should be possible to undertake systematic studies of the precise immunochemical mechanisms governed by individual loci, with the objective eventually of encouraging or altering their immunogenetic effects to provide maximum resistance against cancer.

Proposed Course: (1) Continued studies of the new genes governing response to B-tropic FV, (2) studies of genetic control of the gp 69-71 component of MuLV, (3) studies of virus expression in cultural cells of BALB/c and BALB.B FV-induced tumors, (4) studies of the mechanism of the effect of H-2 antibodies on FV susceptibility, (5) continued studies of FMR antigen: chemical nature and relation to virus.

Date Contract Initiated: May 13, 1965

FLOW LABORATORIES, INC. (N01-CP3-3247)

Title: Studies of Type C Viruses and Herpesviruses in Relation to Oncogenic Potential

Contractor's Project Director: Dr. Raymond V. Gilden

Project Officer (NCI): Dr. Robert J. Huebner

Objectives: To conduct immunological and serological studies of oncogenic type C RNA viruses and herpesviruses associated with neoplasia. To develop immunologic, biologic, and biochemical reagents and techniques for identification of type C viruses and their gene products. To delineate the mechanism of herpesvirus latency and to determine the relevance of herpesviruses associated with human tumors.

Major Findings: (1) Type C viruses. DNA complementary to RD 114 viral RNA was found to hybridize with cellular RNA and DNA from each of a small series of cat tissues, obtained directly at autopsy, and tissue culture cell lines. With the exception of RD 114 producing human cells, non-cat tissues, including the virus-free RD cells, did not contain detectable RD 114 virus-specific nucleic acid sequences. Induction of RD 114-like viruses from "virus-free" cat cells was demonstrated in agreement with earlier results of Sarma and Todaro. Conventional feline type C viruses (FeLV) cross-hybridize extensively among each other but show less than 5% cross-hybridization with RD 114, especially if FeLV probes are prepared from virus grown in human cells. All of the internal virion antigens, including reverse transcriptase, of FeLV and RD 114 can be distinguished by appropriate serological reagents; the major proteins of 30,000 molecular weight (gs antigens) show ~80% sequence homology based on comparison of 25 residues at the amino terminal. Collective evidence suggests that RD 114 is more distantly related to conventional FeLV than the known sub-primate viruses are related to each other. Ease of detection of FeLV gs antigens in cat tumors and embryonic tissue contrasts with general negative findings with RD 114 despite widespread presence of RD 114 RNA in cat tissues. Thus, the viruses exhibit markedly different patterns in spite of both being represented in cellular DNA.

Type C viruses of rat origin (RaLV) also appear to be represented in cellular DNA. These viruses show <5% cross-hybridization with mouse viruses

allowing the demonstration that M-MSV(RaLV) presumed to have been generated by in vivo rescue of M-MSV by RaLV indeed contains nucleic acid sequences homologous to RaLV and M-MSV. The specificity of M-MSV is retained since current probes distinguish readily among mouse virus strains.

Among the large variety of inter-species hybridizations performed, the two primate viruses, woolly monkey and gibbon ape, were unique in showing extensive cross-reaction - at a level comparable to that seen between mouse strains. The gs antigens of the two viruses appear to be ~98% related based on quantitative complement-fixation analyses - again a degree of relatedness only seen in assays with strains within a species. The diversity of the suspected species of origin and the close relationship of gibbon and man suggested that similar material should be present in humans. However, attempts to detect common nucleic acid sequences were negative with human tissue and most strikingly, were negative with gibbon and woolly tissues as well (in collaboration with Dr. David Kohne, Scripps Clinic and Research Foundation, and in agreement with independent results of Dr. E. Scolnick). The origin of these viruses and pattern of spread is thus an open question.

Amino terminal sequence analysis of gs proteins from several virus families has been carried out. In agreement with results of quantitative CF tests and peptide mapping, several mouse strains show single differences (out of 25 residues; i.e., ~10% of the molecule), whereas, at least five differences were seen in comparison of rat and cat viruses with mouse. This initial region is characterized by a common initial tripeptide, prolyl-leucyl-arginyl- a region of hypervariability (positions 4-10), and a region of extensive homology (positions 11-30). To maintain this homology, a gap is needed in the RD 114 sequence at position 5, and 6-7 residues must be inserted in the hypervariable region of the gibbon. The long region of homology contains two tyrosines, of obvious significance for synthesis of potential highly cross-reactive determinants useful for radioimmunoassay. Enzymatic cleavage of the major gs protein has also revealed fragments with retained antigenicity. These studies have shown that multiple reactivities fall under the previous designations: gs-1, species-specific; and gs-3, cross-reactive among mammalian viruses.

Attempts to detect gs-3 antibodies in human sera by radioimmunoassay were negative. The survey included cancer patient and laboratory workers with extensive experience with type C viruses.

Nonproducer mouse cells isolated after transformation by Ki-MSV were treated briefly: BrdU and cloned variants were selected. These were evaluated for viral markers, chromosome complement, and in vivo transplantability. The presence of replicating virus did not appear to correlate with transplantability in vivo.

Sarcoma virus transformed cells exhibit multiple differences in transport of certain sugars. A differential effect of the mold metabolite, cytochalasin B, on transport of 3-O-methylglucose was demonstrated. These findings continue to suggest the possibility that transport sites might be important targets for control of malignant properties.

Transformation of mouse embryo cells by M-MSV(RLV) was inhibited by non-toxic concentrations of cordycepin (3'-deoxyadenosine). The inhibitory effect was maximal between 30-90 minutes post infection and was reversible by adenosine. The site of action of this drug on the transformation process is being investigated.

The DNA cellulose binding method was used to evaluate differences between control and virus transformed cells. Synthesis of several DNA binding proteins which are present in normal growing cells but not in crowded or serum deprived cells are still fully expressed in sarcoma virus transformed nonproducer cells under the latter conditions. Further characterization of these proteins by functional and immunologic procedures is under way. An azauridine resistant mouse cell selected in other experiments was found to produce syncytia when infected with M-MSV(RLV) but not RLV. This effect was neutralized by anti-RLV serum and yields from such cells produced only typical foci on untreated mouse cells. This raises the possibility of analysis of a specific sarcoma virus product independent of helper function.

Virus and reagent production for the type C viruses is a continuing effort. Large amounts of banded virus have been inactivated with formalin and utilized for vaccine and immunization experiments. Certain MuLV preparations have proven useful in eliciting type-specific neutralizing responses in mice. Goat antisera to purified gs antigens have been prepared and have proven most useful as specific and sensitive reagents in gel diffusion and to a lesser degree in complement-fixation tests. Methods of Quality Control for virus production have been established including quantitative particle counting and determination of gs antigen content by radial gel diffusion.

In collaborative studies with Drs. W. P. Parks and E. Scolnick (NCI), and as part of a U.S.S.R.-U.S.A. scientific interchange, human cultures with viral particles were evaluated (derived in the U.S.S.R.). One of these, which lacked reactivity with type C reagents, was found to be indistinguishable from the Mason-Pfizer monkey virus.

(2) Herpesviruses. Studies with the Epstein-Barr virus in collaboration with Dr. Berge Hampar, NCI, VCB, have concentrated on the mechanism of latency and virus activation. Evidence obtained with producer (EB-3) and nonproducer (Raji) cells synchronized by the double thymidine blocking technique indicated that virus activation induced by the thymidine analogue, 5-iododeoxyuridine required incorporation of the drug into DNA during the cells' early S phase (S-1 period) (Hampar et. al., Nature New Biol., 244: 214-217, 1973). Additional studies using DNA inhibitors (ara-C, hydroxyurea, or excess thymidine) indicated that inhibition of DNA synthesis during the S-1 period could result in enhanced virus activation in cell populations already undergoing spontaneous virus activation. The findings with DNA inhibitors and thymidine analogues suggest that some as yet undetermined priming event may be required before cells become susceptible to drug-induced virus activation. Further, the findings suggest that the DNA synthesized during the cells' S-1 period contains unique sequences which control activation of the repressed EB viral genome.

Additional studies using synchronized Raji cells indicate that the repressed resident EB viral genome replicates during the S-1 period, at a time which corresponds temporally to the critical period for activation induced by IdU (in collaboration with Dr. M. Nonoyama). This is consistent with the proposition that EB virus activation occurs at or near the site of the repressed viral genome and suggests that the repressed viral genome may be physically associated with early replicating cell DNA.

Significance to Biomedical Research and the Program of the Institute: The contract is the major source of reagents for typing type C tumor viruses. The expertise developed by the contract for purifying viruses and subviral components and for preparing antisera of known specificity has allowed rapid and reliable identification of viruses isolated from various species. These reagents are especially important in instances where virus isolates have been obtained from human tissues. Since the human type C virus has yet to be isolated and characterized, it is important to have reagents available for testing isolates to exclude contamination by nonhuman viruses. The contractor is also involved in biological and biomedical studies relating to the mechanism of type C virus persistence in cells and the differentiation of events leading to cell transformation. The existing expertise of the contractor and the programs presently under development will prove useful in attempts to develop vaccines for immunoprevention and immunotherapy of human tumors. The herpesvirus studies are directed towards delineating the mechanism of latency in human cells and determining the relevance, if any, of herpesviruses associated with human tumors.

Proposed Course: Continuation of studies summarized above with emphasis on development of highly specific, well-characterized reagents, both of immunological and nucleic acid type. Continuation of attempts to define and control the transformed state; e.g., DNA binding proteins, transport sites. Performance of surveys utilizing immunological and molecular biological techniques on relevant materials. Attempts to describe the natural history of DNA copies of viral genomes in species related to those from which viruses are available. Continued studies on the mechanism of EB virus latency.

Date Contract Initiated: February 1, 1971

HARVARD UNIVERSITY (NO1-CP3-3265)

Title: Primary Structure and Synthesis of Avian Leukosis Virus Proteins

Contractor's Project Director: Dr. David W. Allen

Project Officer (NCI): Dr. Padman Sarma

Objectives: (1) To complete the determination of the primary structure of the gs-a and gs-b antigens of avian myeloblastosis virus (AMV), (2) to isolate and sequence additional group-specific (gs) antigens from

AMV, (3) to compare the structure of these antigens with those of other avian leukosis viruses, (4) to study the distribution of avian leukosis gs antigens in "leukosis-free" chick embryos.

The information obtained from these studies on the detailed chemical structure of the antigens will then be used directly in two further areas: (5) the chemical synthesis of gs antigens and of selected peptides representing specific regions of their primary sequences, and (6) the use of these synthetic peptides in a detailed study of the immunological properties of the antigens, and in the development of sensitive and specific radioimmunoassays for the detection of antigen in embryonic tissues and in the course of oncogenesis.

Major Findings: The amino acid sequence of gs-b (p-15), an avian leukosis group-specific (gs) antigen from avian myeloblastosis virus has been nearly completed. Progress has resulted from a repeated staphylococcal protease digest and fractionation in which six homogenous peptides were found. These were sequences with substantial new information concerning the C-terminal portion of the molecule. In addition, micropeptide mapping is being utilized to isolate peptides which may solve the small portion of the amino acid sequence remaining to be determined.

Progress has been made in studying the antigenic sites of gs-a by fractionating the cyanogen bromide peptides, by gel diffusion, and determining their antigenicity by complement fixation and immunodiffusion.

Significance to Biomedical Research and the Program of the Institute: The determination of the primary structure of the gs-a and gs-b antigens of AMV are a prerequisite to the chemical synthesis of one (gs-b) or both of these antigens. The avian myeloblastosis virus is being used as a model system prior to initiating similar studies with the interspecies antigen (gs-3) of mammalian viruses. The availability of a highly specific and purified antibody against the interspecies antigen would considerably aid in finding antigenic fingerprints of a potentially oncogenic virus in human neoplastic tissue.

Proposed Course: (1) Determine if the N-terminal portion of gs-a, CNBr-3, a 23 amino acid peptide, is antigenic, after isolating it by a gel diffusion column. (2) Synthesize the N-terminal portion of group-specific protein (p-30) from murine leukemia virus, and determine if this portion of the molecule has species or interspecies antigenic activity, and is a suitable antigen for use as a serologic reagent. Such synthetic material would be unlikely to have the same antigenic contaminants as proteins from viruses isolated from animals or cell culture.

Date Contract Initiated: September 18, 1972

HAZLETON LABORATORIES, INC. (NO1-CP3-3212)

Title: The Role of Viruses in Experimental Oncogenesis and Human Cancer

Contractor's Project Director: Dr. Robert C. Good

Project Officer (NCI): Dr. Stuart A. Aaronson

Objectives: The goals of this contract are to develop an understanding of the mechanisms of action of oncogenic viruses and to provide evidence for a viral etiology to human cancer. Work in experimental systems with RNA tumor viruses, including murine sarcoma (MSV) and murine leukemia virus (MuLV) and with the DNA tumor virus, SV40, has led to important findings concerning the oncogenic actions of these viruses and their effects on normal growth. A multidisciplinary approach involving cell biology, virology, biochemistry and immunology is aimed at the eventual development of rational approaches to the prevention and treatment of human tumors.

Major Findings: Studies to determine the role of type C RNA viruses in naturally-occurring tumors have led to several major findings during the past report period. Multiple endogenous type C viruses have been shown to be present in mouse cells of different strains. The findings that biologically distinguishable type C viruses of one cell segregate in mendelian fashion in appropriate genetic crosses indicate that the loci detected represent virus structural information rather than regulatory genes affecting virus expression. Biochemical evidence, obtained by hybridization of cellular DNA with type C virus DNA probes, has shown that the virus-specific DNA is localized within the high molecular weight DNA of the mouse cell. One virus, induced from virus-negative mouse cells in tissue culture, has been shown to cause lymphatic leukemia in a low leukemia incidence strain. These findings establish the oncogenicity of naturally-integrated type C viruses.

In efforts to develop an understanding of the regulatory processes involved in control of potentially malignant endogenous viral genes, it has been shown that one genetic locus, Fv-1, markedly influences the expression of certain endogenous viruses but has little or no effect on others. Further, a new and highly potent class of chemical inducers of type C virus, protein synthesis inhibitors, has been discovered. These inducers differ from halogenated pyrimidines in their ability to activate distinguishable endogenous viruses of the same cell, providing further evidence that cellular regulatory factors specific for each virus, must exist. In BALB/c cells, regulation of the spontaneous activation of two endogenous viruses has also been shown to differ. One virus is spontaneously activated at a very low frequency of around 10^{-6}, a level which is at least 50-fold higher than that of the other. The induced virus fails to persist because of its inability to propagate in normal mouse cells. That activation of this virus also occurs *in vivo* has been demonstrated by the detection of very high-titered naturally-occurring neutralizing antibodies to it in sera of BALB/c mice. Similar antibodies have been shown to be present in a large number of other strains as well, providing evidence for the widespread occurrence of this virus class.

In attempts to define the mechanisms involved in type C virus replication, conditional lethal mutants of MuLV have been characterized into three physiological classes, defective in replication functions at the nonpermissive temperature. Further, one group of mutants has been shown to lack helper functions necessary for fixation of murine sarcoma virus transformation. In studies of the genetic functions of murine sarcoma virus, a selection technique has been devised that allows isolation of large numbers of morphological revertant clones from a line of nonproductively transformed cells. These revertants possess normal _in vitro_ and _in vivo_ growth properties yet contain the MSV genome. One class of revertants has been shown to contain transformation-defective sarcoma viral mutants and should be useful in complementation studies to determine the number of sarcoma viral genetic functions involved in transformation. A sarcoma virus isolate from a tumor of a woolly monkey has been shown to produce transformation of cells in a manner distinguishable from that of known strains of murine sarcoma viruses. However, this new sarcoma isolate, like murine sarcoma virus, is unable to replicate as an infectious virus.

Very sensitive and specific immunological assays have been developed for type C viral proteins, including the 12,000 and 30,000 M.W. polypeptides of type C virus of several different species. In particular, immunoassays for type-specific determinants on these polypeptides have proven very useful as markers for different virus strains for genetic studies and in the identification of new virus isolates. These tests, along with biochemical methods involving DNA-DNA and RNA-DNA hybridization, have been used to search for type C virus information in human cells. Continuous lines of neoplastic cells from a variety of human tumors have also been developed for use in these studies and as resources for the Viral Oncology Program.

Significance to Biomedical Research and the Program of the Institute: In order to develop rational approaches to the prevention and treatment of human neoplasia, a detailed understanding of the mechanisms involved in malignant transformation is extremely important. Tumor viruses in mammalian systems provide excellent models for studying the processes. The knowledge gained from these studies can be directly applied to the search for similar viruses etiologically involved in human cancer.

Proposed Course: Investigations will continue into the mechanisms of viral oncogenesis utilizing model systems developed in this program. There will be expanded emphasis on genetic and biochemical studies to elucidate the mechanisms by which the normal cell regulates expression of its potentially malignant naturally-integrated type C viruses. _In vivo_ studies will be continued to further determine the spectrum of diseases associated with endogenous type C viruses. The mechanisms of type C viral transformation and replication will continue to be worked out utilizing already isolated and partially characterized conditional lethal mutants of mouse leukemia and sarcoma viruses and absolute transformation-defective sarcoma virus mutants. Emphasis will be placed upon application of developed biological, immunological, and biochemical techniques to the search for and

study of viruses potentially involved in human cancer. As new techniques become available through work in model systems, they will be rapidly applied to investigations of human neoplasia.

Date Contract Initiated: September 1, 1972

JACKSON LABORATORY (NO1-CP3-3255)

Title: Natural Occurrence of RNA Tumor Viruses (Genomes) and Host Gene Control of Their Expressions

Contractor's Project Director: Dr. Hans Meier

Project Officer (NCI): Dr. Robert J. Huebner

Objectives: The primary objective of this contract is to achieve an understanding of the mechanisms underlying the genetic determination of susceptibility and resistance to cancer and the RNA tumor viruses. The Jackson Laboratory is a unique source of highly inbred mouse strains. These are used to define specific gene influences on type C RNA virus/genome/tumor expressions under natural conditions, and the influence of environmental and other factors (carcinogens, aging) on host gene controls of oncogene and virus expressions.

Major Findings: (1) Host-Gene Control of Type C RNA Tumor Virus Expression and Tumorigenesis in Inbred Mice. We found a highly significant and predictable association between endogenous viral expression in early life and leukemias and reticulum cell sarcomas with advancing age. Thus, type C RNA tumor virus is the major determinant of these tumors.

(2) Genetic Control of the Group-Specific Antigen (gs-AG) of Murine Leukemia Virus (MuLV). In crosses of AKR/J and C57L/J mice it was found that alleles permissive to gs-AG expression were dominant to their nonpermissive alleles. A second example of single gene control of gs-AG expression in inbred mice was found: presence of gs-AG is recessive to absence, and unaccompanied by complete replicating MuLV.

(3) Transplacental Effects of 1-Ethyl-1-Nitrosourea (ENU) in Inbred Strains of Mice. It was observed that both the ENU-caused teratogenic and carcinogenic effects are strain-dependent but inversely related to one another. The outcome of ENU-induced malformations and tumors in strain-crosses is primarily dependent upon maternal hereditary traits rather than the fetal genotype. In offspring from ENU-treated mothers, a significant association exists between gs-AG, and in some cases, complete viral expression and chemically-induced tumors, but the rate of tumor induction and activation of infectious or subinfectious viral expression is influenced by the strain of mouse. In strains with complete virus expression the induction of tumors was accelerated, whereas in strains with a largely unexpressed viral oncogenic mechanism, ENU induced tumors through a

combined viral-chemical action. A consistently high incidence and development of tumors within less than 10 weeks occurred in certain F_1 mice representing an ideal model for the simultaneous evaluation of anti-type C viral vaccines against both mesenchymal and epithelial tumors.

(4) HRS/J: Hereditary Immunodeficiency and Leukemogenesis. Relative functional defects in the immune system occur in mutants giving rise to a higher incidence of leukemias than in non-mutant mice. Apparently, a deficient "collaboration" among different lymphoid cell types or a deficiency in the proliferative capacity of immunocompetent cells occurs in mutants resulting in an ineffective immunosurveillance against leukemogenesis.

(5) Elevated Sterol Synthesis in Lymphatic Leukemia Cells from two Inbred Strains of Mice. The *de novo* synthesis of digitonin-precipitable sterols in leukemic cells from AKR/J mice is at least 10-fold greater than that of normal cells, and associated with a correspondingly increased activity of the rate-limiting enzyme, 3-hydroxy-3-methyl-glutaryl coenzyme A reductase. Synthesis of fatty acids and production of CO_2 are little or not at all affected. An accelerated sterol synthesis in tumor cells or tissues may reflect their rapid growth and division, two processes that require cholesterol as major cell structural elements.

(6) *In Vivo* Induction of Hepatocarcinoma with Fetal Mouse Liver Cells Spontaneously Transformed in Culture and Activation of Type C RNA Tumor Virus from Carcinoma Cells. A hepatic cell line from C57BL/6J embryos underwent spontaneous transformation after long-term *in vitro* cultivation. It produced rapidly growing carcinomas when inoculated into C57BL/6J-newborns. Reestablishment of tumor cells in culture caused a conversion of gs-AG negativity to gs-AG positivity, and bromodeoxyuridine readily activated complete infectious type C RNA tumor viruses. Early passages yielded B-trop virus and late passages a N-trop virus indicating the presence in C57BL/6J tissue of genetic information for at least two types of biologically distinguishable viruses.

(7) Ly-4, a New Locus Determining a Lymphocyte Cell Surface Antigen in Mice. A new locus was found determining a cell surface alloantigen of bone marrow-derived (b) lymphocytes. A number of positive and negative strains have been identified.

(8) A Micromethod for Lymphoblastic Transformation of Mouse Lymphocytes from Peripheral Blood. A reliable method was developed for culturing mouse peripheral blood lymphocytes that requires only 50 ul of blood, avoids the need for tedious cell separations, and excludes the use of heterologous sera. This method should be useful in investigations of the function of peripheral lymphocytes and allow for study of the genetic control governing responses to phytohemagglutinin or other antigens.

Significance to Biomedical Research and the Program of the Institute: This program has contributed much of the basic data concerning the genetic determinants of oncogenesis and the natural expressions of the endogenous type C virus. It has pointed up the overwhelming influence of genetic predisposition in the development of natural cancer and susceptibility to

environmental carcinogens. The contractor has developed sophisticated systems for defining and locating the genes and loci involved in murine oncogenesis, and virus and antigen expression, and has rescued complete virus through gene complementation by hybridization of two virus-free mouse strains.

Proposed Course: (1) Further development, characterization, and uses of recombinant inbred lines. (2) Study of genetic control of endogenous murine leukemia virus. (3) Study of association of the viral group-specific antigen with tumor development. (4) Genetic analysis of a dominant gene inhibiting expression of the group-specific antigen. (5) Mapping of viral structural genes by use of defective sarcoma virus mutants: complementation classes. (6) Additional marker studies for mapping structural genes of viral components. (7) Induction of complete or partial virus synthesis by drugs. (8) Chemical co-carcinogenesis studies. (9) Marker studies (immunogenetic and biochemical). (10) Study of genetic control of embryonic and post-natal, normal and abnormal cell proliferation. (11) Study of the effect(s) of hormonal stimulation of RNA metabolism in dwarf mice, and (12) host genetic rescue of murine leukemia virus.

Date Contract Initiated: May 2, 1967

MICROBIOLOGICAL ASSOCIATES, INC. and CHILDREN'S HOSPITAL OF AKRON (NO1-CP4-3240)

Title: The Roles of Viruses and Chemicals in the Etiology of Cancer and its Prevention

Contractor's Project Directors: Dr. Riley Housewright (Tech. Manager)
Dr. Mina Lee Vernon
Dr. Paul Price
Dr. Ilan Shif
Dr. Johng S. Rhim
Dr. Kiyoshi Higuchi
Dr. Carrie Whitmire
Dr. Richard E. Kouri
Dr. John C. Parker
Dr. Aaron E. Freeman (Children's Hospital of Akron)
Dr. Howard Igel (Children's Hospital of Akron)

Project Officer (NCI): Dr. Robert J. Huebner

Objectives: Primary emphasis is placed on the following major tasks: Task A: Studies related to a search for human type C virus. Task B: Studies related to the prevention and treatment of tumors. Task C: Carcinogen assays and cell repository. Task D: Diagnostic and Testing Functions.

Within the framework of certain of these tasks, the contractor provides extensive service and collaborative research functions to other contracts and NCI supported scientists.

Major Findings: Task A. (1) Type C particles have been seen in 17/20 normal human placentas examined by electron microscopy to date, although there is no definitive evidence of type C particles in the few tissue cultures examined so far. Extensive efforts are in process to isolate the type C particles from human placentas and/or human tissue using co-cultivation, various culture techniques and chemical treatment. Near term placentas from three strains of mice (DBA/2, C57BL/6Cum and AKR/J) were examined in the hope of finding a readily available model system with which to work. These murine placentas have shown only intracisternal A particles in 13 placentas examined. Type C particles were readily found in one of two blocks of placenta from rhesus monkeys. The material in these blocks was not suitable for detailed studies. Examination of tissue from baboon placentas is planned for the near future. The only interest in the subhuman primates is one of comparative morphology and implications for continuing human studies. (2) Electron microscopic studies of the human paralytic disease, Guamanian amyotropic lateral sclerosis (ALS), have required modifications of fixation and transport procedures to assure getting suitable tissue for ultrastructural study. These difficulties seem to be under control now and the studies will continue. The purpose is to explore the possibility that pantropic wild mouse viruses which cause an ALS-like disease in mice may be involved. (3) Virus isolation from human tumors (ATS-treated NIH Swiss mice passaged tumors, Dr. Paul Arnstein, Berkeley, Calif.) and human lymphoid cells (Sloan-Kettering Inst.) was attempted by co-cultivation with human cells, lymphoid cells or cells of heterologous origin and by chemical treatment. No positive results were obtained and no further work of the type is planned. (4) Replication of Ki-MSV in human cell lines of normal and neoplastic origin are in process attempting to isolate human nonproducer (NP) cells. Such isolates may be useful in turning on a human type C virus.

Task B. (1) In vitro studies have shown that the antibiotic streptonigrin (SN) at nontoxic levels can protect Fischer rat embryo cells from transformation by 3-methylcholanthrene, 7,12-dimethylbenzanthracene or benzpyrene. The mechanism must still be defined. The same dose of SN which inhibits chemical transformation also inhibits 5'-IdU-induction of the endogenous rat type C virus by over 80%. The conditions for in vitro activation have been optimized. (2) Electron microscopic studies of serially transplanted BALB/cf/CD mammary tumors have continued through Passage 12. Particle counts have confirmed our earlier impression that the production of type B particles was gradually decreasing in numbers with passage. Intracytoplasmic type A particles increased in numbers through several passages, but may be beginning to decrease in numbers at the 12th passage. These studies will continue through an estimated 20 passages. The purpose is to select tumors with high numbers of B particles for anti-mammary cancer viral vaccines. (3) Studies to determine the effect of tissue culture derived interferon on MCA tumor induction in neonatal CF-1, C57BL/6, NIH Swiss and weanling CF-1 mice were completed. The MCA dose level used was one giving 50% tumor incidence in 4 months.

Interferon treatment was initiated 3 days prior to MCA and continued for 4 months. At 4 months interferon significantly reduced tumor incidence in all groups except the CF-1 neonates; however, at 10 months the reduction in tumor incidence was less significant. Since earlier studies demonstrating that interferon reduced MCA tumor induction in CF-1 neonates were done with mouse sera interferon and these last studies were with mouse tissue culture interferon, one more series of studies will be undertaken to compare the two sources of interferon under the same conditions. (4) Two derivatives of the antibiotic rifampicin have been studied against MCA carcinogenesis *in vivo*. Preliminary evidence suggested that treatment with o-n'-octyloxine could decrease MCA tumor incidence in NIH Swiss mice while demethylrifampicin derivatives had only a small effect on latency. Additional studies in NIH Swiss and C57BL/6 Cum mice were undertaken using several treatment schedules and two dose levels. The results from these studies indicate MCA-induced carcinogenesis could not be altered by rifampicin treatment. (5) Recent studies of the effects of type C RNA viral vaccines on MCA carcinogenesis have failed to demonstrate satisfactory development of CF antibody due to the poor quality of the vaccine. Insufficient envelope antigens were available to elicite an adequate immune response. Initial tumor development was delayed with citrate controls, normal muscle controls and viral vaccines with adjuvants. In some instances AKR viral vaccines given with FCA both delayed tumor induction and reduced the incidence. (6) Seventeen days after RLV infection of BALB/c mice, the mice were treated subcutaneously with either 150 μg MCA or 150 μg DMBA and observed for 6 months. MCA induced 83% sarcomas while DMBA induced 43% and the latency period was longer for DMBA tumor induction. Mice receiving MCA and RLV (10^{-2}) developed 14% sarcoma, 39% leukemia and 43% both leukemia and sarcoma. When less RLV (10^{-3}) was given, 34% developed sarcoma, 21% leukemia and 31% both leukemia and sarcoma. Mice receiving DMBA and RLV (10^{-2}) died early with leukemia. Only one mouse had both leukemia and sarcoma. When less RLV (10^{-3}) was given, 9% developed sarcomas alone, 28% leukemia alone, and 15% both leukemia and sarcoma. Although there was no reduction in incidence of leukemia in this group (RLV 10^{-3} + DMBA) there was a reduction in the total incidence of sarcomas from 43% to 24%. This reduction in tumor incidence may either be due to the early death from leukemia thereby reducing the mice at risk to sarcoma induction or to some protective effects to sarcoma induction by the RLV infection as was seen earlier with RadLV.

Task C. (1) Utilized the well-tested Fischer rat embryo cell transformation system to test the carcinogenic potential of coded fractions of smog condensates collected at different locations in the Los Angeles Area. Four of the five unknowns were carcinogenic; three known controls done blindly behaved as expected. (2) Isolated, characterized, stored and shipped cell cultures and viruses of special interest to the VCP. Ninety-six cultures were shipped to 16 different groups during the last 6 months. Three type C viruses were isolated from human tumor cells which had been passed through immunosuppressed rats and mice and characterized as genetically transmitted xenotropic rat and mouse viruses. (3) Two possible *in vitro* transformation assay systems for studying the effects of chemical carcinogens on human cells have been developed and are being studied further. Several animal cell transformation systems were screened in an attempt to develop a more

rapid and more quantitative system for identifying chemical carcinogens and for studying the role of endogenous as well as exogenous type C RNA viruses in chemical carcinogenesis. (4) Transformation of hamster fetal tissue culture cells treated with 1-B-D-arabinofuranoxylcylosine (ara-C) appears to occur during the S-phase of cell growth. It may be that what is considered induction of transformation by ara-C is purely a selective process since the number of transformed foci does not increase in kinetic studies, rather there is a reduction in the number of normal colonies. Transformation was observed only in hamster fetal cells which express a certain level of spontaneous transformation. Certain of the transformed cells derived from these ara-C studies release low levels of type C RNA viruses as demonstrated by EM, H^3-uridine incorporation, banding at 1.16, low levels of reverse transcriptase activity and gs antigen and BrdU pretreatment enhances the levels of all these parameters. This study was undertaken in an effort to develop a more sensitive *in vitro* assay system but is not sufficiently promising for further work for this purpose although the system may be useful in other respects and the hamster virus will be more fully characterized. (5) The effect of expression of the endogenous RNA viral genome on chemical carcinogenesis is being evaluated in DBA/2 (D2) and C57BL/6 (B6) mouse strains and the backcross and intercross animals derived from these parental strains. The B6 mice have little gs antigen expression, the D2 mice are 100% positive for gs antigen and the F1, F2 and B6xF1 mice have intermediate levels of gs antigen expression suggesting the presence of at least two genes regulating expression in this cross. MCA tumor induction was randomly distributed between the gs+ and gs- animals. Infectivity was not determined. The alleles regulating AHH inducibility also segregate in this cross. Of 251 inducible mice treated with MCA, 202 developed tumors with an average latency of 131 days. Of 85 noninducible animals treated with MCA, nine developed tumors with an average latency of 195 days. These data suggest a close relationship between AHH inducibility and MCA tumorigenesis. (6) Studies on an RNA type C virus from tumors resulting from transplantation of rat kidney cells transformed by DMBA are in progress. The tumor cells were found to contain rat leukemia virus gs antigen. The sera of tumor-bearing rats developed antibodies reactive with a rat leukemia virus pseudotype. (7) Hepatic adenocarcinoma was produced in C57BL/6J mice inoculated with liver cells spontaneously transformed in culture. The isolate was BALB tropic. This work is being terminated. (8) A number of Mason-Pfizer monkey virus (M-PMV) infected human and simian lines were established. The RNA-dependent DNA polymerase assay was found to be a sensitive method for the detection of M-PMV replication. This work is being terminated.

Task D. (1) During the past 6 months, approximately 5,600 serodiagnostic tests were performed on 3,900 specimens. Over 90% of the specimens were wild or colony mouse sera tested for antibodies to Sendai, polyoma or lymphocytic choriomeningitis. The remaining specimens tested included antigens and antisera for determination of anticomplementary activity, commercial rat colony sera for Sendai and polyoma antibodies and various mouse sera for the presence of antibodies to 11 indigenous murine viruses. The following two studies were performed in collaboration with Contract NO1-CP3-3288 (Microbiological Assoc., Inc.). (2) Using this laboratory's isolate of Casitas disease virus, *in vivo* and *in vitro* neutralization tests

were attempted using anti-Gross and anti-AKR serum. Neutralization was not demonstrated. Experiments were undertaken to study the host-range susceptibility of Casitas virus with respect to N-type and B-type strains of mice. Paralytic disease was induced in N-type NIH Swiss mice. No paralytic disease was observed in the other N- or B-type mouse strains. Further experiments were initiated to clarify the N-tropic behavior of the isolate. (3) As a result of a major Sendai epizootic at the animal facility of an NIH contractor, involving mostly geriatric mice, several parameters of Sendai virus infection have been studied. Four inbred mouse strains were compared to determine if the genetics of the mouse influences resistance or susceptibility. Their susceptibility (determined from infectivity based on serologic response) was similar and the mortality rates were not significantly different. To study the effect of age on susceptibility to Sendai infection, 100 mice are being held in germ-free isolators until they are 18 months old. At that time their susceptibility to Sendai virus will be compared to that of younger mice. An experiment studying the effect of Sendai virus infection on 3 MC tumors in three strains of weanling mice was terminated when controls being held at NIH became infected with Sendai. (4) Host range studies of Ki-MSV indicate a wider host range than hitherto believed. NP clonal lines of guinea pig embryo (GPE) cells isolated from transformed foci induced by Ki-MSV produced neither virus nor viral antigens. However, the sarcoma virus genome was rescued in these NP cells by co-cultivation with "helper" murine leukemia virus releasing GPE cells. Particles resembling guinea pig leukemia virus were activated from guinea pig NP cell following chemical treatment. These particles, approximately 100 mμ in the mature form, possessed a density of 1.16-1.17 g/ml and contained reverse transcriptase activity. There were also numerous intracytoplasmic type A particles rather than the intracisternal A particles previously associated with guinea pig leukemia. These particles are more similar to type B particles than to type C, but are not truly typical of either. Banding materials from BrdU-treated guinea pig NP cells were inoculated into newborn guinea pigs and are under observation for induction of leukemia and tumors. (5) A total of 429 specimens have been examined by electron microscopy for the presence of viral particles. These tissues were derived from the following Tasks or Groups: Task A (23.1%), Task B (6.1%), Task C (3.5%), Task D (1.0%), Task E (14.9%), Contract NO1-CP3-3248, Microbiological Assoc., Inc. (4.9%), Contract NO1-CP4-3254, Microbiological Assoc., Inc. (20.3%) and the Poolesville facility or any direct referral of the Project Officer (28.2%). Many of the observations are contained in other sections of this report. (6) Performed over 1,900 reverse transcriptase (RT) assays for other groups within the Program. Optimized the conditions for the *in vitro* activation of an endogenous rat virus and detection of the activated virus by the viral RNA instructed DNA polymerase assay. (7) Preliminary studies in the development of a sensitive neutralization test using the reverse transcriptase assay has shown this procedure to be possible and may be useful in evaluating sera in vaccine studies. The test must now be correlated with other test systems such as XC tests and focus reduction.

Subcontract, Children's Hospital of Akron, Ohio: (1) In collaboration with Dr. Zimmerman of MA, this group studied the possibility that type C viruses might be slow transforming agents. Six pairs of cultures derived

from BALB/c embryos were used; one served as a control and the other was inoculated with a B-tropic virus. Around P 20-30, the four surviving control cultures became heteroploid but were contact inhibited and did not produce tumors in animals. The five surviving virus-infected cultures converted to wild, disoriented, non-contact inhibited hereroploid fibroblasts interdispersed with multinucleated giant cells and produced tumors in mice. It was concluded that the B-tropic virus enchances malignant transformation of BALB/c embryo cells. The possible tumorigenicity of the heteroploid cell lines at high passage and the possible implications of a sarcoma genome are under study.

(2) Two AKR negative clones obtained from Dr. Hartley consistently grew as contact inhibited fibroblasts passage after passage; did not produce transformed foci and did not produce virus when kept as log phase cultures, but could be activated to produce infectious virus after BrdU treatment. However, if untreated cultures were allowed to stand after becoming confluent, macroscopically visible areas of morphological alteration appeared. These foci, consisting of non-contact inhibited, criss-cross fibroblasts, had a higher plating efficiency than the surrounding cells which were considered normal. The transformed cells produced transplantable tumors in AKR mice; the normal cells did not. All cell lines were heteroploid. The transformed cells often produced murine gs-1 antigen and infectious virus. Seventeen transformed and 14 normal colonies picked from cultures maintained in a confluent contact inhibited state were subsequently kept in log phase with frequent subdivision. After 24 doublings, three transformed and one normal colony became gs positive and two transformed and one nontransformed cell line contained infectious virus by XC test. Thus, it would seem that activation of virus is neither a necessary prerequisite nor an absolute result of transformation. The triggering mechanism is being studied.

Significance to Biomedical Research and the Program of the Institute: This contract is a core part of a coordinated, comprehensive, targeted research program to define the role of the type C RNA genome as determinant of oncogenesis, to develop diagnostic systems for screening carcinogens, and most importantly to continue studies with vaccines, interferon, and other potential candidates for cancer prevention and control. This program has contributed profoundly to the development of new approaches and concepts, which in turn have provided new insights into the nature of the oncogene, and mechanisms of viral and chemical carcinogenesis. Specifically, the in vitro tissue culture systems for assaying environmental carcinogens make possible large scale, rapid screening of environmental compounds, drugs, food additives, etc., at a fraction of the cost of equivalent tests in vivo. In view of the screening burden posed by literally thousands of new compounds each year, the newly developed tissue culture systems represent the only feasible hope of recognizing possibly dangerous carcinogens before the public has been harmed.

The development of viral vaccines capable of immunizing against chemical carcinogenesis, and optimistic results with mouse interferon against chemically induced tumors in animals, have important implications for cancer prevention and control in the near future. The initial tests have

established that vaccines which confer greatest protection are those derived from "natural" type C viruses, leading to the conclusion that immunity is antigen-specific. These results are particularly encouraging with the advent of new methods for inducing human type C viruses. The finding of type C viruses in human placentas has added considerable impetus to the search for other human type C virus candidates.

Proposed Course: Work will be continued on (1) the development, evaluation and standardization of *in vivo* and *in vitro* systems for studying the effects of known and suspected environmental carcinogens; (2) development and application of sensitive *in vitro* assay systems for studying the natural history of type C tumor virus infection and its relationship to carcinogenesis in a variety of mammalian systems with major emphasis on the isolation and characterization of type C viruses from human material; (3) development and testing of type C virus vaccines and interferon to evaluate their efficacy in prevention of spontaneous and chemically-induced tumors in genetically defined mouse strains; and (4) electron microscopic studies, serological studies and reverse transcriptase assays in support of the above and related projects within the VCP.

Date Contract Initiated: February 1, 1970

MICROBIOLOGICAL ASSOCIATES, INC. (NO1-CP4-3254)

Title: Studies of Type C RNA Tumor Viruses

Contractor's Project Directors: Dr. Damodar Deshmukh
Dr. Nirmal Mishra

Project Officer (NCI): Dr. Padman Sarma

Objectives: This project is concerned with basic studies on the isolation and characterization of type C RNA tumor viruses from various avian and mammalian species, including man. A large portion of the effort is directed towards the development and use of sensitive *in vitro* systems for studying the prevalence and behavior of overt as well as covert type C viruses. Various approaches are being intensively applied to animal and human tumor systems having covert ("switched off") viral genomes in efforts to "turn on" or rescue overt expressions of the viral genomes.

Major Findings: (1) Studies on the prevalence of endogenous type C virus RD 114 in cats: Previous reports from this laboratory discussed the induction and isolation of a virus identical with RD 114 from virus-free sublines of an established cat kidney cell culture (Crandell). In more recent studies, evidence was obtained to suggest that the RD 114 viral genome is widespread in apparently normal cats. Thus, virus-free cultures of diverse cat cells derived from normal or tumor tissues contained convert RD 114-like viruses inducible with 5-iododeoxyuridine (IdU). Infectious virus was isolated from each of 16 cat cell cultures derived from nine cats,

which included six cultures of diverse, normal embryonic tissues from six cats, cultures of tumor (osteosarcoma) of two adult cats, one subline CRFK of Crandell cat cell line and seven single cell clones prepared from subline C-C of the same Crandell cat cell line. It was found that RD 114 virus occurs in an infectious form in certain fetal cat tissues. Thus, RD 114 virus as well as feline leukemia virus (FeLV) were recovered from the spleen and bone marrow of cat XC 114B through which McAllister et. al. passed RD cells immediately prior to the original discovery of the RD 114 virus. RD 114-like virus was also isolated from a thymus of a cat fetus. In addition, cat lymphosarcoma specimens examined contained FeLV, but none of these specimens contained infectious RD 114. These studies provide conclusive evidence on the original mode of infection of RD cells with RD-114 and suggest the widespread prevalence of an inducible RD 114 virus genome in the cat population.

(2) A survey of cats and humans for prevalence of feline leukemia-sarcoma virus neutralizing serum antibodies: Sera of adult domestic cats and humans (veterinarians and laboratory workers) were surveyed for the presence of virus neutralizing antibodies against feline leukemia-sarcoma viruses of subgroups A, B and C. A focus neutralization test was used based on the neutralization of feline cell transforming effects of approximately 100 focus forming units of feline leukemia pseudotypes of Harvey strain of murine sarcoma virus (Harvey strain of murine sarcoma virus with the viral envelopes of the described serotypes of feline leukemia virus). Virus neutralizing envelope antibodies against one or more envelope antigenic types were found in the sera of 13 of 59 (22%) cats without neoplasia and in 9 of 38 (23.7%) cats with neoplastic disease but not in the sera of 36 veterinarians or in 33 laboratory personnel working in two laboratories engaged in feline leukemia virus research. These findings on the prevalence of virus neutralizing antibodies against feline type C viruses confirms the suspected widespread distribution of these viruses in domestic cats, evidenced by the regular occurrence of lymphosarcoma and other neoplastic diseases in a proportion of cats and the observations of the prevalence of demonstrable levels of the group-specific antigen of FeLV in a proportion of apparently normal adult and fetal cat tissues (Sarma, P.S., Gardner, M., Parks, W.P., and Huebner, R.J., unpublished observations). Also, it was found that cats seldom contain serum antibodies against the gs-1 antigen of FeLV, presumably as a consequence of immunological tolerance to this antigen resulting from prenatal exposure to this antigen. Thus, these studies suggest that cats with neoplasia as well as cats without discernible neoplastic disease are capable of responding immunologically to the viral envelope antigens of FeLV and is in agreement with similar findings made in other laboratories.

(3) *In vitro* host range of feline leukemia virus: Viral envelope antigens play an important role in permitting avian leukosis and sarcoma to infect cells of avian and certain mammalian species. Cellular susceptibility or resistance to the avian viruses is a genetically determined property of the avian cells and is believed to be governed by the presence or absence of cell receptor sites which permit the entry of a particular antigenic type of virus. It was found that cat cells from diverse sources are uniformly susceptible to the three known antigenic types of feline leukemia and

sarcoma viruses. However, it was found that cells of other mammalian species show a differential pattern of susceptibility or resistance to the three antigenic types of feline leukemia viruses. Results of three consecutive experiments gave similar results. FeLV of subgroup A showed the narrowest host range; virus derived from infected feline cultures established productive infection of feline and canine cells. MAH (Sarma) strain of FeLV derived from a naturally occurring feline lymphoma after serial propagation in vitro in FEF culture failed to infect human cultures. However, FeLV present in the original MAH cat tumor was able to infect human cell line RD. FeLV of subgroup B showed the widest host range. The virus was able to establish productive infection of a wide variety of mammalian cells including human, monkey, canine, bovine, porcine, and hamster cells. The subgroup C virus showed an intermediate host range. The virus caused productive infection of feline, canine and human cells. In addition, this virus was able to infect guinea pig cells which were nonsusceptible to both A and B subgroup viruses. Heterologous host cells productively infected with FeLV continuously released infectious FeLV capable of establishing productive infection of homologous host cells (FEF). The virus reisolated in FEF cultures was identical with the original FeLV by viral interference tests. The dog cells were not highly susceptible to infection with the A and C subgroup viruses as revealed by the fact that gs-1 antigens of FeLV were only detectable by CF test 6 weeks after virus infection. Preliminary asaays of subgroup B FeLV in human embryo, vero, bovine embryo, porcine embryo, and feline embryo cultures and subgroup C FeLV in guinea pig embryo and feline embryo cultures have shown that the heterologous cultures are as susceptible as homologous feline embryo cultures to productive infection with these strains of FeLV. In other studies it was shown that the selective resistance of human, pig and bovine cells to the A subgroup virus can be used to practical advantage for the "purification" of the B component of virus mixtures of A and B viruses including the cell transforming feline sarcoma viruses. Hardy, Jarrett and their associates found that FeLV has a capacity to undergo horizontal transmission to uninfected cats under natural and experimental conditions. The current studies, and the recent similar studies of Jarrett on A and B and C subgroup FeLV in human and dog cells, establish the important role of viral envelope antigens of feline leukemia and sarcoma viruses in permitting or restricting infection across species barrier. Possible implications of these findings in natural spread of virus to other mammalian species remains to be verified.

(4) Studies on the etiologic agent of naturally occurring turkey leukosis: In collaborative studies with Dr. Prem Paul and Dr. Benjamin Pomeroy of the University of Minnesota, it was shown that naturally occurring turkey leukeosis (two outbreaks) can be experimentally transmitted to turkey poults with cell free filtrates of turkey tumors. The inoculated turkeys develop reticuloendotheliosis within 20 days and eventually succumb to the disease. A type C virus was isolated from turkey, chick and duck cell cultures. The virus is not a member of the avian leukosis group but does contain RNA-dependent DNA polymerase. In preliminary studies, it was possible to transmit the disease to turkey poults utilizing virus derived from clarified culture fluids of infected cultures. These studies suggest that the type C viruses isolated from two turkey leukeois outbreaks may be closely related to the reticuloendotheliosis virus (RE virus, T virus) originally isolated

by Twiehaus from a turkey with leukosis-like lesions. In addition, these studies demonstrate, for the first time, the prevalence and etiological role played by the reticuloendotheliosis or turkeys.

Significance to Biomedical Research and the Program of the Institute: It is becoming increasingly apparent that many vertebrates, including man, carry information in their genomes for complete RNA tumor virus expression, including oncogenic potential. These studies provide further information on the natural occurrence and spread of oncogenic type C viruses in homologous and heterologous species, including man.

Proposed Course: (1) Demonstration, isolation and characterization of endogenous type C viruses of rat, cat, turkeys, and man will be continued; (2) the natural history of turkey reticuloendotheliosis virus will be studied; and (3) attempt to isolate and characterize bovine type C virus.

Date Contract Initiated: October 23, 1973

MICROBIOLOGICAL ASSOCIATES, INC. (NO1-CP3-3248)

Title: Immunoprevention of Spontaneously Occurring Neoplasms

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

Project Officer (NCI): Dr. Gary J. Kelloff

Objectives: The primary objective is to study immunoprevention of spontaneously occurring tumors in laboratory mice by the use of viral and cellular vaccines. The contractor has extensively studied the parameters involved in spontaneous neoplasia, including the incidence, progression and histological types of neoplasms in several strains of mice. Additionally, the relationship of the occurrence of the neoplastic state to the host's indigenous type C virus expression has been well defined. The important parameter remaining, the host's cellular and humoral immune response to its neoplasms and type C virus, has been under study in the past contract year. The additional methodologies required to pursue these objectives have been developed. These include a footpad assay for measurement of delayed type hypersensitivity in the mouse, techniques for lymphocyte culture and the development of a lymphoblast transformation assay for type C viruses and tumor cells.

Major Findings: Immunoprevention studies to date have included a determination of the optimal means of virus production and characterization and the optimal fixation and storage of viruses. These preparations have been evaluated by the XC test, protein determinations, CF titer, polymerase assay, ability to induce neutralizing antibodies and most importantly SPAT assay to rule out residual infectivity in the vaccine. These studies are completed and have led to a standardization of the vaccine preparation so that each vaccine lot can be adequately evaluated. The optimal dose,

route and schedule of immunization of the BALB/cCR mouse with banded type C virus has been evaluated by the host's response as measured by the footpad assay, the lymphoblast transformation assay, by neutralization tests, and ultimately by the host's ability to resist challenge by live murine leukemia viruses. The sensitivity and specificity of the footpad assay and the lymphoblast transformation assay have been worked out. Both assays can distinguish type C viruses varying in the envelope sub-type (e.g., RLV and AKR-LV) and show no reactivity with the host cells that the viral immunogens have been grown in. The development of these immunological tests will permit the contractor to more satisfactorily design and monitor experiments whose ultimate objectives will be the prevention of spontaneous neoplasms. Shorter term experiments involving viral vaccine immunization and challenge with exogenous virus or tumor cell lines carrying virus or tumor transplant lines are in progress. These experiments will also provide important information in designing experiments to prevent spontaneous neoplasms. The tumor dose kinetics of a highly malignant transformed BALB/cCR cell line containing B/C-LV has been determined, and this line will be used to challenge B/C mice immunized with AKR-LV vaccine. This cell line has been used to prepare cellular vaccines by both formalin fixation and by X-ray inactivation. These cellular vaccines have been shown to confer immunity against challenge with homologous live tumor cells, and it appears that preparations of these cell vaccines can be used in the footpad and the lymphoblast transformation assays to establish the immune status of these animals. Primary and secondary transplants of spontaneous tumors of various histologic types (e.g., sarcomas, carcinomas, leukemia, and Hodgkin's disease) are being prepared for use as cellular vaccines to determine the importance of their histologic specificity as well as the importance of their endogenous type C virus expression.

Significance to Biomedical Research and the Program of the Institute: This contract has contributed significantly to (1) experimental findings from which the oncogene hypothesis was formulated, (2) demonstrating the prevalence of the type C viral genome during normal embryogenesis, and (3) to an understanding of the endogenous type C virus relationship with its host and its genetic and epigenetic relationship to the host's naturally occurring neoplasm. The current studies on immunoprevention of spontaneously occurring tumors are designed to answer the question of whether neoplastic disease can be prevented or treated by classical virological vaccine or cellular vaccine techniques.

Proposed Course: (1) Completion of studies designed to develop usable techniques for monitoring host cellular immunity including the footpad assay, the lymphoblast transformation assay, and a cell-mediated and humoral cytotoxicity assay. (2) Immunoprevention studies with viral vaccines will include challenge with exogenous leukemia viruses; challenge with tumor cell and tumor transplant lines, and holding animals for prevention of spontaneous tumors. (3) Immunoprevention studies with cellular vaccines will include challenge with exogenous leukemia viruses; challenge with tumor cell and tumor transplant lines and holding animals for prevention of spontaneous tumors. The importance of the immunogenicity of type C virus in cellular vaccines will be examined.

Date Contract Initiated: November 15, 1961

ST. LOUIS UNIVERSITY (NO1-CP4-3359)

Title: Search for Viral-Specific Genetic Material in Human Cancers and Studies on the Mechanism of Oncogenesis by RNA and DNA Tumor Viruses

Contractor's Project Director: Dr. Maurice Green

Project Officer (NCI): Dr. Robert J. Huebner

Objectives: This research program is aimed at understanding in detail the mechanism of cell transformation by RNA and DNA tumor viruses, applying new information on viral carcinogenesis and on the molecular biology of human cells directly to the problems of human cancer, and searching for inhibitors of polymerase that may control the expression of cancer specific genetic information.

Major Findings: Poly 2'-O-methylcytidylate-oligodeoxyguanylate is a highly sensitive and specific template for the detection of RNA-directed DNA polymerase in RNA tumor virus particles of avian, murine, feline, and primate origin and transformed murine cells in which four distinct DNA polymerases have been detected, as shown by studies designed to evaluate the potential of poly(2-O-methylribonucleotides) as templates and inhibitors. Based on these findings, we are now developing the methodology to detect the possible presence of RNA-directed DNA polymerase in human neoplastic tissues.

The single (α) and two subunit (αβ) forms of RNA-directed DNA polymerase from AMV have RNase H activity with different modes of action. Both enzymes have exoribonuclease activity which degrades the RNA of an RNA-DNA hybrid in both the 5'→3' and 3'→5' directions, but αβ RNase H is a processive exoribonuclease which digests a polynucleotide chain to completion before attacking a second chain, while α RNase H is a random exoribonuclease which releases the polynucleotide substrate after each chain scission.

The polyhomoadenylate and adjacent nucleotides at the 3'-terminus of 30-40S RNA in the genome of MSV(MLV). Adenosine is the major 3'-OH terminal nucleoside of the 60-70S RNA genome of murine sarcoma-leukemia virus [MSV(MLV)], its 30-40S RNA subunits, and the poly(A) segments derived from 60-70S or 30-40S RNA by RNase A + T_1 treatment. The 3'-terminal nucleotide sequence of the 30-40S RNA subunits is $-G(C,U)A_{100}$-OH.

Free and membrane bound polyribosomes of the MSV(MLV)-producing, transformed rat cell line, 78A1, contain virus-specific 35S RNA while membrane-bound polyribosomes contain, in addition, virus-specific 20S RNA. Nonvirus-producing MSV transformed mouse cells contain a single virus-specific RNA species sedimenting at 26-27S which could be the product of a restricted

transcription of the integrated viral genome or could represent a smaller subunit specific for the MSV genome. The protein product of in vitro translation by preloaded free and membrane-bound polyribosomes was partially characterized.

The induction of virus in MSV transformed, nonproducer cells was optimized with regard to time and treatment with halogenated pyrimidine derivatives. The activation of the MSV genome following infection with MLV was followed by measurements of cell agglutination, and interpreted in terms of an independent effect of specific sarcoma virus genes on the cell surface.

The early events during the rapid transformation of murine cells by MSV was analyzed. By hybridization, parental RNA was detected on polyribosomes at 2 hours after infection; by inhibition studies, a requirement for protein synthesis was demonstrated at 2-4 hours for maximal virus replication; by autoradiography and biochemical analysis, the synthesis of virus-specific DNA was detected in the cytoplasm at 3-4 hours post-infection; by cytological hybridization, virus-specific DNA was detected in the nucleus and on chromosomes by 5 hours after infection.

Rifamycin SV derivative with 3-substituted side-chains are specific inhibitors of nucleic acid polymerases, and are inactive against a variety of non-polymerizing enzymes. The interaction of purified RNA-directed DNA polymerase of AMV and MSV(MLV) with 2,5-dimethyl-4-N-benzyl demethyl rifampicin (AF/ABDMP) was studied in detail. The drug interacts with DNA polymerase, and not on RNA or DNA template, and is a non-competitive inhibitor of the enzyme with respect to template and deoxyribonucleoside triphosphate substrates. The mechanism of inhibition may involve cooperative binding to a hydrophobic site(s) on the polymerase molecule that influences the initiation of DNA synthesis. A series of polycyclic derivatives were examined with regard to the effect of the side chain on DNA polymerase inhibitory activity. These derivatives bind with very high affinity to natural and synthetic polynucleotides and in this way block the template activity of DNA polymerase. Inhibition of purified AMV DNA polymerase by several 2'-O-methyl and 2'-O-ethyl polyribonucleotides, was demonstrated at low polymer concentration. These compounds apparently compete with the template for binding of the active site on the enzyme and are potent inhibitors of DNA polymerase activity.

Adenovirus replication and cell transformation. Adenovirus DNA sequences were detected by cytological hybridization in cells transformed by adenovirus types 2, 7, and 12, members of three human adenovirus families. By new mathematical treatment of data from reassociation kinetic analysis, it was determined that multiple copies (over 100) of a segment of the viral genome was present in cells transformed by adenovirus 7. By hybridization with radioactive L and H strands of adenovirus DNA, the fraction of the viral genome integrated was measured and the mode of transcription regulation was analyzed in transformed cells and early and late after productive infection. By the use of separated strands, the individual early mRNA molecules were isolated in highly purified form from adenovirus 2 infected cells for use in defining the early functions of the viral genome involved

in cell transformation and growth control. A nuclear membrane complex which released adenovirus-specific RNA and has poly(A) polymerase activity has been studied.

Analysis of human cancer and normal tissue for RNA and DNA tumor virus-specific nucleotide sequences. RNA from normal and neoplastic human tissues was analyzed for RNA sequences specific for RD 114 virus and MSV(MLV) with non-definitive results. DNA from normal and neoplastic human tissues are being analyzed for sequences specific for the simian sarcoma virus. Based on our detection of viral RNA and DNA sequences in herpesvirus 1 and 2 transformed cells, and the ease of detection of single copies of a portion of the viral genome in adenovirus transformed cells, we have developed procedures utilizing (1) Berg's nick translation method, and (2) the use of separated viral DNA strands which can unequivocally detect a fraction of a copy of the viral genome in human cells. These methods are presently being applied to the analysis of normal and neoplastic human tissue.

Significance to Biomedical Research and the Program of the Institute: Within the 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 (d) verification of oncogenicity for man. The basic research on the molecular biology of normal and virus infected cells may provide the basis for understanding the mechanism of animal virus infection and carcinogenesis, and for developing a rational chemotherapy for viral diseases and cancer.

Proposed Course: The following investigations are planned: (1) The analysis of human cancers for DNA sequences specific for RNA tumor viruses and for RNA-directed DNA polymerase activity; (2) analysis of human tumors for adenovirus - and herpesvirus - specific DNA sequences; (3) properties of purified RNA-directed DNA polymerase of RNA tumor viruses and their associated RNase H activity; (4) properties and synthesis of virion and intracellular viral RNA and nascent DNA of oncornaviruses; (5) mechanism of virus replication and cell transformation by RNA tumor viruses; (6) synthesis of RNA tumor virus-specific proteins and their regulation - *in vivo* and *in vitro* studies; (7) inhibitors of viral and cell DNA and RNA polymerase molecules - an approach to the chemotherapy of viral diseases and cancer; and (9) human adenoviruses - mechanism of virus replication and cell transformation.

Date Contract Initiated: March 20, 1967

SALK INSTITUTE (NO1-CP4-3243)

Title: Interactions Between RNA Tumor Viruses and Other Viral Agents

Contractor's Project Director: Dr. Walter Eckhart

Project Officer (NCI): Dr. Stuart A. Aaronson

Objectives: To determine whether infection with a DNA virus induces the appearance of RNA tumor virus RNA in infected cells.

Major Findings: Considerable variability in the amounts of RNA homologous to R-MLV in uninfected BALB/3T3 cell preparations was noted. Polyoma-infected BALB/3T3 do not produce RNA tumor viruses assayed by reverse transcriptase in the medium of infected cells. An endogenous type C virus from JLS-V9 cells (derived from a BALB mouse) was induced and a radioactive DNA probe to use in searching for RNA synthesis in polyoma-infected cells was prepared. We have infected two lines of rat cells with polyoma (F111 and F1706). One of the infected F111 cultures has undergone morphological transformation. The size classes of RNA present in JLS-V9 cells was examined by assaying for protection of the R-MLV probe. The 35S and 20S species were found but no 70S was observed.

Significance to Biomedical Research and the Program of the Institute: The expression of genes (viral or cellular) causes an alteration of cell growth. If it is possible to understand the factors that govern expression of RNA virus genes in the mouse system (where the molecules involved can be identified), much of that information should be applicable to human cells. Thus, these studies serve as a model for the human situation.

Proposed Course: (1) To characterize the type C/viral RNA in uninfected and polyoma-infected 3T3 cells to see whether size changes occur after polyoma infection.

Date Contract Initiated: June 5, 1967

SCRIPPS CLINIC AND RESEARCH FOUNDATION (NO1-CP4-3375)

Title: Immunologic Study of RNA Tumor (Type C) Viruses

Contractor's Project Director: Dr. Frank J. Dixon

Project Officer (NCI): Dr. Robert J. Huebner

Objectives: This project offers a multidisciplinary study of oncorna viruses, with special emphasis on the situation in the New Zealand (NZ) mouse, and the nature of their interaction with their host which may lead to the formation of tumors or to the development of immunologic diseases, or both. The disciplines involved are virology, molecular biology, biochemistry and immunopathology. The immunologic emphasis in this work relating both to the immunopathologic consequences of oncorna virus infection and to the use of immunologic markers of viral presence are perhaps a unique aspect of this proposal. A better understanding of the

immunologic consequences of oncorna virus infection is needed if we are to understand viral oncogenesis and host defense mechanisms, and to achieve effective therapeutic and/or prophylactic immunotherapy, and also to avoid the possible immunopathologic complications of such therapy.

Numerous reports have identified immune responses to many persistent viruses, including the oncorna viruses, with associated immunological diseases of varying severity. The manifestations of these immune responses; i,e, antibodies and sensitized cells, can serve as indicators of the presence of such infections and, in the case of oncorna viruses, provides one of the best and at times the only evidence of their existence. The fact that antibody responses to oncorna viruses may be associated with particular immunologic diseases, such as immune complex nephritis, prompts a search for oncorna viruses in patients with these conditions with particular emphasis on placentas from lupus patients. In addition, there is strong evidence linking the oncorna viruses to human immunologic disease and suggesting that nuclear antigens may be present in a particularly immunogenic form as a result of oncorna virus infection. Thus, the pathologic package offered by oncorna virus infection may include tumors for the immunologically unresponsive host and immunologic disease for the most responsive, with appropriate combinations for those in between.

The approaches taken are designed to determine how the virus acts to initiate either tumors or self destructive immune responses, and what factors in the host determine the pathological outcome of such an infection, with the objective of developing methods of preventing or interrupting the infectious process and its consequences.

Major Findings: Effects of neonatal infection of mice and rats with SLV: A major focus of the program is to determine the oncogenic and autoimmunogenic potential of SLV, the oncorna virus isolated from NZB mouse lymphoblasts. SLV was inoculated into 16 strains of mice, selected to include both B and N-tropic types and a variety of H-2 types, so that the influence of genetic factors in the propagation of virus and causation of disease could be observed. The mice were monitored monthly for serum gs levels and anti-nuclear antibodies (ANA), and examined pathologically at autopsy. Results to date revealed an interesting discrepancy between the two presumed manifestations of autoimmunity--ANA and glomerulonephritis; i.e., the C57BL/6J (low leukemic strain) have a 100% incidence of ANA with no glomerulonephritis, while the C57Br (high? leukemic strain) have a high incidence of glomerulonephritis and low ANA. The specificity of the ANA in several strains may well give a clue as to how oncornavirus infections initiate ANA, and the nature of antibodies in glomeruli of glomerulonephritic animals without ANA should also aid in understanding how these viruses may operate as autoimmunogens. Studies have just been initiated in nude mice infected with SLV to determine the role of the thymus in the pathologic events associated with this infection. To date these studies suggest that the SLV oncorna virus isolated from the NZB mice is highly oncogenic in all murine strains except NZBxW, NZWxB and C57BL/6. However, the way in which the NZ hybrids and the C57BL/6 handle SLV appears to be quite different since in the former there is little

elevation of serum gs, while in the latter there is a marked elevation. While all three strains develop ANA when infected with SLV, only the NZ hybrids also develop glomerulonephritis within the first 6 months of life. Thus, it seems possible that in developing a means of controlling the oncogenic effect of SLV, the NZ hybrids develop immunologic disease. These studies are continuing in mice, as well as in two strains of rats. To date the SLV has caused lymphoma in the rat, but has not as yet induced either ANA formation or glomerulonephritis.

Vaccine studies: Immunization of NZBxW female mice with fomalinized SLV vaccine either produced no effect on the incidence of glomerulonephritis or appeared to accelerate the disease. However, since the SLV is probably not the xenotropic or natural type C RNA virus of the mouse, this probably represents an increase in host response to enhanced infection. It points up the absolute necessity of (1) making vaccines to the precise virus(es) involved in oncogenesis and (2) the necessity to work out vaccine protocols in well-defined systems.

Isolation and characterization of an oncorna virus surface glycoprotein: This group has successfully isolated and purified the major glycoprotein which appears on lymphocytes transformed by oncorna viruses. Similar results were also recently reported by Dr. T. August. In both laboratories, the results indicate that this protein is one of the major targets of neutralizing antibody and as such is ideal to illustrate the relationship between vertically inherited viral genes and naturally occurring late life diseases. This protein is available not only on the surface of the cell, but also on virion surfaces, a fact which is important for an understanding of the relationship between the host immune response and oncogenesis. Thus, one factor which must determine the fate of the tumor in the host is the balance between the mass of antibodies and the mass of tumor cells. Since the major neutralizing antigen is present on two replicating structures, cells and virions, the mass of virus produced could compete for cytotoxic antibody (neutralizing antibody is cytotoxic for transformed cells), and thus may be an important factor in the host-tumor balance even if newly synthesized virus fails to transform and thus recruit additional cells. This work is important because of the many phenotypic expressions of inherited viral genes; those which result in alterations of the cell surface are among the most important for the following reasons: (a) altered or unique cell surface polypeptides are potential targets for the host's immune defenses, (b) changes in the cell surface may indicate that an oncogenic viral genome is present whether or not it is completely expressed, and (c) changes in the macromolecular composition of the cell surface may lead to "asocial" behavior of transformed cells.

Taxonomy of SLV: SLV was compared in cross neutralization tests to AKR and Moloney viruses and was found to be distinguished from both, but much closer to the Moloney. Studies on genotypic relationship of SLV to other murine leukemia viruses are well underway and will be reported on in more detail at a later date.

Somatotrophic variation of the molecular properties of oncorna viruses: In prokaryotic systems it has been known for some time that the phenotype of a virus can in a number of ways be controlled by the genotype of the host cell. In eukaryotic systems there is growing evidence that, at least as far as leukemogenesis is concerned, a similar phenomenon may be important. The evidence indicates that cells of thymic origin may play a key role. The Scripps group has a number of continuously growing murine thymocytes which produced a large amount of virus and thus were able to propagate the virus on fibroblasts derived from a number of mouse strains. They are utilizing these to study the molecular differences between "thymocyte derived" and fibroblast derived" viruses which might account for their difference in leukemogenicity. They found a dramatic difference in the molecular properties of viruses derived from thymocytes or passaged on fibroblasts. In summary, their findings indicated that, by the standard criteria used by virologists, their isolate of SLV from thymocytes would be considered to contain "defective" virions; whereas, after passage on fibroblasts it would be considered to contain almost exclusively effective virions. Furthermore, upon dilution below the fibroblast infectivity dose, SLV derived from thymocytes was highly oncogenic in vivo. It would thus appear that the most threatening virus from the point of view of oncogenesis is the defective agent since it may possess sufficient information to code for polypeptide(s) which render host cells oncogenic, but may lack that portion of the genome which codes for surface antigens, etc., which allow the host defenses to recognize cells as neoplastic. These concepts may directly bear upon where one should search for "candidate" human tumor viruses.

Lack of viral expression in spontaneous tumors of $NZBxWF_1$ and NZB mice: In contrast to the lymphoblastoid cells established from the spleens of the NZ mice, spontaneous tumors do not produce infectious virus, nor is virus inducible by IdU or BrdU. It is now of extreme importance to determine why these tumors lack the complete expression of the viral genome and because of the work done during the last year aimed at developing representative "probes" it will be possible to determine by reannealing kinetics if the "producer" lymphocytes and "nonproducer" tumors have the same or similar numbers of oncorna virus genome equivalents or if the tumors lack a full gene complement.

Biophysical properties of plasma membrane associated DNA: By reannealing kinetics and a number of other physical properties, this group was able to show that cytoplasmic membrane associated DNA is a unique species and clearly is not due to contamination of cells with either microplasma or viruses. Furthermore, a cytoplasmic membrane associated DNA could represent the potential genetic information for as many as 100,000 diverse genes of 1,000 nucleotide pairs each. In addition to its genetic potential and its possible role in the origin of diversity in immunoglobulin producing cells, the physical location of cytoplasmic membrane associated DNA suggested the possibility that it might be incorporated into any virion which would bud from the plasma membrane. This is in fact the case; this group has shown that at least some oncorna viruses pick up cytoplasmic membrane associated DNA during the process of budding. These results were confirmed by showing that another RNA containing virus, vesicular stomatitis,

also picked up cytoplasmic membrane associated DNA during the maturation process. Suprisingly, the DNA is present in the core of both the "B" and "T" virion particles. It is not known as yet whether this "passenger" DNA has biological significance, but this raises the possibility for rearrangement of genetic information via either vertically or horizontally transmitted viruses because of their passive pick up of genes from a genetic compartment with an analytical complexity of at least 100,000 genes. They are also exploring the possibility of generalized transduction.

Immunoelectron microscopic study of the structure of nucleic acids: One of the early aims of the contract was to determine if antibodies to a variety of nucleic acids could be useful for isolation and/or determination of the structure of nucleic acids. This group had earlier found that these antibodies were very useful in studying the fate of a variety of nucleic acids during the cell cycle of eukaryotic cells. Taking advantage of the high specificities of these antinucleic acid antibodies and different nucleases and the resolution of the electron microscope, this group developed a highly sensitive method for probing the "ultrastructure" of nucleic acids. The method has a high degree of resolution (approximately 25 nucleotides) and may, in combination with specific nucleases, allow them to probe very small regions of nucleic acid molecules.

Quantitation and isolation of plasma membrane associated immunoglobulin: Plasma membrane associated immunoglobulin both gives the specificity to the immune process and is involved in the early stages of activation of lymphocytes. Before being able to determine if these key molecules are altered in amount and/or structure in immunodeficiency states and/or cancer, it was necessary to determine their nature on normal lymphocytes. During the past contract year, this group has continued to refine the methods for quantitation of the number of plasma membrane associated immunoglobulin molecules per cell and has succeeded in isolating and characterizing the structure of these molecules. The results, since confirmed in other laboratories, suggest that plasma membrane associated immunoglobulin may have a unique structure which may be important for its role as a receptor molecule. These studies provide an excellent framework against which to determine whether the amount or structure of plasma membrane associated immunoglobulin is altered in the neoplastic state.

Fusion of lymphocytes by feline leukemia virus: One difficulty in utilizing the technique of cell fusion in the study of immunologic problems has been the fact that lymphocyte-lymphocyte heterokaryons are not easily formed with the usual methods to fuse cells. During the past contract year, this group conducted a survey of a number of viral agents and found that some of the feline oncorna viruses, both FeLV and RD 114, caused lymphocytes to fuse within 1.5 to 3 hours. In addition to overcoming a technical hurdle, there were several implications of this study. It is now known that FeLV can be transferred horizontally, at least among cats; and therefore, any effect on human cells may be of importance. In addition, they were able to show that expression of the endogenous EB virus genome was induced in the human cells fused by FeLV. Thus, although it has been shown by Old, et. al, that FeLV was not detected in the cells of pet owners, the possibility that FeLV might play a role in human disease by activating an endogenous viral genome should not be dismissed.

Search for type C viruses in humans: Type C viruses were reported recently in the syncytial trophoblasts of primates and humans. Because of the possible role of similar viruses in the etiology and pathogenesis of systemic lupus erythematosus (SLE), this group decided to attempt to isolate a type C virus from the placentas of patients with SLE. To date four placentas have been obtained, and numerous particles easily seen in the electron microscope in the one specimen examined to date. These materials are being fixed for histology and electron microscopy and the trophoblasts are being co-cultivated with a variety of tissues. These materials will be studied intensively with the objective of getting out a human type C virus capable of replication.

Significance to Biomedical Research and the Program of the Institute: This program is relevant to the goals of the National Cancer Institute in relation to (1) determination of cause, (2) early detection of risk, and eventual (3) effective prevention of cancer. The etiological association between type C RNA viruses and cancer has been firmly established in chickens, mice, hamsters, rats and cats and is strongly implicated in cows, gibbon apes, woolly monkeys and baboons as well. In addition, the particle has been seen in human placentas to a titer suggesting that it is universally present. Expression of this apparently universal oncogenic potential, however, is dependent on the immunologic responses of the host. Although these immunologic controls are well recognized, the mechanisms are poorly understood. Characterization of the viruses, viral antigens and host cell responses will provide important insights into the etiological role of the viruses under varying immunologic conditions, and the means of detecting and interfering with their oncogenic properties through the use of viral vaccines.

Proposed Course: The project will continue to focus on several interrelated aspects of the oncogenic and immunopathologic consequences of oncorna virus infection with particular emphasis on the situation in NZ mice. (1) The possible role of SLV, an oncorna virus(es) obtained from NZB lymphoblast cultures, in the genesis of spontaneous tumors and immunologic diseases of the NZ mice. (2) Using SLV as a model oncorna virus, attempts are being made to find if an how in vivo infection of a variety of murine and rat strains with this agent will cause tumors and immunologic diseases. (3) Isolate and characterize the glycoprotein surface antigens of oncorna viruses and use this antigen as one of the yardsticks in evaluating the host-virus relationship. (4) Analyze the number and nature of the viruses produced by continuous thymoblast lines derived from NZB and related mice. (5) Correlate a variety of virion coded or induced phenotypic markers of infected lymphoblasts with their oncogenic or immunologic potential in vivo. (6) Study the incorporation of exogenous nucleic acids into oncorna and other RNA viruses and attempt to determine whether this process may play a role in the induction of "autoimmune" responses following infection with their viruses. (7) Search for human type C viruses in the placentas of humans with lupus erythematosus.

Date Contract Initiated: June 29, 1972

SOUTHERN CALIFORNIA SCHOOL OF MEDICINE, UNIVERSITY OF and CHILDREN'S HOSPITAL OF LOS ANGELES (NO1-CP5-3500)

Title: A Comprehensive Field and Laboratory Research Program on the Etiology and Epidemiology of Human Cancer

Contractor's Project Directors: Dr. Murray B. Gardner (USC)
Dr. Robert M. McAllister (Children's Hosp.)

Project Officer (NCI): Dr. Robert J. Huebner

Objectives: To mount a multifaceted, highly interrelated program designed to determine the roles of viruses, physical and chemical carcinogens, and other factors in the etiology of human and animal cancer in a natural urban ecology. These studies are carried out at USC School of Medicine and at Children's Hospital of Los Angeles.

Viral studies: Human, pet and feral animal cancer and fetal materials are under intensive study for RNA tumor virus expression, utilizing all the modern in vitro as well as in vivo test systems. Extensive field studies and procurement efforts provide large numbers of tissues derived from cancer patients, genetically defective individuals, and spontaneous and therapeutic abortions. These materials are utilized for in vitro and in vivo biological studies and are subjected to serological, immunological, biochemical and electron micrographic analyses designed to detect, isolate and characterize the RNA viruses and virus-specific antigens associated with naturally occurring animal and human neoplasms.

Epidemiological studies: This program is providing, through hospital record surveys and community questionnaire surveys, up-to-date information of the natural occurrence of human cancer as it may be influenced by genetic, viral, environmental or other factors, including exposure to variable smog components in differing ecological areas of Los Angeles County, industrial and household carcinogens, and pets with and without cancer. Other factors such as occupation, aging, genetic defects, smoking, hormone therapy, and immunosuppressants are being studied using classical epidemiological methods combined with virological and serological surveillance.

Environmental studies: This program is concerned with monitoring focal environmental areas for levels of carcinogens and other air pollutants. Materials collected are characterized and supplied to laboratories at USC as well as to NCI and other VCP contract programs; e.g., Contract NO1-CP4-3240, for studies to determine the carcinogenic activities of such pollutants in tissue culture and in animals.

Major Findings: The Cancer Surveillance Program is a mechanism for rapid reporting of all cancer cases in Los Angeles County. Through the voluntary cooperation of all major hospitals (165 in number), approximately 22,000 incident cases annually are made available for collaborative epidemiologic, immunologic and virologic studies. Higher lung cancer mortality occurred in Caucasian males living in certain heavily industrialized areas of Los Angeles County. These areas were characterized by elevated levels of benzo(a)pyrene

and certain other polycyclic aromatic hydrocarbons of primarily industrial origin in the air and soil. The most likely explanation for these excess lung cancer deaths would be a long-standing synergistic action between smoking and neighborhood air pollution. A new class of carcinogenic compounds which are methanol soluble was isolated from particles in Los Angeles air. The transforming activity of this extract approaches that of 3-MC in rodent cell culture systems.

Geometric mean antibody titers to EBV were elevated in patients with three histologic subtypes of Hodgkin's disease and were normal in patients with the lymphocyte predominant subtype. Antibody titers to three other herpes viruses (varicella, CMV, herpes 1) were normal. These results suggest that EBV is not important in the etiology of at least the lymphocyte predominant form of Hodgkin's disease. Patients with Hodgkin's disease had an increased frequency of HL-A1. We have observed three clusters of Hodgkin's disease in Los Angeles County. In a collaborative case control study in two geographic areas (Los Angeles and New Orleans) no risk associated with prior tonsillectomy, appendectomy or infectious mononucleosis was found, although an increased risk for dexedrine users was found.

Epidemiologic surveys designed to obtain evidence of a milk transmitted human breast cancer virus showed no evidence of an increased risk of breast cancer associated with being breast fed, regardless of the age at diagnosis, and excess familial risk of breast cancer occurred equally in both maternal and paternal lines. A marked protective effect of hormones administered in menopause was found. Virus particles were not found by EM in milk specimens and there was no correlation between detection of reverse transcriptase activity in milk samples and family history of breast cancer. These results fail to support the possibility of a milk agent as an etiologic factor in human breast cancer.

Cell strains derived from childhood tumors have been characterized and tested for their capacity to form tumors in ATS-treated hamsters. Tumors that formed have not released hamster or other type C viruses. Attempts to induce or rescue type C virus in our established human sarcoma or lymphoma cell lines, in fresh human leukemia cells, in lymphocytes from patients with autoimmune disease and in human placentas have been negative. Type C virus-like particles, few in number, were seen by EM in 10 of 33 human placentas. An enzyme (DNA polymerase) substrate (possibly RNA) complex has been isolated from the cytoplasm of RD cells. Transfection of RD cells with DNA from RD 114 cells has probably been achieved.

Although wild mice from most trapping areas in Southern California were "switched-off" for type C virus expression and were resistant to spontaneous cancer until old age, young wild mice from two particular trapping areas were found to be unusually "switched-on" for type C virus activity and to be remarkably prone to spontaneous lymphoma and/or lower limb paralysis. Transmission and neutralization studies have established unequivocally the indigenous type C viruses as etiologic agents of both diseases. The lower limb paresis results primarily from a direct neurotropic effect of type C virus upon motor neurons in the lower spinal cord. In these two genetically susceptible colonies of wild mice, type C virus is spread epigenetically

via milk and transplacentally. Several of these viral isolates also show an unusually wide _in vitro_ host range, including mouse (N-tropic) and human cells. Virus expression is largely "switched-off" in the progeny of these mice bred with C57/BlSn mice as a result of introducing a dominant gene for repression of gs antigen production. Thus, type C RNA viruses must be considered as potential etiologic factors in humans as well as mice, not only for cancer and possibly immunologic disorders, but also for unexplained neurologic diseases.

Endogenous type C viruses were isolated from rats of five different strains, from established cell lines and secondary embryo cultures. Natural history studies of FeLV and RD 114 showed an excellent correlation between detection of FeLV and RD 114 gs antigen by CF in fetal and postnatal cat tissues and the detection of type C particles by EM and isolation _in vitro_ of the corresponding virus. The results confirm that RD 114 is an endogenous type C virus with a translational block in most cats to production of detectable gs antigen or infectious virus. FeLV behaves more as an exogenous virus, detectable in high prevalence in young cats with lymphoma or unexplained anemia, in moderate prevalence in cats with infectious peritonitis and in normal fetal cats, and in low prevalence in normal postnatal cats and in old cats with carcinoma and lymphoma. The oncogenicity of RD 114 remains undetermined.

Significance to Biomedical Research and the Program of the Institute: This program searches for causes of human, pet and other animal cancers in a natural ecology, utilizing (1) experimental animal systems; (2) basic viral and chemical carcinogenesis studies; and (3) epidemiological profiles of cancer incidence in Los Angeles Area humans and animals in relation to exposure to environmental carcinogens.

In addition, the program continues as a prime resource for supplying human and animal materials to the viral oncology in-house and VCP contract programs, particularly to Microbiological Associates (NO1-CP4-3240), Flow Laboratories, Inc. (NO1-CP3-3247), St. Louis University (NO1-CP7-0692), University of California, Naval Biomedical Research Laboratories NO1-CP3-3237), and the California State Department of Public Health (NO1-CP4-3209).

Proposed Course: Continuation of (1) efforts to rescue a human candidate type C virus and eventual development of vaccines for trials in cancer patients; (2) studies of the type C virus isolated from a feral house mouse which is responsible for naturally-occurring early lymphoma incidence and amyotrophic lateral sclerosis-like paralysis in the colony under study and in other mice as well under experimental conditions; (3) epidemiological studies of factors influencing cancer incidence and type; (4) characterization of air pollutants; and (5) procurement, growth and distribution of human and animal materials with the VCP.

Date Contract Initiated: June 26, 1968

SOUTHERN CALIFORNIA SCHOOL OF MEDICINE, UNIVERSITY OF (NO1-CP4-3242)

Title: Conditional Lethal Mutants of RNA Tumor Viruses

Contractor's Project Director: Dr. Peter K. Vogt

Project Officer (NCI): Dr. Gary J. Kelloff

Objectives: (1) To continue the isolation of temperature-sensitive mutants of avian sarcoma and leukosis viruses; the goal of this effort is to build a collection of about 300 well-characterized mutants of different avian tumor virus subgroups. Special emphasis is being placed on mutants which are affected in their transforming ability. (2) To characterize each newly isolated mutant with physiological and genetic techniques. (3) To determine the number of viral genes involved in transformation and identify the products of these viral genes.

Major Findings: The isolation of temperature-sensitive mutants of avian sarcoma viruses was continued. At present the contractor is concentrating on their collection of class T mutants (viruses which cannot induce nor maintain transformation at 41°). This collection is to be used primarily in complementation studies to see how many viral genes are involved in the transformation process.

The two early coordinate mutants ts335 and ts337 have been studied further. Unequivocal proof that they have a temperature-sensitive reverse transcriptase was obtained by purifying the mutant enzymes and studying their activities in vitro. Genetic revertants of ts335 and ts337 to wild type and to recombinants with wild type virus strongly indicate that temperature-sensitivity in replication and transformation: whenever the temperature-sensitive enzyme becomes wild type by recombination or genetic reversion, the replicative and transforming properties of the virus also assume wild type characteristics.

Several additional coordinate temperature-sensitive mutants have also been analyzed; i.e., viruses which neither transform nor replicate at 41°. Some of these mutants could be shown to carry at least two mutations, and the two mutations were separated by recombination experiments.

Significance to Biomedical Research and the Program of the Institute: The work on avian sarcoma virus ts mutant has provided and will provide detailed information on the function of individual viral genes, on their timetable of action and their location on the viral genome. Of particular value will be the characterization of viral transforming genes, their number, position on the genome and sequence of activity. Eventually, this work should lead to the identification and isolation of the primary gene products of transforming genes, the hypothetical transforming proteins. The characterization of such proteins will be a major step towards understanding viral carcinogenesis.

Proposed Course: Assuming 50 complementation groups in RNA tumor viruses, one would need about 200-300 mutants randomly distributed over the genetic

map to achieve the degree of map saturation required for a rough genetic definition of individual viral genes. The work, therefore, will proceed with the isolation of 200-300 mutants which will require 2 to 3 years. Physiological and genetic studies will be conducted in parallel with mutant isolation and can be expected to require 5 years.

Date Contract Initiated: October 15, 1971

STANFORD UNIVERSITY (NO1-CP4-3244)

Title: (A) Study of Human Tumor Cell Cultures; (B) Operation of a Central Mycoplasma Diagnostic Laboratory

Contractor's Project Directors: Dr. Leonard Hayflick

Project Officer (NCI): Dr. James T. Duff

Objectives: Part A is for studies on the cultivation and characterization of human tumor tissue, growth of human tumors in mice treated with anti-lymphocytic serum, and transformation of normal human cells by chemical carcinogens. Part B serves as a central diagnostic facility for the detection and identification of mycoplasma contamination in virus preparations, sera, cell cultures, and clinical materials submitted by other VCP contractors. Upon request, identification of isolates is made as to species; and mycoplasma antigens are distributed to those investigators requiring these materials.

Major Findings: Study of human tumor cell cultures: Using cytochalasin B and centrifugation, this group has succeeded in enucleating 100% of mass cultures of human and animal cells. Enucleated normal and cancer cells are now being hybridized to reciprocal whole cells to determine the control for malignancy and activation of endogenous oncogenic viral genomes. Malignancy is assessed by inoculating cells into antithymocyte treated and thymectomized mice. Several fundamental questions in cancer biology are being approached using this technique.

Studies on the transformation of cultured normal human cells to malignant cells with chemical carcinogens have continued and several chemical carcinogens have been tested. Only one, 4-nitroquinoline 1-oxide has shown activity in that WI-38 grown in the presence of this compound has apparently escaped from control on its finite lifetime. Tests for malignancy are now under way.

A method for detecting histocompatibility antigens on cultured human cells was developed. Direct cytotoxicity testing of SV40 transformed cells showed no loss or gain of HL-A specificities with the exception of cell strain JTM where controls were negative for HL-A5, but SV40 infected cells reacted positively to three different alloantisera. By quantitative micro-absorption, no loss of HL-A specificities was recorded. However, in

all cases, with the exception of a Down's strain, the SV40 infected cells became positive for HL-A5.

Operation of a central mycoplasma diagnostic laboratory: During a 5-1/2 month period, the contractor received and tested 899 samples from VCP contractors for mycoplasma. Eighty-three positive isolations were made. A new biochemical procedure for detecting microbial contamination of cell cultures was developed. Studies are in progress on a mycoplasma inhibitor found in cell cultures that upon dilution reveals the presence of mycoplasmas which are masked in undiluted cell culture fluids and therefore yield negative results.

Significance to Biomedical Research and the Program of the Institute: The mycoplasma diagnostic facility is a testing and monitoring service available to all VCP contractors and viral oncology intramural staff. Many of the most important viral specimens, cell cultures, sera, etc., used in the viral oncology program are sent to this facility for mycoplasma testing. Transformation of a human diploid cell line by chemicals could lead to a more direct testing of the oncogene theory, provide an *in vitro* assay system for testing the carcinogenic effect of chemicals on human cells, and could lead to a "switch-on" of a human tumor virus. The system of C57/L mice treated with anti-lymphocyte serum is being used to tell whether cells from neoplastic tissues, grown *in vitro*, are "tumor cells or fibroblasts."

Proposed Course: (1) Continuation of mycoplasma testing of samples received from other VCP laboratories. (2) Continuation of studies on (a) effects of various chemical carcinogens on normal human cells *in vitro*, alone and in combination with oncorna viruses; (b) human tumor growth in immunosuppressed mice; (c) histocompatibility expression of human cells transformed by oncogenic viruses; and (d) enucleation of human normal and cancer cells with Cytochalasin B.

Date Contract Initiated: June 19, 1969

WASHINGTON, UNIVERSITY OF (NO1-CP3-3372)

Title: Studies on Tumor-Specific Transplantation Antigens

Contractor's Project Directors: Dr. Karl Erik Hellström
Dr. Ingegerd Hellström

Project Officer (NCI): Dr. Robert J. Huebner

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

Major Findings: (1) Demonstration that sera from certain tumor patients can increase the specific cytotoxic effect on tumor cells of lymphocytes from the respective patients (and from other patients with the same tumor) and can often also bestow a tumor cell cytotoxic effect on lymphocytes from control donors.

(2) Demonstration that lymphocyte mediated cytotoxic reactions to Rous sarcoma cells in Japanese quails can be blocked by the tumor-bearers' serum, and an analysis of this blocking effect. Evidence was obtained indicating that antigen (in the absence of detectable antibody) can also block cytotoxicity and that antibodies can facilitate the formation of blocking complexes by releasing antigen from the tumor cells. The data are compatible with the hypothesis that the most efficient blockers are antigen-antibody complexes.

(3) A continued analysis of allograft tolerance in rats, which we feel provides a model to understand some aspects of tumor immunity. Evidence for a role of blocking factors in operationally defined allograft tolerance was obtained.

Significance to Biomedical Research and the Program of the Institute: This important and productive study of human and animal tumor antigens, as well as cell-mediated and humoral immune responses to the antigens, has already given, and promises to continue to give, insights to the understanding of the body's immune response to tumors, and ultimately may lead to immunotherapy of established tumors in man.

Proposed Course: Continued investigation of interaction between lymphocytes and serum factors in animals and patients with tumor and attempts to prevent and treat tumors by immunological means.

Date Contract Initiated: April 14, 1969

WEIZMANN INSTITUTE OF SCIENCE (NO1-CP4-3241)

Title: Study of Virus-Induced Tumor-Specific Transplantation Antigens

Contractor's Project Director: Dr. Leo Sachs

Project Officer (NCI): Dr. Robert J. Huebner

Objectives: To investigate the differences between the structure of the surface of normal cells and of cells transformed by viral and non-viral carcinogens by studying the differential binding of plant lectins.

Major Findings: Mobility of surface membrane lectin sites on normal and transformed cells: The results of these studies indicate that the carbohydrate-containing structures on the cells that bind Con. A are mobile. The experiments have provided direct evidence that in cells that are in

suspension *in vivo*, malignant transformation is associated with reduction in mobility of these sites and that in cells that form a solid tissue, malignant transformation is associated with an increase in mobility of these sites.

Viscosity of lipids in the surface membrane of normal lymphocytes and leukemic cells: The results of these studies have shown that the lymphoma cells which have a lower mobility of Con. A sites than the normal lymphocytes showed an increased fluidity of the surface membrane lipids. This increased fluidity of the lipids was also found in the cells of eight patients with chronic lymphatic leukemia compared to normal peripheral blood lymphocytes from eight normal humans. These results suggest that the amount of cholesterol present in the cell surface membrane may play a significant role in malignancy.

Membrane changes in the cell cycle: In order to determine possible differences between normal and transformed cells in the cell cycle, cells in interphase and mitosis were compared. It was found that transformed fibroblasts in interphase and normal fibroblasts in mitosis were agglutinated by Con. A and the lectin from wheat germ; whereas, normal fibroblasts in interphase and transformed fibroblasts in mitosis were not agglutinated by these lectins. These data suggest that there is a reversed cyclic change in the mobility of specific surface membrane sites in normal and transformed cells.

Surface membrane glycopeptides correlated with tumorigenesis: In order to determine other chemical differences between normal and malignant cells, the fucose-containing glycopeptides on the surface membrane of normal, transformed and tumor cells have been studied. In all cases, the gel filtration profiles of the fucose-containing glycopeptides were similar to those obtained from normal fibroblasts. In contrast, the profiles derived from the tumors formed after inoculation into animals of all of these cell lines showed the appearance of a specific group of glycopeptides which was not found in the original cells. These results indicate the existence of a correlation between surface membrane glycopeptides and tumor formation.

Significance to Biomedical Research and the Program of the Institute: Compounds that interact differentially with the surface membrane of normal and tumor cells are of value in elucidating the chemical nature of the differences in the surface that are associated with cell malignancy. They are also of potential value for tumor chemotherapy.

Proposed Course: The contract will terminate at the end of the current contract year.

Date Contract Initiated: April 22, 1969

WISTAR INSTITUTE OF ANATOMY AND BIOLOGY (NO1-CP3-3250)

Title: Extraction and Characterization of Virus-Induced Transplantation Antigen and Rescue of Virus from Sarcomas and Leukemias

Contractor's Project Director: Dr. Anthony J. Girardi

Project Officer (NCI): Dr. James T. Duff

Objectives: (1) Studies on attempts to isolate viruses from human sarcoma and leukemia patients. (2) Localization of the integrated SV40 genome on chromosomes of transformed human cells.

Major Findings: Sarcoma cells are being employed in cell fusion experiments with human leukemia cells; this is being followed by induction (IDU and dexamethasone) in attempts to activate leukemia-sarcoma viruses. The assay cells used for the hypothetical agents include normal human, marmoset, avian and murine species as well as normal cells derived from sarcoma patients.

Hybrid cells resulting from fusion of mutants of "normal" mouse and transformed human cells are being analyzed for SV40 traits. The SV40 genome appears to be specifically integrated on the human chromosome C7 as indicated by expression of T antigen, TSTA antigen and rescuable infectious SV40 virions. Loss of the C7 chromosome is accompanied by loss of the above mentioned activities. These studies are being expanded in attempts to localize the EBV genome, the Rous sarcoma genome and the murine sarcoma genome in human cells which were transformed by these agents but which are now producer cells.

Significance to Biomedical Research and the Program of the Institute: In order to establish the viral etiology of human cancer, it is essential to pursue new techniques and methodology for the isolation of a potential candidate human tumor virus. These studies are attempts to isolate this elusive agent. The identification of the integration sites of SV40 hybrid genomes in transformed cells and the determination of their linkage relationship with other chromosome markers may explain the mechanisms of virus-induced transformation.

Proposed Course: Continuation of the studies on the isolation of a human cancer virus and initiate studies involving the relationship among murine sarcoma virus transformed cells that differ in the degree of viral genome expressed.

Date Contract Initiated: February 1, 1971

7. TUMOR VIRUS DETECTION SEGMENT

CONTRACT SUMMARY REPORT

Human Studies

Reverse transcriptase purified from three cases of human acute myelogenous leukemia has antigenic properties closely related to those of the woolly monkey and gibbon ape type C viruses. (Contract #43207)

Terminal transferase activity has been found in six (out of eight studied) cases of childhood acute lymphoblastic leukemia. It has not been found in cells from patients with chronic lymphatic leukemia (five cases), lymphosarcoma cell leukemia (four cases) and acute myeloblastic leukemia (six cases). It is likewise absent from a variety of cells in culture and from normal human spleen and circulating lymphocytes. Fractionation of normal human infant thymocytes has allowed identification of a sub-population of thymocytes which contain this enzyme. They are small, buoyant, mitotically active cells, the population which predominates during fetal life and may be T lymphocyte precursor cells. (Contract #33348)

No DNA polymerase activity in human milk comparable to that shown in the milk of mice producing type B or C viruses was observed by phosphocellulose chromatography. In addition, there was no correlation between the presence of endogenous activity and positive simultaneous detection assays for reverse transcriptase on the one hand and a positive family cancer history on the other. HBT-3, a human breast tumor cell line, contains an enzyme strikingly similar to the reverse transcriptases found in type C viruses with respect to size, template and cation preferences, and adsorption to phosphocellulose and oligo-dT-cellulose. (Contract #43249)

The human papovavirus, BK virus, can transform BHK_{21} clone 13 cells and these cells can induce tumors in adult hamsters. BK virus has also transformed a marmoset fibroblast cell line. (Contract #43318)

The Immunodeficiency Cancer Registry has continued and increased leading to 164 known patients with immunodeficiency who developed malignancies and nine cases known to be alive with tumors which may be available for procurement. The bulk of tumors in children with primary immunodeficiency diseases are lymphoreticular. Persons with several forms of primary immunodeficiency (and high cancer risk) almost invariably have decreased numbers of T lymphocytes and increased numbers of B lymphocytes. Patients with X-linked (Bruton's) agammaglobulinemia lack lymphocytes with surface immunoglobulins but have adequate numbers of lymphocytes with receptors for complement (C3), suggesting that B lymphocytes are present but defective in immunoglobulin expression. (Contract #33357)

Membrane lipids of human and mouse lymphocytes were studied following interaction with the mitogens PHA and Con A. Using a fatty acid spin label and electron paramagnetic resonance, it was found that membrane

fluidity is significantly increased within 5 minutes with return to normal values at 60 minutes, a time course closely following that of the rise in cGMP. (Contract #33357)

Primate Studies

Type C viruses have been isolated from baboon tissues. The first isolate was obtained from a baboon placenta and the virus was found to grow well in a variety of heterologous cells, but especially in a dog thymus line. Several additional viruses were isolated from baboon spleen, lung, and testes. The DNA product of an endogenous reaction hybridizes to normal baboon cell DNA and also to the cellular DNA of other primates (patas, African green, and two species of macaques--rhesus and stumptail). Viral expression (RNA, gs antigen) can be detected in normal baboon spleen, testes, and placenta. Eight to 12 DNA copies are found per diploid genome in all normal baboons tested. This is the first demonstration of endogenous type C virus in primates. (Contract #43207)

There is partial sequence homology between the endogenous cat type C (RD-114/CCC) virus group and the endogenous primate virus group; the gs antigens and reverse transcriptases of viruses of these two groups share antigenic determinants with one another and their pseudotypes interfere with one another. The results indicate a closer evolutionary relationship between these two groups of viruses that would not have been expected based on the extensive genetic divergence between the species. (Contract #43207)

The 30,000 dalton gs proteins purified from woolly monkey and gibbon ape type C viruses were found to be highly related immunologically, although they cross-reacted very little with other mammalian type C viral gs antigens. (Contract #43236)

In contrast to the results with baboon type C virus, no nucleic acid sequences homologous to ^{3}H-DNA transcripts prepared from woolly monkey and gibbon ape type C viruses could be detected in any primate tissue examined (including woolly monkey and gibbon ape). Thus these viruses do not seem to be endogenous primate viruses. (Contract #43207)

Spider monkeys have been shown to be latent carriers of *H. ateles*. Ten *H. ateles* strains have been obtained by co-culturing spleen derived lymphocytes with permissive cultures; all have been determined to be related to the prototype *H. ateles* strain by serum neutralization and fluorescent antibody techniques. (Contract #33390)

In owl monkeys (*Aotus trivirgatus*), *H. saimiri* lymphoma is a contagious disease. An epizootiological trip to the zone of Iquitos and Pucalpa, Peru indicated that the *Aotus* species is practically free of *H. saimiri*, and is most likely that this agent is transmitted horizontally by the *Saimiri* species. (Contract #33390)

H. saimiri has been passed serially more than 32 times in dog fetal lung (DFL) cultures, bringing the titer of H. saimiri for OMK cells from 5.5/ml to ≤ 0.5/ml. The incubation period and incidence of malignant lymphoma in Aotus species inoculated with DFL-H saimiri passed in DFL cells is less than that observed with the wild parent virus. (Contract #33390)

CV-1 cells, with a change to arginine-deficient medium after infection were found to be useful in obtaining higher titers of Herpesvirus saimiri (HVS). CV-1 cells, overlayed with methocel and arginine-deficient medium were found to be the best plaque titration system. Methanol precipitation of HVS was found to be useful to concentrate virus prior to further purification. HVS DNA probably occurs as a single molecule with a density in CsCl of 1.729 g/cm^3. (Contract #22068)

The cAMP concentration in contact-inhibited cells reproducibly drops two-fold within three hours after infection with SV40, then gradually rises to the original level over the following 8-10 hours. This is the earliest known effect of SV40 infection upon the host cell. (Contract #43249)

Murine Studies

A new host range class of endogenous murine type C viruses has been identified which are unable to replicate in any mouse cell line tested so far, but replicate well in a variety of other mammalian cells, including rhesus monkey, dog, mink and rat cells. Because of the sensitivity of the rabbit cell line, SIRC, for detecting this group of viruses, they have been called "S-tropic" viruses. By host range properties and by nucleic acid hybridization studies, this new class of viruses can be distinguished from both "N-" and "B-" tropic murine type C viruses. The S-tropic virus is preferentially induced by halogenated pyrimidines from the Balb/3T3 line, although low levels of N-tropic type C virus are also produced. S-tropic virus has also been shown to be spontaneously released from certain Balb/3T3 derived cell lines. Balb/c splenocytes preferentially release S-tropic type C virus after induction and also after graft-versus-host or mixed splenocyte reactions. Spleens from older Balb/c animals generally contain S-tropic virus, whereas young animals do not. Normal weanling mice of C57BL, CBA, DBA, NZB, C58, and AKR strains have S-tropic virus. The S-tropic viruses from Balb/c cells and the S-tropic viruses from NIH Swiss cells are related but different. (Contract #43207)

Murine leukemia viruses and vesicular stomatitis virus (VSV) will phenotypically mix producing pseudotypes in which the VSV genome is enveloped by a membrane containing MuLV-specific protein. VSV pseudotypes of both N-tropic and B-tropic murine leukemia virus show very little restriction in cell lines derived either from animals homozygous for Fv-1^n or Fv-1^b. Therefore, it would appear that the Fv-1 restriction against murine tumor viruses does not take place at the cell surface but rather is due to an intracellular restriction. (Contract #33348)

By RNA:^{3}H-DNA hybridization, Kirsten sarcoma virus was shown to possess two distinct sets of nucleic acid sequences. One set is contained in murine type C helper virus and the other set is contained in rat type C helper viruses. Moloney sarcoma virus had sequences of murine type C virus but not of rat type C helper virus. The results indicated that Kirsten sarcoma virus arose through a process of recombination between Kirsten leukemia virus and nucleic acid sequences found in rat cells. (Contract #43236)

The mouse cell line Balb/c 3T3 and its derivatives transformed either spontaneously or by treatment with a variety of agents including methylcholanthrene and X-irradiation were analyzed for cytoplasmic RNA complementary to DNA products from the Kirsten strain of murine sarcoma-leukemia virus and from an endogenous type C virus of Balb/c 3T3. While none of these clonal lines spontaneously releases virus they all contained RNA which was partially homologous to a portion of the 35S RNA isolated from these viruses. The parental cell line, Balb/c 3T3 contained a low level of viral-related RNA. There was an increased amount of this RNA in some of the transformed cells. (Contract #43236)

Mice of the C57L x (C57L x AKR)F_1 backcross were examined for genetic segregation of infectious leukemia virus (MuLV), MuLV gs-1 antigen, and the MuLV-induced cell surface antigens G_{IX}, GCSA, G_L, and G_T. Segregation ratios indicated that (1) virus production was controlled by two non-linked dominant genes, (2) expression of gs-1 antigen was controlled by three non-linked dominant genes, (3) expression of G_{IX} and GCSA antigens was controlled by one dominant gene, and (4) expression of G_L and G_T antigen was controlled by two non-linked dominant genes. (Contract #22022)

When 3T3 cells were infected with MSV at an m.o.i. of 20, the gal NAc transferase activity was reduced by about 50% while levels of other glycosyl transferases either stayed the same or increased. This decrease in gal NAc transferase activity, which is similar to that seen in the polyoma and SV40 transformed mouse cells, was observed two days after morphological transformation by MSV was complete. Chemically transformed mouse cells do not show altered gal NAc transferase activity, nor do murine leukemia virus infected 3T3 cells. S+L- cell lines continue to show decreased gal NAc transferase activity six months or more after the original MSV infection. (Contract #43249)

AKR cells have a cytoplasmic receptor protein for glucocorticoids; Kd for dexamethasone is approximately 3×10^{8} M. Hydrocortisone modulates the rate of synthesis of the viral proteins in AKR cells after activation by IdU has occurred and does not affect the activation process *per se*. (Contract #20208)

Reversed phase column chromatography revealed (a) the 70S-associated 4S RNA of both AKR murine leukemia virus and the RD-114 virus can be resolved into at least 30 peaks at the salt concentrations characteristic of tRNA elutions; (b) the chromatographic pattern of the "free" 4S RNA

isolated from the AKR murine virus is best interpreted as a mixture of the 70S-associated 4S and of cellular 4S RNA; (c) comparison of the 70S-associated 4S RNA preparations from different RNA tumor viruses showed an apparent similarity in position (salt concentration) but considerable differences in relative quantity of the eluted peaks; (d) no peak has been detected unique to the virus 4S RNA preparation (and not found in the cellular 4S preparation); and (e) different species of 4S RNA are dissociated from the 70S RNA at different temperatures. (Contract #20208)

Immune precipates formed by reaction of ^{3}H-labeled MLV and serum from BC3F1 mice indicate that the endogenous reaction is against three virus antigens, of apparent molecular weights 60,000, 43,000, and 17,000 with titers of 1:750, 1:80, and 1:100 respectively. Since the serum titer against intact virus was about 1:500, these results suggest that the 60,000 molecular weight component is the determining component. Immunoglobulin and endogenous MuLV antigen(s), presumably in the form of immune complexes, became localized within kidney glomeruli with age. (Contract #20208)

At 100 μg/ml, both poly(adenylic acid) [poly(A)] and poly(2'-O-methyladenylic acid) [poly(Am)] inhibited the uptake of radioactively labeled leukemia virus by Swiss mouse embryo cells, but neither had a similar effect on Sindbis virus absorption. At 10 μg/ml, poly(Am) did not inhibit the uptake of leukemia virus but did inhibit virus replication by 85%; in contrast, the replication of Sendai virus and Sindbis virus was not inhibited significantly at this concentration. Both compounds were effective only when added prior to or early during virus infection. Poly(Am) was a much more effective inhibitor than poly(A), probably due to the nuclease resistance of the former compound. Poly(Am) at 5 μg/ml also inhibited transformation of 3T3 cells by Moloney sarcoma virus. However, neither poly(A) nor poly(Am) at high concentrations inhibited the activation of endogenous leukemia virus by iododeoxyuridine in AKR mouse embryo cells. (Contract #20208)

When poly(Am) at 10 μg/ml was given to newborn mice at least four hours before inoculation with MSV there was significant protection against tumor development and death. Poly(Am) enhanced the humoral immune response of adult mice to sheep red blood cells and to MSV. It stimulated the response of newborn mice to MSV, and also stimulated a premature antibody response to the endogenous leukemia virus. It did not effect the transplantation and tumor induction by the (K)MSV transformed Balb/3T3 cells. (Contract #20208)

Employing an ^{3}H-DNA copy of mouse mammary tumor virus (MTV) RNA or the Kirsten strain of murine leukemia virus RNA, it was shown that the DNA from a variety of mouse cells, even those not producing virus, gave readily detectable hybridization reactions. With the type C probes, the DNA from NIH 3T3 and feral mouse cultures had lower levels of hybridization than seen with DNA from RIII, Balb/c or C57 Bl/6 mice. These differences might represent partial homology between the viral

genomes present in NIH Swiss and feral mouse cells and the Kirsten murine leukemia probes, and/or might be related to the fact that neither of these cells are inducible to form a complete type C virus, and may lack an essential viral component necessary for complete viral expression. (Contract #43236)

L8A clone 11 and L8A clone 6 derived from Sykes CCL-51 mouse mammary tumor cell line, although morphologically similar differed significantly in their expression of type B viral information. Clone 11 contained numerous type B particles and high levels of type B specific S1 antigen and cellular RNA which hybridized with MTV ^{3}H-DNA product. Clone 6 did not have detectable particles by electron microscopy, had antigen levels below sensitivity and very little RNA hybridizable to MTV DNA. Both clones contained comparable levels of MTV DNA sequences. Type C viral expression (gs antigen and RNA levels) in both clones were comparable. (Contract #43236)

Oligo-dT-cellulose chromatography appears to be a promising tool for the separation of polymerases of type C viruses from those of type B viruses. It is known that enzymes in Mason-Pfizer virus and R3 mouse milk prefer Mg ++ to Mn ++ in copying poly rA:oligo-dT. These enzymes do not bind to oligo-dT-cellulose. A minor enzyme activity from R3 milk binds to oligo-dT-cellulose and prefers Mn ++ to Mg ++. It is believed that it represents polymerase from type C virus which is known to be present in R3 milk. (Contract #43249)

Dexamethasone, a synthetic glucocorticoid, and cortisol increase both the size and number of polyoma plaques when virus particles are plated on monolayers of either primary or secondary mouse embryo or 3T3 cells. The hormones appear to work via the cytoplasmic receptors which mediate other glucocorticoid-sensitive functions. It appears that the steroid does not promote virus uptake or adsorption but participates rather in early viral functions. Experiments on transformation of primary and secondary hamster embryo cells using polyoma virus showed that dexamethasone and cortisol increased the frequency of transformation 3 to 6-fold while inactive steroid, epicortisol, was ineffective. A "sub-optimal inducer," progesterone, had intermediate effects but, as expected, prevented maximal stimulation by dexamethasone. The optimum dexamethasone concentration is approximately 10^{-8} M, consistent with the amount required to saturate the glucocorticoid receptors. (Contract #33332)

A skin fibroblast cell line from an adult marmoset has been transformed by a small plaque variant of polyoma virus. The transformation of cell species other than rodent had not been previously described. (Contract #43318)

Avian Studies

It was found that in formamide as in aqueous buffers, the electrophoretic mobility of the a subunit of nondefective avian sarcoma viruses is lower than that of the b subunit of transformation defective or leukosis viruses, indicating that the a subunit has a higher molecular weight. Comparative studies by oligonucleotide fingerprinting and RNA-DNA hybridization on the RNAs of corresponding (=same subgroup and strain) avian sarcoma and leukosis viruses have indicated that the a subunit of avian sarcoma viruses is related to the b subunit of leukosis virus by the formula, $a = b + x$, wherein x presumably represents a sequence concerned with transformation of fibroblasts. (Contract #43212)

Two mutants of Rous sarcoma virus which are temperature sensitive in a function which is necessary only during the very early stage of the viral growth cycle, both had a reverse transcriptase which was three-fold more rapidly inactivated by heat than was wild type DNA polymerase. The ribonuclease H activity of both mutant DNA polymerases was as much as ten-fold more heat labile than wild type activity. In a series of recombinants between avian leukosis virus and mutant sarcoma virus, in all cases the mutants which showed temperature sensitivity for growth, also showed temperature sensitivity of DNA polymerase indicating that the two characteristics segregate together. Furthermore, revertants of one of the mutants showed a normal DNA polymerase and ribonuclease H. (Contract #33348)

Density gradient sedimentation in alkaline cesium chloride of DNA from normal chicken embryos or leukemic myeloblasts fragmented to a size of 13S revealed that the DNA sequences complementary to 70S avian myeloblastosis virus RNA sedimented in the high guanine plus cytosine region ahead of the main peak of cellular DNA. An increase in the cellular content of viral DNA was detected as early as one hour after infection with AMV or RSV. Early after infection most of the newly synthesized viral DNA consists of free small molecular weight molecules. Within 24 hours, the viral DNA becomes covalently linked to host cell DNA. (Contract #33283)

H^3-labeled 35S AMV RNA was exhaustively hybridized with excess of normal chicken DNA to remove all sequences which the virus DNA has in common with normal cells. The residual RNA hybridized to leukemic chicken DNA but did not rehybridize with normal chicken DNA. It is concluded that DNA from leukemic cells contain viral specific sequences which are absent in DNA from normal cells and that an RNA probe prepared in this fashion can be used to screen DNA for leukemic specific sequences. (Contract #33283)

TUMOR VIRUS DETECTION SEGMENT

Dr. George J. Todaro, Chief, VLLB, VO, DCCP, Chairman
Dr. Bernard Talbot, VLLB, VO, DCCP, Vice Chairman

ATOMIC ENERGY COMMISSION (Y01 CP 20208)

Title: NCI-AEC Viral Carcinogenesis Program

Contractor's Project Directors: Dr. F.T. Kenney
Dr. R.W. Tennant
Dr. W.K. Yang
Dr. M.G. Hanna

Project Officer (NCI): Dr. Bernard Talbot

Objectives: In January 1963 an interagency agreement was established between the National Cancer Institute and the Oak Ridge National Laboratory for carcinogenesis studies. In September 1972, the Viral Oncology funded portion was split out as this separate Interagency Agreement. It is divided into 4 main areas, studying Regulation of Gene Expression (Kenney); RNA Tumor Virus Cell Biology (Tennant); Enzymology of Viral Carcinogenesis (Yang); and Immunology of Viral Carcinogenesis (Hanna).

Major Findings: The effect of hydrocortisone on virus activation by IdU treatment of AKR cells has been examined. It appears that hydrocortisone modulates the rate of synthesis of the viral proteins after activation has occurred and does not affect the activation process per se.

AKR cells were found to have a cytoplasmic receptor protein for glucocorticoids; Kd for dexamethasone is approximately 3×10^8 M. The receptor is similar to other glucocorticoid receptors in all respects tested so far.

34S RNA subunits of MLV can be fractionated into poly (A)-containing and nonpoly (A)-containing fractions by chromatography on poly (U)-Sepharose. Rates of synthesis and of packaging of RNA into virus particles indicate small but significant differences between the two fractions.

Immune precipitates formed by reaction of ^{3}H-labeled MLV and serum from BC3F1 mice indicate that the endogenous reaction is against three virus antigens, of apparent molecular weights 60,000, 43,000, and 17,000 with titers of 1:750, 1:80, and 1:100 respectively. Since the serum titer against intact virus was about 1:500, these results suggest that the 60,000 molecular weight component is the determining component. Similar results were obtained with sera from C3H mice. Experiments involving reaction of intact virus with BC3F1 serum indicate that the same antigens are reactive as those found in assays of disrupted virus.

Using poly (Um) affinity chromatography, when extracts of IdU-activated AKR cells are passed over these columns, the bulk of the cellular DNA polymerase

passes through. A gradient elutes the virus reverse transcriptase as an apparently single species eluting at 0.37 M NaCl.

Nuclei from a steroid insensitive rat hepatoma did not take up steroid bound to its cytoplasmic receptor but did take up the receptor-steroid complex formed in normal liver cytoplasmic extracts. These results suggest a defect in the receptor protein, or that some other cytoplasmic component is required for nuclear uptake of the steroid-receptor complex.

Reversed phase column chromatography (RPC-5) revealed (a) the 70S-associated 4S RNA of both AKR murine leukemia virus and the RD-114 virus can be resolved into at least 30 peaks at the salt concentrations characteristic of tRNA elutions; (b) the chromatographic pattern of the "free" 4S RNA isolated from the AKR murine virus is best interpreted as a mixture of the 70S-associated 4S and of cellular 4S RNA; (c) comparison of the 70S-associated 4S RNA preparations from different RNA tumor viruses showed an apparent similarity in position (salt concentration) but considerable differences in relative quantity of the eluted peaks; (d) no peak has been detected unique to the virus 4S RNA preparation (and not found in the cellular 4S preparation); and (e) different species of 4S RNA are dissociated from the 70S RNA at different temperatures. In addition, turnover rate studies suggest that two populations of RNA species are present in the 4S RNA derived from virus 70S RNA.

Immunoelectron microscopy (IEM) demonstrated that $B6C3F_1$ serum possesses significant levels of free antibody specific for MuLV envelope antigens. This reactivity was detected on budding viruses of EG♂2, AKR high passage, K36 and plasmacytoma cells. Immunoglobulin and endogenous MuLV antigen(s), presumably in the form of immune complexes, became localized within kidney glomeruli with age; this deposition correlated well with the chronic development of glomerulonephritis. Immunoglobulin eluted from kidneys of aged animals also was shown by IEM to be specific for virus envelope antigen(s).

At 100 μg/ml, both poly(adenylic acid) [poly(A)] and poly(2'-O-methyladenylic acid) [poly(Am)] inhibited the uptake of radioactively labeled leukemia virus by Swiss mouse embryo cells, but neither had a similar effect on Sindbis virus absorption. At 10 μg/ml, poly(Am) did not inhibit the uptake of leukemia virus but did inhibit virus replication by 85%; in contrast, the replication of Sendai virus and Sindbis virus was not inhibited significantly at this concentration. Both compounds were effective only when added prior to or early during virus infection. Poly(Am) was a much more effective inhibitor than poly(A), probably due to the nuclease resistance of the former compound. Poly(Am) at 5 μg/ml also inhibited transformation of 3T3 cells by Moloney sarcoma virus. However, neither poly(A) nor poly(Am) at high concentrations inhibited the activation of endogenous leukemia virus by iododeoxyuridine in AKR mouse embryo cells.

When poly(Am) at 10 μg/ml was given to newborn mice at least four hours before inoculation with MSV there was significant protection against tumor

development and death. Poly(Am) enhanced the humoral immune response of adult mice to sheep red blood cells and to MSV. It stimulated the response of newborn mice to MSV, and also stimulated a premature antibody response to the endogenous leukemia virus. It did not effect the transplantation and tumor induction by the (K)MSV transformed Balb/3T3 cells.

The reciprocal host-range restriction of N- or B-type mouse embryo cells for B-tropic or N-tropic mouse leukemia viruses was analyzed by cell fusion. Fusion of nonpermissive cells in the presence of the restricted virus did not alter the relative resistance, and radioactively labeled virus adsorbed at the same rate to permissive (N-type) and nonpermissive (B-type) cells. The fate of N- or B-tropic virus in heterokaryons of N- and B-type cells was analyzed by simultaneous autoradiography and fluorescence microscopy, which allowed virus-induced proteins to be scored specifically in heterokaryons or in cells of either parental type. Heterokaryons restricted both N- and B-tropic virus, but did not restrict N/B tropic virus, which infects either type cell with equal efficiency. Fusion of permissive (N-type) cells with nonpermissive (B-type) cells at intervals after infection with N-tropic virus indicated that the restriction possibly affected some virus function generally occurring within 12 hours after infection.

Significance to Biomedical Research and the Program of the Institute:

This effort is focused on the phenomenon of viral carcinogenesis; the problem is investigated in terms of enzymology, immunology, cell biology and control of gene expression. This multifaceted approach studies primarily the mouse leukemia system, but increasingly the findings are being carried over into work with human tumor cells in an attempt to understand, and ultimately deal with, the problem of cancer in man.

Proposed Course: Continue to develop a concerted, interdisciplinary research program in the central aspects of viral carcinogenesis.

Date Contract Initiated: July 1, 1963 (Separate Viral Oncology Contract: September 1, 1972)

BIOLABS, INC. (NO1 CP 22068)

Title: Development and Evaluation of Methods for Large Scale Preparation of Purified Oncogenic Herpesviruses

Contractor's Project Director: Dr. Clyde R. Goodheart

Project Officer (NCI): Dr. Dharam V. Ablashi

Objectives: The development and evaluation of methods for large scale production of purified oncogenic herpesviruses (especially *Herpesvirus saimiri*) for biochemical, immunologic and virologic studies.

Major Findings: 1. CV-1 cells, with change to arginine-deficient medium after infection, found to be useful in obtaining higher titers of *Herpesvirus saimiri* (HVS). 2. CV-1 cells, overlayed with methocel and arginine-deficient medium, found as best plaque titration system. 3. Methanol precipitation of HVS found to be useful to concentrate virus prior to further purification. 4. Sucrose gradient and controlled-pore glass column chromatography attempted for purifying HVS, but with inadequate results so far. 5. HVS DNA probably occurs as a single molecule with a density in CsCl of 1.729 g/cm^3. 6. HVS DNA, like HSV2 DNA, transcribes poorly with *E. coli* transcriptase.

Significance to Biomedical Research and the Program of the Institute:

Herpesvirus saimiri induces a fatal lymphoma and/or leukemia in various primates. Mortality is almost 100% even in animals inoculated as adults; this is most unusual for any oncogenic virus. Long term lymphoblastoid cell lines have been established in suspension culture, derived from primary lymphoid cells of diseased animals. These cell lines resemble the EBV-carrying human lines both with regard to growth and because only a few cells produce viral antigens. Another point of analogy between the disease in non-human primates produced by HVS and that of Burkitt's lymphoma in man is the *in vivo* immunological response to early and late antigens. HVS has more in common with the EBV system than any other existing animal model system. Extensive studies to continue to follow these leads require large amounts of purified and infectious virus and virus nucleic acid which exceed the present capacity for virus production with the Program. These materials can be utilized to find new antigens which may be similar to EBV. Eventually this may determine contributions of herpesvirus to neoplastic processes in man.

Proposed Course: 1. Continue with present procedures for growing HVS to be used in hybridization and DNA experiments. 2. Continue to attempt to improve growth conditions and yields. 3. Finish characterization of HVS DNA. 4. Continue to study transcription of HVS DNA, especially using transcriptase from permissive host cells.

Date Contract Initiated: December 20, 1971

FOUNDATION CURIE-INSTITUT DU RADIUM (NO1 CP 43219)

Title: Molecular and Genetic Studies of Rous Sarcoma Virus

Contractor's Project Director: Dr. R. Laterjet

Project Officer (NCI): Dr. Robert Bassin

Objectives: (1) To pursue the study of the infectious DNA which has been found in permissive and non-permissive cells transformed by RSV and in cells infected by lymphomatosis viruses. Some basic questions are: When is this DNA made and integrated? Can it exist in episomal form? Can it infect non-permissive cells as well as permissive cells? This study facilitates research of cryptic viruses in other systems, notably human. (2) To study, in synchronized permissive cells, the events required for successful infection and transformation. This study may help to define optimal conditions for successful infection and transformation by other oncornaviruses. (3) To pursue the study of the reasons for non-permissiveness of RSV-transformed mammalian (hamster BHK21) cells and of the mechanism of virus induction following fusion of these cells with permissive cells. (4) To pursue the genetic study of information carried by the RSV genome, especially that information on which successful infection and cell transformation depends. (5) To study further the factors which elicit cell transformation; i.e., virus-dependent factors produced in infected cells and other factors (medium components, etc.).

Major Findings: This is a new contract and major findings have not been reported.

Significance to Biomedical Research and the Program of the Institute:

This work will aid in the goal of determining the relationship of viruses to the etiology of human cancer. Because of the profound similarities between oncornaviruses, Rous sarcoma virus (RSV) is an excellent model system for the study of these viruses, including those which can be expected to be found in human leukemias and sarcomas or other tumors. Rous sarcoma virus is, today, the best known and most easily studied oncornavirus.

Date Contract Initiated: October 15, 1973

HERBREW UNIVERSITY (NO1 CP 33310)

Title: Studies on Herpesvirus (EBV) and its Role in Human Cancer

Contractor's Project Director: Dr. Yechiel Becker

Project Officer (NCI): Dr. Berge Hampar

Objectives: The proposed work will concentrate on four main areas: (1) The composition and internal organization of EBV-DNA. The molecular composition and properties of EBV-DNA will be investigated (the presence of alkali sensitive bonds, denaturation properties, G + C rich regions in the DNA, electrophoresis in acrylamide gels). (2) The state of EBV-DNA in Burkitt's lymphoblasts. The relationship between EBV-DNA and the host DNA

genome will be studied utilizing electrophoresis in acrylamide gels to separate viral DNA from host cell DNA. The nature of the cellular DNA synthesized in Burkitt's lymphoblasts after arginine deprivation, at the time of EBV-DNA replication, will be studied. (3) Transcription of EBV-DNA in the lymphoblasts. The nature of EBV mRNA synthesized in Burkitt's lymphoblasts will be studied using cells prior and after induction of virus synthesis with BrdU or arginine deprivation. Poly-A-containing mRNA species will be isolated and their specificity studied by hybridization to EBV-DNA. (4) Translation of EBV genetic information in Burkitt's lymphoblasts. The viral structural peptides synthesized in virus producing and nonproducing cell lines and their intracellular localization will be investigated. Virus specific surface antigens will be characterized.

Major Findings: This is a new contract and major findings have not been reported.

Significance to Biomedical Research and the Program of the Institute:

EBV appears to be associated with Burkitt's lymphoma. Since the members of the herpesvirus group are usually viruses which cause lytic infections in sensitive cells, the relationship between EBV and its host, the lymphoblast, represents a new type of cell-virus interaction. The Burkitt's lymphoblasts in human tumors contain EBV-DNA in a repressed state. Only when the cellular mechanisms are changed, expression of the viral genes takes place and EB virions are synthesized. The nature of the mechanisms in the lymphoblasts that control viral gene functions are not yet known. The proposed studies will shed light on the nature of the association of EBV-DNA with the lymphoblast's genome. The studies on the molecular processes involved in the regulation of the Herpesvirus DNA genomes in permissive and transformed cells will provide basic information on the nature of the EB virus induced antigens and their role in the ability of the tumorigenic cells to proliferate and develop tumors in humans. These studies may help to assess the usefulness of these viral antigens for immunodiagnosis and immunotherapy of cancer in humans.

Date Contract Initiated: June 29, 1973

LITTON BIONETICS (NO1 CP 43249)

Title: Applications of Animal Virus Model Systems to Human Neoplasia

Contractor's Project Director: Dr. Alan Rein

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

Objectives: To study the nature of MSV defectiveness and the role of helper leukemia virus and to assess the applicability of this model system to human tumors. To establish and characterize new human tumor cell lines and develop

methodology to use in the detection of viruses and subviral products. To study the human cell line HBT-3 and its DNA polymerases.

Major Findings: When S+L- cells are infected with MuLV they undergo a dramatic morphological change, which has been used as the basis of a rapid MuLV assay. MuLV causes the S+L- cells to round-up and become extremely non-adhesive. The growth of the culture slows considerably and the fraction of cells synthesizing DNA declines two-fold by two days after infection. When individual cells are seeded in small culture wells after infection, it is found that 90 - 95% of them only complete one or a few cell divisions. They do not seem to undergo lysis, but merely terminate their growth. There is no loss of "viability" as determined by trypan blue staining. These changes represent a synergistic interaction between the MuLV and the S+L- genomes. A few S+L- cells after infection with MuLV do continue to grow and yield clones, which appear to be producing large quantities of completely noninfectious virus and smaller amounts of infectious viruses.

When normal 3T3 cells are infected with similar high multiplicities (50) of MuLV there is a small, but highly reproducible reduction in the growth rate of the culture within two days after infection. One important difference between the effect of MuLV on normal cells and that on S+L- cells is that while MuLV significantly reduces the cloning efficiency of S+L- cells, it does not have this effect on normal cells, but merely decreases temporarily the rate at which the individual cells can multiply. These results imply that MuLV alone is not as "neutral" in its action on normal cells as has generally been considered.

Studies of the S+L- MSV genome in normal rat kidney cells reveal that the rat S+L- cells resemble mouse S+L- cells in several respects: they display a transformed morphology, but do not release infectious MSV unless they are superinfected with a helper leukemia virus. They contain mouse but not rat gs-1 antigen. They release virus-like particles which have a density of 1.16 and contain RNA and reverse transcriptase. The RNA is not a discrete high molecular weight species. The reverse transcriptase resembles that found in mouse S+L- particles and MuLV virions with respect to molecular weight (6.7-7.0 x 10^4 daltons), binding to oligo-dT-cellulose, and chromatography on phosphocellulose. The enzyme shows the template preferences characteristic of viral reverse transcriptases and can copy heteropolymeric regions of RNA extracted from rat S+L- cells or, less efficiently, RNA from avian myeloblastosis virus. However, rat S+L- cells also differ from mouse S+L- cells in several ways: the RNA in the rat S+L- particles can easily be copied in situ in an RNase-sensitive endogenous reaction; and the S+L- particles are produced in greater quantity than in mouse S+L- cells. Superinfecting the rat S+L- cells with MuLV, while rescuing infectious MSV, does not increase the total production of physical particles by these cells.

The 3T3 mouse cell FL line differs from almost all other mouse cell lines in supporting the growth of both N-tropic and B-tropic viruses.

The process of cellular transformation by MSV was shown to be completely insensitive to the host-range restriction mechanism. This may mean that the target of the restriction mechanism is specific for MuLV's and may be concerned with late stages of viral replication. An alternative interpretation would be that S+L- MSV may resemble some MuLV's ("NB-tropic" MuLV) in being equally infectious in both N- and B-type cells.

When 3T3 cells were infected with MSV at an m.o.i. of 20, the gal NAc transferase activity was reduced by about 50% while levels of other glycosyl transferases either stayed the same or increased. This decrease in gal NAc transferase activity, which is similar to that seen in the polyoma and SV40 transformed mouse cells, was observed two days after morphological transformation by MSV was complete. Chemically transformed mouse cells do not show altered gal NAc transferase activity, nor do murine leukemia virus infected 3T3 cells. S+L- cell lines continue to show decreased gal NAc transferase activity six months or more after the original MSV infection.

Flat revertants of S+L- 3T3 cells with one exception contained MuLV gs antigen(s) without demonstrable S+L- virus production and reverse transcriptase activity in supernatant fluids. Revertants did not show rescuable murine sarcoma virus.

The cAMP concentration in contact-inhibited cells reproducibly drops two-fold within three hours after infection with SV40, then gradually rises to the original level over the following 8-10 hours. This is the earliest known effect of SV40 infection upon the host cell.

No DNA polymerase activity in human milk comparable to that shown in the milk of mice producing type B or C viruses was observed by phosphocellulose chromatography. In addition, there was no correlation between the presence of endogenous activity and positive simultaneous detection assays for reverse transcriptase on the one hand and a positive family cancer history on the other.

HBT-3, a human breast tumor cell line, contains an enzyme strikingly similar to the reverse transcriptases found in type C viruses with respect to size, template and cation preferences, and adsorption to phosphocellulose and oligo-dT-cellulose. Attempts to induce virus from this line have been unsuccessful. The HBT-3 enzyme which binds to oligo-dT-cellulose can use heteropolymeric regions of RNA from avian myeloblastosis virus, but not from yeast, as a template for DNA synthesis. This is a further similarity between this enzyme and type C viral enzymes.

Oligo-dT-cellulose chromatography also appears to be a promising tool for the separation of polymerases of type C viruses from those of type B viruses. It is known that enzymes in Mason-Pfizer virus and in R3 mouse milk prefer Mg^{++} to Mn^{++} in copying poly rA:oligo-dT. These enzymes do not bind to oligo-dT-cellulose. A minor enzyme activity from R3 milk binds to oligo-dT-cellulose and prefers Mn^{++} to Mg^{++}. It is believed that it represents polymerase from type C virus which is also known to be present in R3 milk.

Significance to Biomedical Research and the Program of the Institute:

An understanding of viral defectiveness and the role "helper" viruses play is of value in determining the occurrence and mechanism of viral oncogenesis by type C viruses in man. Identification of viruses or viral products in human breast tumor cells is of value in assessing the role of viruses in human breast cancer and, ultimately, in developing techniques of both diagnostic and therapeutic significance.

Proposed Course: Additional S+L- lines will be isolated from wild-type stocks of MSV and their genetic inter-relationships will be determined. Aspects of quantitative transformation of 3T3 cells by MSV will be studied. The viral-like reverse transcriptase present in HBT-3 cells will be further analyzed, and the applicability of dT cellulose column chromotography to detection of virus-specific polymerase in human cells will be ascertained.

Date Contract Initiated: June 27, 1969

MASSACHUSETTS INSTITUTE OF TECHNOLOGY (N01 CP 33348)

Title: Studies of Leukemia Virus DNA Polymerases

Contractor's Project Director: Dr. David Baltimore

Project Officer (NCI): Dr. Edward Scolnick

Objectives: Characterization of DNA polymerases associated with oncorna viruses and normal and neoplastic cells; to investigate their function, mechanisms, and products.

Major Findings: Two mutants of Rous sarcoma virus which are temperature sensitive in a function which is necessary only during the very early stage of the viral growth cycle were examined. Both mutants had a reverse transcriptase which was three-fold more rapidly inactivated by heat than was wild type DNA polymerase. The ribonuclease H activity of both mutant DNA polymerases was as much as ten-fold more heat labile than wild type activity. In a series of recombinants between avian leukosis virus and the mutant sarcoma virus, in all cases the mutants which showed temperature sensitivity for growth, also showed temperature sensitivity of DNA polymerase indicating that the two characteristics segregate together. Furthermore, revertants of one of the mutants showed a normal DNA polymerase and ribonuclease H.

The DNA polymerase in the virions of hamster leukemia virus (HaLV) was unable to carry out DNA synthesis directed by the endogenous RNA template. The 60-70S RNA of the virus could, however, act as template for added avian myeloblastosis virus DNA polymerase. The enzyme could efficiently

utilize exogenously added ribo- or deoxyribohomopolymers as templates for the synthesis of complementary DNA. Both disrupted virions and purified HaLV DNA polymerase showed a preference for certain ribohomopolymers as templates over the homologous deoxyribohomopolymers. Poly(A)• oligo(dT) was a poor template for the purified enzyme and globin messenger RNA primed with oligo(dT) as well as 60-70S HaLV RNA were inactive as templates. Neither HaLV DNA polymerase nor the murine leukemia virus enzyme exhibited RNase H activity. Electrophoresis of the HaLV DNA polymerase in SDS-containing polyacrylamide gels revealed equimolar amounts of two polypeptides of molecular weight 68,000 and 53,000. The sedimentation rate of the active enzyme in glycerol gradients was consistent with a structure containing one each of the two polypeptides.

Virus-specific RNA sequences were detected in mouse cells infected with murine leukemia virus by hybridization with radioactively labeled DNA complementary to Moloney murine leukemia virus RNA. Approximately 0.3% of the cytoplasmic RNA in MuLV infected JLS V-11 cells was virus-specific and 0.9% of MuLV infected SCRF 60_A cell RNA was virus-specific. Uninfected JLS V-9 cells contained approximately 10-fold less virus-specific RNA than infected JLS V-11 cells. Moloney leukemia virus DNA completely annealed to JLS V-11 or SCRF 60_A RNA but only partial annealing was observed with JLS V-9 RNA. This difference is ascribed to non-homologies between the RNA sequences of Moloney virus and the endogenous virus of JLS V-9 cells. Virus-specific RNA was found to exist in infected cells in three major size classes: 60-70S RNA, 35S RNA, and 20-30S RNA. Agents which remove material from the cell surface were effective in removing a majority of the 60-70S RNA. The 35S and 20-30S RNA is relatively unaffected by these procedures. Approximately 35% of the cytoplasmic virus-specific RNA in infected cells is contained in the membrane-bound material; this RNA consists of some residual 60-70S RNA and 35S RNA, but very little 20-30S RNA. The virus-specific messenger RNA appeared to be mostly 35S RNA.

Murine leukemia viruses and vesicular stomatitis virus (VSV) will phenotypically mix producing pseudotypes in which the VSV genome is enveloped by a membrane containing MuLV-specific protein. Such pseudotypes of murine viruses will grow in murine cells but not, for instance, in Chinese hamster cells. VSV pseudotypes of both N-tropic and B-tropic murine leukemia virus were produced by growing VSV in cells infected by N- and B-tropic viruses. These pseudotypes show very little restriction in cell lines derived either from animals homozygous for $Fv\text{-}1^n$ or those homozygous for the $Fv\text{-}1^b$ allele. Therefore, it would appear that the Fv-1 restriction against murine tumor viruses does not take place at the cell surface but rather is due to an intracellular restriction.

Two new kinds of DNA polymerase activity have been identified in the chick bursa of Fabricius which have not previously been seen in any mammalian tissues. When any dNTP is supplied individually, incorporation into acid-insoluble material is seen. But when all four dNTP's are supplied together, inhibition, of a competitive type, is seen; the opposite of what is normally seen with DNA polymerases.

Observations on terminal transferase activity have been extended to 23 patients with various leukemias and lymphomas. Detectable terminal transferase activity has been found in six (out of eight studied) cases of childhood acute lymphoblastic leukemia. It has not been found in cells from patients with chronic lymphatic leukemia (five cases), lymphosarcoma cell leukemia (four cases) and acute myeloblastic leukemia (six cases). It is likewise absent from a variety of cells in culture and from normal human spleens and circulating lymphocytes. Fractionation of normal human infant thymocytes has allowed identification of a sub-population of thymocytes which contain this enzyme. They are small, buoyant, mitotically active cells, the population which predominates during fetal life and may be T-lymphocyte precursor cells, which are in the early stages of "thymic processing" after entering the thymus from the bone marrow.

Significance to Biomedical Research and the Program of the Institute:

The characterization of the enzyme that produces DNA from the tumor virus's genetic material (RNA) has a very high priority in the VCP. It may provide much more sensitive techniques for locating cancer virus genetic information in human tissues.

Proposed Course: In the coming year, the following specific studies will be undertaken: (1) further study of mutants of Rous sarcoma virus which have a temperature sensitive DNA polymerase; (2) studies on temperature activation of DNA polymerase from RSV; (3) comparison of murine versus avian reverse transcriptase; (4) studies of protection of the enzyme against heat inactivation; (5) study of intracellular reverse transcriptase; (6) reverse transcription of globin messenger RNA to study its base composition; (7) study of VSV pseudotypes of mammalian tumor viruses; (8) cloning of Moloney leukemia virus in NRK cells and search for defective genomes; (9) characterization of new DNA polymerase activities in the chick bursa of Fabricius; (10) screening of human tumors for terminal transferase; and (11) study of terminal transferase in murine leukemia.

Date Contract Initiated: May 1, 1971

JOHNS HOPKINS UNIVERSITY (NO1 CP 43266)

Title: Studies of New Papovaviruses Isolated from Man

Contractor's Project Director: Dr. Richard T. Johnson

Project Officer (NCI): Dr. Bernard Talbot

Objectives: Study the papovaviruses recently isolated from man. (1) Attempts to isolate new papovaviruses, to characterize them, and to study if viral antigens are present in human cerebral neoplasms. (2) Epidemiological studies of the presence of these viruses and their antigens in human sera and urine. (3) Nucleic acid hybridization to look for the presence of papovavirus information in human pediatric tumors.

Major Findings: This is a new contract and major findings have not been reported.

Significance to Biomedical Research and the Program of the Institute:

Papovaviruses are known to cause natural or experimental tumors and to transform cells to tumorigenesis in culture. Therefore, human viruses of this group should be studied as possible cancerogenic agents. Since there is now serological evidence that human infection by certain small papovaviruses is rather common, it is important to assess the role of these viruses in human cancer. As a first step, the viruses must be isolated and characterized biologically and at the molecular level. With this basic information it should be possible to determine whether human tumors contain viral genes or viral gene products.

Date Contract Initiated: February 1, 1974

MELOY LABORATORIES (NO1 CP 43236)

Title: Immunological and Biochemical Studies of Mammalian Viral Oncology

Contractor's Project Director: Dr. John E. Verna

Project Officers (NCI): Dr. Wade Parks
Dr. Edward Scolnick

Objectives: To study biochemically and immunologically the role of viruses in mammalian cancer.

Major Findings: The 30,000 dalton gs proteins were purified from monkey fibrosarcoma and gibbon ape lymphosarcoma type C viruses and a sensitive radioimmunoassay developed. The woolly and gibbon gs proteins were found to be highly related immunologically. However, they cross-reacted very little with other mammalian type C viral gs antigens and antisera. The original woolly monkey and gibbon ape tumors contained high levels of woolly viral antigens. This indicated that the natural tumors from distinct species contained immunologically related viral antigens.

The mouse cell line Balb/c 3T3 and its derivatives transformed either spontaneously or by treatment with a variety of agents including methylcholanthrene and X-irradiation were analyzed for cytoplasmic RNA

complementary to DNA products from the Kirsten strain of murine sarcoma-leukemia virus and from an endogenous type C virus of Balb/c 3T3. While none of these clonal lines spontaneously releases virus they all contained RNA which was partially homologous to a portion of the 35S RNA isolated from these viruses. The parental cell line, Balb/c 3T3 contained a low level of viral-related RNA. There was an increased amount of this RNA in some of the transformed cells.

Employing an ^{3}H-DNA copy of mouse mammary tumor virus (MTV) RNA or the Kirsten strain of murine leukemia virus RNA, it was shown that the DNA from a variety of mouse cells, even those not producing virus, gave readily detectable hybridization reactions. With the type C probes, the DNA from NIH 3T3 and feral mouse cultures had lower levels of hybridization than seen with DNA from RIII, Balb/c or C57 Bl/6 mice. These differences might represent partial homology between the viral genomes present in NIH Swiss and feral mouse cells and the Kirsten murine leukemia probes, and/or might be related to the fact that neither of these cells are inducible to form a complete type C virus, and may lack an essential viral component necessary for complete viral expression.

The ^{3}H-DNA transcript of the type C viruses isolates from gibbon or woolly monkey tumors readily hybridized to the DNA from cells infected with these viruses, indicating multiple copies of virus in these exogenously infected cells. Unlike the murine systems, however, the DNA from natural uninfected primate tumors and a variety of lower mammalian tissues failed to hybridize to these ^{3}H-DNA transcripts, indicating less than one complete copy of either of these type C viruses in these tissues. The lack of reaction with the uninfected primate DNAs examined raises questions as to the origin of these type C viruses in primates.

By RNA:^{3}H-DNA hybridization, Kirsten sarcoma virus was shown to possess two distinct sets of nucleic acid sequences. One set is contained in murine type C helper virus and the other set is contained in rat type C helper viruses. Moloney sarcoma virus had sequences of murine type C virus but not of rat type C helper virus. The results indicated that Kirsten sarcoma virus arose through a process of recombination between Kirsten leukemia virus and nucleic acid sequences found in rat cells. A model for the formation of transforming type C viruses involving the transduction of oncogenic information was proposed.

L8A clone 11 and L8A clone 6 derived from Sykes CCL-51 mouse mammary tumor cell line, although morphologically similar differed significantly in their expression of type B viral information. Clone 11 contained numerous type B particles and high levels of type B specific S1 antigen and cellular RNA which hybridized with MTV ^{3}H-DNA product. Clone 6 did not have detectable particles by electron microscopy, had antigen levels below sensitivity and very little RNA hybridizable to MTV DNA. Both

clones contained comparable levels of MTV DNA sequences. Type C viral expression (gs antigen and RNA levels) in both clones were comparable. These cell lines thus provide an in vitro system for studying the factors that regulate independently both type B and type C viral gene expression.

Other work with MMTV has: (1) standardized MMTV production with chemical, physical and immunological assays as a function of strain and parity; (2) developed and/or improved immunoassays for MMTV proteins by complement fixation, hemagglutination-inhibition and radioimmunoassays; (3) developed procedures for the purification of two of the major structural virion polypeptides, the s1 antigen and a major internal nucleoid polypeptide with a MW of 27,000; (4) prepared antiserum to MMTV and to the purified s1 and P27 in goats and pigs; (5) demonstrated that s1 is a glycoprotein and probably not a core or nucleoid protein; (6) determined the amino acid content of purified MMTV proteins; (7) separated and characterized the MMTV RNA-dependent DNA polymerase from milk demonstrating for the first time its unique features; (8) demonstrated an unexpected biochemical relationship between MMTV and MP-MV of rhesus monkeys. Both prefer Mg^{++} as a divalent cation with synthetic templates and elute ahead of type C polymerases by gel chromatography; (9) demonstrated that all mouse strains examined express MMTV in their milk as a function of genetic control and parity; (10) documented wide-spread expression of MMTV infection throughout the organs of the mouse; (11) confirmed and extended earlier observation that both high and low incidence strains contain comparable amounts of MMTV DNA; (12) consistent with the concept that genetic regulation is the important regulator of MMTV expression, demonstrated that F1 off-spring from reciprocal high and low mammary tumor incidence crosses, resemble the low expressor and show little or no maternal influence; (13) demonstrated that the corticosteroid, dexamethasone, stimulates MMTV in tissue culture thereby providing a possible tissue culture source of the virus; and (14) described for the first time a perirectal papillary hireadenocarcinoma occurring in RIII female mice which is highly associated with MMTV production.

Significance to Biomedical Research and the Program of the Institute:

The ability to detect viral information in transformed cells is basic to establishment of etiological association, and to an ultimate approach to prevention or treatment of cancer.

Proposed Course: Continuation to achieve the objectives described.

Date Contract Initiated: May 25, 1965

UNIVERSITY OF CALIFORNIA (NO1 CP 43212)

Title: Comparative Studies on the Structure and Replication of Murine and Avian RNA Tumor Viruses

Contractor's Project Director: Dr. Peter Duesberg

Project Officer (NCI): Dr. Bernard Talbot

Objectives: To study the RNA structure and the replication of avian and murine RNA tumor viruses.

Major Findings: The RNAs of four replication-defective, transforming murine viruses, Kirsten, Moloney, Harvey and Friend virus, were examined and compared to those of murine leukemia viruses. All four contain an RNA component that is smaller than the RNA of their respective helper leukemia virus. In addition, the replication-defective sarcoma viruses contain an RNA component which is physically indistinguishable from that of leukemia helper virus; it is thought to be the RNA of the respective helper virus present in each stock of replication-defective transforming virus investigated. The smaller RNA components found only in transforming viruses are thought to contain genetic information required for cell transformation and to lack at least some of the genes present in the RNA of helper virus.

It was found that in formamide as in aqueous buffers, the electrophoretic mobility of the a subunit of nondefective avian sarcoma viruses is lower than that of b subunit of transformation defective viruses. This indicates that the a subunit of nondefective avian sarcoma viruses has a higher molecular weight than the b subunit of leukosis or transformation defective viruses.

Comparative studies on the RNAs of corresponding (=same subgroup and strain) avian sarcoma and leukosis viruses have indicated that the a subunit of avian sarcoma viruses is related to the b subunit of leukosis virus like $a = b + x$, wherein x presumably represents a sequence concerned with transforming ability for fibroblasts. This relationship was deduced from comparative oligonucleotide fingerprinting and RNA-DNA hybridization using DNA transcribed from viral RNA by viral DNA polymerase.

Preliminary evidence suggested that a 30-40S precursor could not be demonstrated in murine virus harvested at 3-minute intervals. The failure to demonstrate a 30-40S RNA precursor in murine virus, so far, may reflect a difference in the kinetics of viral release or in the kinetics of 30-40S to 60-70S RNA aggregation between Rous sarcoma and murine leukemia virus.

The 5' endgroup of the 30-40S RNA species of PR RSV-C was determined to be A.

The purified DNA polymerase of two murine viruses, Kirsten MSV (MLV) and Moloney MSV (MLV) was compared to that of avian sarcoma viruses. It was found that the purified DNA polymerase of the murine viruses differs from the avian counterpart in two distinctive properties: (1) It lacks detectable RNase H. (2) It has a low template activity with viral RNA and other natural DNA and RNA templates.

Significance to Biomedical Research and the Program of the Institute:

Basic research on RNA tumor virus structure and replication may provide the basis for understanding viral carcinogenesis which may ultimately lead to the control of human cancer. Studies on the a subunit of 60-70S RNA may pinpoint the localization of the virally transmitted oncogenic information.

Proposed Course: The following will be studied: (1) The genetic relationship between the small RNA components found in replication-defective, transforming murine viruses and the RNA components of helper leukemia viruses. (2) The DNA polymerase of Friend virus and Harvey virus to determine whether it is associated with RNase H and to determine its ability to transcribe viral RNA and natural DNA templates. (3) Avian tumor virus RNA to see if the poly A is at the 3' end. (4) Whether the 60-70S RNA complex of avian and murine tumor viruses is segmented haploid, consisting of different 30-40S subunits, or polyploid. (5) Electronmicroscopy to study the subunit structure of 60-70S tumor virus RNA. (6) Analysis of the RNA of tumor virus recombinants.

Date Contract Initiated: June 29, 1971

UNIVERSITY OF CALIFORNIA (NO1 CP 33332)

Title: Hormonal Control of Gene Expression in Tumor Viruses

Contractor's Project Director: Dr. Gordon M. Tomkins

Project Officer (NCI): Dr. Bernard Talbot

Objectives: To study (1) the mechanisms by which viral transformation disrupts cellular growth regulation, and (2) cellular mechanisms which control the expression of viral genes.

Major Findings: Using cultured S49 mouse lymphoma cells, it was shown that cyclic AMP-activated kinase is responsible for the pleiotypic effects seen. Added cyclic GMP blocks the inhibitory actions of cAMP on membrane transport suggesting that the two regulatory nucleotides have opposing effects: cAMP blocking the growth cycle at a critical point in G1, and

cGMP tending to maintain cell proliferation. Considerations suggest that "oncogene" products block the membrane of transformed cells in a "growth" conformation, which cannot produce pleiotypic mediator (e.g., cAMP) in response to physiological signals instructing the cells to stop dividing.

Dexamethasone, a synthetic glucocorticoid, and cortisol increase both the size and number of polyoma plaques when virus particles are plated on monolayers of either primary or secondary mouse embryo or 3T3 cells. The hormones appear to work via the cytoplasmic receptors which mediate other glucocorticoid-sensitive functions since the specificity of the viral response resembles that of the other responses (e.g., enzyme induction) and anti-inducing steroids can antagonize glucocorticoid stimulation. Steroid pre-treated cells showed no increase in viral function. However, adding dexamethasone later in infection showed that the steroids exert their effects five to ten hours after exposure of cells to the virus. It appears that the steroid does not promote virus uptake or adsorption but participates rather in early viral functions.

Experiments on transformation of primary and secondary hamster embryo cells using polyoma virus were performed in which cells were treated with both virus and hormone for 24 hours. The virus was then diluted and the cells plated in agar. The number of clones appearing under these conditions was used to score for transformation. Dexamethasone and cortisol increased the frequency of transformation 3 to 6-fold while inactive steroid, epicortisol, was ineffective. A "sub-optimal inducer," progesterone, had intermediate effects but, as expected, prevented maximal stimulation by dexamethasone. Dose-response curves show that the optimum dexamethasone concentration is approximately 10^{-8} M, consistent with the amount required to saturate the glucocorticoid receptors. Pretreatment of cells with dexamethasone (followed by steroid removal before exposure to the virus) did not increase the number of transformants, suggesting that the hormone acts only while viral genes are in the cells. Since transformation probably requires expression only of early gene functions the hormones may act to promote the expression of these genes.

Significance to Biomedical Research and the Program of the Institute:

There is considerable evidence that the entire gencme of both DNA and RNA oncogenic viruses may be integrated into the chromosomes of host cells, whether or not such cells show evidence of their presence. Malignant transformation appears to depend, to a large extent at least, on the degree to which viral "oncogenes" are expressed, which means that a major aim of viral oncology must be to understand the biological factors which regulate virus gene expression. A closely related problem concerns the mechanisms which control the cellular specificity both of viral transformation and productive infection. That is, what factors determine the types of cells in multicellular organisms which are

susceptible to transformation or infection by particular oncogenic viruses? Hormones are tissue-specific effectors which selectively control gene expression in a variety of cell types. It is thus an important facet of the VCP to investigate viral gene expression and cellular specificity in the light of the present (and rapidly evolving) information about the mechanisms of hormone action.

Proposed Course: (1) Investigation of the mechanism of growth regulation in transformed and untransformed cells and in cultured S49 Balb/c mouse lymphoma cells. Do the cyclic nucleotides and the glucocorticoids inhibit cell proliferation at exactly the same point in the cell cycle, and if so, by affecting the same underlying process? The relation between membrane receptors, adenylate cyclase and cAMP production. The effects of serum and insulin on cAMP concentrations in S49 cells and the influence which various membrane mutations have on this interaction. The effects of serum starvation and readdition and insulin treatment on cGMP levels. Attempts to achieve cell-free reconstruction of functional membranes containing components from transformed and normal cells with the aim of identifying regulatory factors which differ between membranes from transformed and normal cells (2) Continuation of work on the effects of the glucocorticoids on productive infection and transformation by polyoma virus. Definition of the steroid sensitive period of the cell cycle and attempt to discover the precise nature of the reactions which allow the hormones to promote transformation. Following of the effects of the glucocorticoids on cyclic nucleotide concentrations, enzyme induction, DNA synthesis and the appearance of T antigen. Investigation of whether the transformation of a variety of non-permissive mouse cells (e.g., 3T3) by SV40 is affected by the glucocorticoids. Determination of which serum components are responsible for affecting steroid stimulation of polyoma infection. Determination of the portion of the polyoma life cycle which is steroid-sensitive in one-cycle MEF.

Date Contract Initiated: April 25, 1972

UNIVERSITY OF CALIFORNIA (NO1 CP 33283)

Title: Oncogenesis by RNA Tumor Viruses in Experimental Systems and in Humans

Contractor's Project Director: Dr. Marcel Baluda

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

Objectives: (1) To characterize the nature of the genome of RNA tumor viruses using AMV and SSAV as models, (2) to search for the presence of virus specific DNA in human tumor cells, and (3) to investigate whether oncornavirus specific RNA sequences are expressed in some human tumor cells.

Major Findings: Density gradient sedimentation in alkaline cesium chloride of DNA from normal chicken embryos or leukemic myeloblasts fragmented to a size of 13S revealed that the DNA sequences complementary to 70S avian myeloblastosis virus RNA sedimented in the high guanine plus cytosine region ahead of the main peak of cellular DNA. When the DNA was fragmented into pieces of 6.6S there was a broader distribution of the DNA sequences complementary to the viral RNA.

DNA-RNA hybridization studies between 70S RNA from avian myeloblastosis virus and an excess of DNA from 1) AMV-induced leukemic chicken myeloblasts, or 2) a mixture of normal and of congenitally infected K-137 chicken embryos producing avian leukosis viruses revealed the presence of fast and slowly-hybridizing virus specific DNA sequences. However, the leukemic cells contained twice the level of AMV specific DNA sequences observed in normal chicken embryonic cells. The fast reacting sequences were two to three times more numerous in leukemic DNA than in DNA from the mixed embryos. The slowly reacting sequences had a reiteration frequency of approximately 9 and 6, in the two respective systems. Both the fast and the slowly-reacting DNA sequences in leukemic cells exhibited a higher T_m (2°) than the respective DNA sequences in normal cells. In normal and leukemic cells the slow hybrid sequences appeared to have a T_m which was 2° higher than that of the fast hybrid sequences. Individual non-virus producing chicken embryos, either gs-antigen positive or negative, contained 40 to 100 copies of the fast sequences and 2-6 copies of the slowly hybridizing sequences per cell genome. Normal rat cells did not contain DNA that hybridized with AMV RNA whereas non-virus producing rat cells transformed by B-77 avian sarcoma virus did, but only the slowly-reacting sequences. The results demonstrate that leukemic cells transformed by AMV contain new AMV specific DNA sequences which were not present before infection.

An increase in the cellular content of viral DNA was detected as early as one hour after infection with AMV or RSV. Early after infection most of the newly synthesized viral DNA consists of free small molecular weight molecules. Within 24 hours, the viral DNA becomes covalently linked to host cell DNA. In cells transformed for a long time and in normal cells all the virus specific DNA sequences appear to be integrated.

Denatured DNA from leukemic myeloblasts or uninfected chicken embryos, immobilized on nitrocellulose filters, was hybridized to a vast excess of ^{3}H 70S RNA from purified avian myeloblastosis virus. The viral RNA was eluted from the RNA-DNA hybrids, purified and then rehybridized in solution to an excess of either leukemic or normal chicken embryonic DNA. This study revealed that all the slow and the fast hybridizing viral RNA sequences detectable by liquid hybridization in DNA excess, had hybridized to the filter-bound DNA. Both techniques also gave similar values for the number of 28S ribosomal RNA genes contained in a chicken cell genome.

the GLV endogenous reverse transcriptase system and exhaustively hybridized with normal human DNA or RNA will be used as a probe to look for oncornavirus specific RNA sequences in human tumor cells. Similar experiments will also be carried out with the primate oncornavirus SSAV-1 since this virus might be expected to exhibit a greater homology than GLV with putative human oncornaviruses. (3) In addition, new isolates of promising candidate human oncornaviruses will be characterized and tested. Once a good RNA or DNA probe is obtained, human tumors will be screened according to their content of oncornavirus specific DNA sequences and the degree of transcription of those sequences. Concomitantly, different normal tissues from cancer patients will be tested to determine (a) whether the oncornavirus DNA sequences are localized in the neoplasia, or uniformly distributed in all tissues, and (b) whether transcription of virus specific DNA sequences is limited to the tumor cell.

Date Contract Initiated: June 8, 1972

UNIVERSITY OF ILLINOIS (NO1 CP 43318)

Title: Studies on the Molecular Mechanism of Carcinogenesis by Oncogenic Viruses

Contractor's Project Director: Dr. Giampiero di Mayorca

Project Officer (NCI): Dr. Bernard Talbot

Objectives: The work continues to focus on 1) temperature sensitive mutants of polyoma virus and 2) the human papovavirus, BK virus.

Major Findings: Seventy-five different temperature sensitive mutants of polyoma virus have been characterized from the point of view of lytic cycle and transformation.

Mutant Py235, a non-transforming mutant, was found to be an absorption mutant, especially defective for BHK_{21} cells. Gel electrophoresis revealed no detectable difference in molecular weight or the species of polypeptides between Py235 and the wild type. Also "fingerprint" peptide maps revealed no difference.

A "cold" mutant for transformation was found which is only slightly ts for the lytic cycle. Preliminary reversion experiments of cells transformed by this mutant at the permissive (high) temperature were positive; when shifted at the low temperature the cells reverted to normal.

A skin fibroblast cell line from an adult marmoset, HF-250, has been transformed by a small plaque variant of polyoma virus (PySP). Under

H^3-labelled 35S AMV RNA was exhaustively hybridized with excess of normal chicken DNA to remove all sequences which the virus DNA has in common with normal cells. The residual H^3-RNA which failed to hybridize was fractionated by hydroxylapatite column chromatography to separate hybrids from unpaired H^3-RNA. The residual RNA hybridized to leukemic chicken DNA but did not rehybridize with normal chicken DNA. It is concluded that leukemic cells' DNA contain viral specific sequences which are absent in DNA from normal cells and that an RNA probe prepared in this fashion can be used to screen DNAs for leukemic specific sequences.

In normal chicken cells, oncornavirus DNA appears to be associated with cell sequences reiterated 1200 times, and each integration unit appears to have a maximal size approximately equivalent to the 35S RNA subunit of the virion. In infected cells some viral DNA also appears to be integrated among similarly reiterated cellular DNA sequences. However, there are additional viral sequences in infected cells which behave as if they were integrated adjacent to unique sequences.

Hybridization of 70S GLV RNA with an excess of DNA from normal and leukemic human tissues revealed that some of the neoplastic human tissues contained viral specific DNA sequences equivalent to 15% of the viral genome and that normal tissue DNA lacked homology with GLV RNA. Also, the DNA sequences homologous to GLV RNA could be separated from the bulk human DNA in alkaline CsCl because they have a higher G+C content.

SSAV-1 viral RNA contains 50-60S RNA and also RNA molecules with sedimentation coefficients of 28S, 18S, and 4-10S. Unlike the 50-60S RNA, these smaller RNA molecules lack poly(A) sequences. Upon heat denaturation, the native 50-60S RNA yields distinct 28S and 18S subunits which both appear to contain poly(A) sequences. The G+C content of the SSAV-1 50-60S RNA is 55.5%. The 28S RNA from purified virions has a G+C content identical to 28S ribosomal RNA and unlike the 28S RNA obtained from the melted 50-60S viral genome, it contains essentially no poly(A) sequences. In preliminary experiments, 60-70S SSAV-1 RNA did not hybridize significantly with DNA from either normal or cancerous tissues.

Significance to Biomedical Research and the Program of the Institute:

This program will elucidate the mechanism of oncogenesis by RNA tumor viruses in experimental animal systems, e.g., avian myeloblastosis virus in chicken cells, and simian sarcoma associated virus in primate cells and will investigate whether some human malignancies are induced by putative human RNA tumor viruses.

Proposed Course: (1) Studies will continue using AMV as a model system for oncornaviruses. (2) Gross leukemia virus (GLV) RNA will be used to look for DNA sequences in various human tumors by hybridization of DNA trapped on filters with an excess of GLV-RNA and by liquid hybridization of viral RNA in an excess of cellular DNA. Also, DNA made *in vitro* using

soft agar conditions (0.35%) described by MacPherson, the HF-250 cells formed discrete colonies by 16 days after infection with 100 PFU/cell of purified PySP. The transformation of cell species other than rodent, e.g., BHK, has not been previously described.

Rat fibroblasts and sister cells persistently infected by RLV each transformed by polyoma virus with the same frequency when colony growth in soft agar was used as the method for assaying viral cell transformation.

BK virus, a human papova virus, has been successfully grown in high titers in human embryonic cells and has been purified. A quantitative, reproducible plaque assay has been developed for BK virus using early passage HEK cells whose monolayers remain flat and chiefly fibroblastic.

The ability of BK virus to transform BHK_{21} clone 13 cells and the ability of the BK transformed BHK_{21} clone 13 cells to induce tumors in adult hamsters has been established. In addition to BHK cells, BK is capable of transforming a primate cell type, Marmoset fibroblast, designated as HF-250 cell line. A dose response relationship of BK transformation has been established with the BHK_{21} clone 13 cells in which the multiplicity of infection was determined by the plaque assay.

Hemagglutination and hemagglutination inhibition indicated that the distribution of antibodies to the BK virus is consistent with the published figures for human populations. To date, 39 sera from 29 individuals have been tested with 78 percent of the individuals tested positive. In addition, a survey of sera from adult, colony-born, and newly imported Marmosets has also been conducted with a majority of sera tested giving positive results.

BK virus proteins have been examined by SDS-polyacrylamide gel electrophoresis and peptide mapping of tryptic digests. Preliminary results indicate that BK virus contains fewer peptides than either polyoma or SV40. Preliminary studies indicate that the BK virion contains two species of DNA which co-sediment with forms 1 and 11 of polyoma virus DNA. The data indicate that the DNA of the BK virion is a covalently closed, circular molecule of 3×10^6 daltons.

A recent report from this laboratory described a conditional state of malignant transformation induced by chemical carcinogens in several subclones of the BHK_{21}/13 hamster cell line. All subclones tested, whether transformed by dimethylnitrosamine (DMN) or nitrosomethylurea (NMU), exhibited the transformed phenotype only at a high temperature (38.5°C) and not at a lower temperature (32°C). It was now found that when these conditionally transformed subclones are infected with either hamster sarcoma virus or polyoma virus, they can express the transformed phenotype at 32°C.

Significance to Biomedical Research and the Program of the Institute:

Studies on small icosahedral DNA viruses as SV40 and polyoma have

contributed an impressive body of information in recent years on the interactions between these viruses and cells both at the level of the vegetative growth of these viruses and of their transforming activity. The genetic analysis of the genome of these viruses, by the use of ts mutants may provide a major advance in our knowledge of the mechanism of transformation at the molecular level, and could if the right kind of mutants became available, bring information on the nature of the viral gene responsible for the maintenance of the transformed state of the cell.

These studies have recently become of great practical importance in the light of recent reports on BK virus, a new human papova transforming virus. BK virus is important as a potential candidate "oncogenic" virus for the human population, for the following reasons: (1) BK virus is capable of malignant transformation of BHK_{21} clone 13 cells and marmoset fibroblast, HF-250, cells. (2) BK virus is of human origin presumably not closely related antigenically to polyoma or SV40. (3) The BK virus appears as a widespread infectious agent in the human population based upon hemagglutination inhibition serum studies.

Proposed Course: (1) Continuation of the characterization of the contractor's large collection of temperature sensitive mutants of polyoma virus. The most important questions to be answered are: a) Is the genetic information responsible for the maintenance of transformation "viral" or "cellular" (in such case the virus would act as a mutagen)? b) If the information responsible for the maintenance of transformation is viral, is the gene involved necessary for the vegetative growth of the virus or not (as in the case of Rous sarcoma virus)? c) How many genes constitute the genome of polyoma virus as determined by complementation? (2) Investigations on the status of polyoma genome in hamsters, rat, and marmoset cells. Patterns of oligonucleotides obtained by digestion of polyoma DNA will be obtained and the oligonucleotides used for hybridization experiments to learn what portion of the viral genome are preferentially integrated. (3) Continuation of the biological characterization of BK virus, a human papovavirus; its antigenic relation to SV40 and polyoma. Host range studies, transformation attempts with human cells. EM structural studies both of the viral particle and its DNA. Biochemical characterization of the viral DNA, its size, base composition, buoyant density, and Tm. Biochemical characterization of the proteins of the viral particle in comparison with SV40 and polyoma. (4) Hybridization experiments between BK DNA and human cell DNA from a variety of human tumors to find out if BK virus genome is integrated in the genome of human tumor cells and in what tumors and how frequently. (5) Studies of the extent of complementarity between BK, SV40, and polyoma DNA to find out if they are related from a genetic point of view. (6) Investigation of the restriction enzyme patterns of BK DNA and comparison with the patterns of polyoma and SV40.

Date Contract Initiated: December 9, 1971

UNIVERSITY OF MINNESOTA (N01 CP 33357)

Title: The Cell Surface in Lymphoid Malignancies and Virus-Transformed Cells

Contractor's Project Director: Dr. John Kersey

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

Objectives: To search for viruses in cancer patients with immunodeficiency diseases, and to study the role of the cell surface in malignant transformation.

Major Findings: The Immunodeficiency Cancer Registry has continued and increased leading to 164 known patients with immunodeficiency who develop malignancies and nine cases known to be alive with tumors which may be available for procurement. The bulk of tumors in children with primary immunodeficiency diseases are lymphoreticular.

One patient with combined (T and B) system immunodeficiency developed a lymphoreticular malignancy following use of transfer factor. This lymphoid malignancy is now in cell culture, was sent to investigators within the Virus Cancer Program, and is available to others. Tumor material will probably soon be available from several other known patients with immunodeficiency syndromes who are currently bearing cancers for immunologic and virologic analysis.

An antihuman thymocyte serum has been developed in goats which after absorption with human red cells and B lymphocytes (from a patient with CLL) was found to be very useful for detecting human thymocytes and peripheral blood T lymphocytes using a cytotoxicity assay.

Ten of sixteen children with acute lymphoblastic leukemia (ALL) were found to have lymphoblasts with T cell markers, and thus the apparent origin of these leukemias was in T lymphocytes. In most cases not all lymphoblasts bound sheep erythrocytes or carried thymocyte antigen suggesting that dedifferentiation may be a significant aspect of the disease. One child was found with extramedullary (subcutaneous) origin of lymphoma with T lymphocyte markers. Another child with acute leukemia had leukemic cells with both T (E-binding) and B (EAC-binding) markers. Preliminary indications are that children with T cell leukemia have a worse prognosis than those whose leukemias have no detectable differentiative markers.

In a study of 30 cases of acute leukemia using immunofluorescence to detect surface immunoglobulins, an acute leukemia with a B cell origin was found twice. Monoclonal B cell proliferation was observed in peripheral blood of a child who presented with non-African Burkitt's

lymphoma with leukemic transformation. The other patient was 75 years old and presented as acute lymphoblastic leukemia with 96% of blasts in peripheral blood. All these cells were bearing one type of heavy (mu) and one type of light (lambda) chain.

Fifteen cases of untreated Hodgkin's lymphoma revealed normal or low normal percentages of B lymphocytes with normal distribution among classes. Approximately 70% of these patients showed a deficiency in cell mediated immunity when measured by several parameters *in vivo* and *in vitro*. Studies of patients with untreated non-Hodgkin's malignant lymphoma (not CLL) indicated evidence of monoclonal B cell proliferation in peripheral blood of 7 of 24 cases.

Persons with several forms of primary immunodeficiency (and high cancer risk) almost invariably have decreased numbers of T lymphocytes and increased numbers of B lymphocytes. Patients with X-linked (Bruton's) agammaglobulinemia lack lymphocytes with surface immunoglobulins but have adequate numbers of lymphocytes with receptors for complement (C3), suggesting that B lymphocytes are present but defective in immunoglobulin expression.

In a patient with congenital absence of the thymus (Di George syndrome) who lacked the T cell population, B cells were increased in percentage and also in absolute number both in peripheral blood and in the removed lymph node. This child was successfully treated with a fetal thymus transplant and showed a gradual decrease of B lymphocytes in the peripheral blood parallel with the restoration of the T cell population and T cell function. There is a similar over-proliferation of B cells in patients with lepromatous leprosy who regularly show a depression of cellular immunity but have well preserved humoral immunity.

In persons with X-linked agammaglobulinemia low percentages of immunoglobulin-bearing lymphocytes were found in the peripheral blood. In one patient with X-linked agammaglobulinemia lacking serum immunoglobulins and B lymphocytes of three major immunoglobulin classes, there was a normal level of serum IgE and a normal percentage of IgE-bearing lymphocytes.

Binding of sheep erythrocytes (SRBCs) to human T lymphocytes is temperature-dependent with maximum binding at 4–21°C. Temperature dependence is abolished by pretreatment of lymphocytes with *Vibrio cholera* neuraminidase. Binding is inhibited by the membrane-active drug cytochalasin B but not by colchicine. Electron micrographs (scanning and transmission) show binding of SRBCs to microvilli projecting from the surface of T lymphocytes. The results suggest that active membrane metabolic processes, probably associated with some degree of receptor motility is necessary for antigen binding to T lymphocytes.

Cyclic AMP and its analog, dibutyryl cAMP, inhibit SRBC binding to human T lymphocytes. Cyclic GMP and its analog, 8 bromo cGMP, did not influence

binding. cAMP inhibited binding of SRBCs to mouse spleen cells, while cyclic GMP alone had no effect. Cyclic GMP was effectively able to prevent the cyclic AMP induced inhibition of antigen binding.

Membrane lipids of human and mouse lymphocytes were studied following interaction with the mitogens PHA and Con A. Using a fatty acid spin label (6 doxyl heptadecanoate) and electron paramagnetic resonance, it was found that membrane fluidity is significantly increased within 5 minutes after mitogen binding. Maximum increases in fluidity occur at 15-30 minutes with return to normal values at 60 minutes. These data suggest that enhanced membrane fluidity is a very early event in the lymphocyte triggering process with a time course closely following that of the rise in cGMP.

Significance to Biomedical Research and the Program of the Institute:

The main premise behind initiation and continuation of this contract is the idea that there should be a much better chance of recovering a complete human tumor virus from a tumor in an immunologically incompetent patient than in an immunologically competent one. The contract provides information and materials from patients either with immunodeficiency diseases or iatrogenically immunosuppressed, which are investigated for the presence of tumor viruses. The recovery of a complete human tumor virus would greatly aid the study of the relationship of viruses to human cancer, leading towards an effective means of prevention and control.

Basic studies on (a) the role of the cell surface in malignant transformation and (b) the modulating influences of membrane structure and fluidity and virus release will aid our understanding of the role of the cell surface in cancer. This may ultimately lead to ways to control the process of malignant metastases, and may aid in the development of anti-cancer vaccines and in immunotherapy.

Proposed Course: 1. Continuation of studies linking immunodeficiency, cancer, and oncogenic viruses. (a) Continuation of the immunodeficiency-cancer registry. (b) Procurement of tumors arising in immunodeficient individuals for direct virologic analysis and establishment in tissue culture to search for tumor viruses. (c) Continuation of clinical studies of tumors arising in immuno-deficient individuals. (d) Studies of antitumor immune responses to tumors in immunodeficient individuals. 2. Continuation of studies on the role of the cell surface in malignant transformation, including studies of: (a) Membrane markers found on human T cell and B cell leukemias and lymphomas. (b) Use of antithymocyte serum to detect thymocytes and peripheral T lymphocytes. (c) Cyclic nucleotides (cAMP and cGMP) as intracellular regulators of differentiation of mouse T lymphocytes, human leukemias and human lymphomas. (d) Influence of vinca alkaloids and glucocorticoids on the induction of differentiation in leukemia. 3. Studies of modulating influences of membrane structure and fluidity and virus release, specifically: (a) to examine the effect

of membrane active agents vinblastine, colchicine, lumicolchicine, cytochalasin B, and phorbol myristate acetate on the distribution of the intramembranous particle-intrinsic membrane protein complex, the organization of the plasma membrane lipids, and the extent and pattern of viral release from known producer transformed 3T3 cells, (b) to determine the influence of changes in the distribution of intrinsic membrane proteins and the order of membrane lipids on virus release during the cell cycle and in log phase growth cultures, (c) to determine if increased membrane fluidity which accompanies malignant transformation is in some manner related to the state of differentiation of the cell.

Date Contract Initiated: May 13, 1971

UNIVERSITY OF WISCONSIN (N01 CP 22022)

Title: Studies on the Role of RNA Tumor Viruses and Related Genetic Information in Induction of Tumors by Chemicals

Contractor's Project Director: Dr. Robert C. Nowinski

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

Objectives: To investigate the role of MuLV and its gene products in spontaneous leukemia and in chemically mediated oncogenesis. Both in vivo and cell culture oncogenesis systems are studied by a variety of immunological, enzymological, hybridization, and genetic techniques.

Major Findings: Twelve chemically transformed cell lines and twelve non-transformed cell lines were classified according to morphological type and were observed for over 300 days in tissue culture. There were no alterations in the transformed cell lines which suggested a progression to a more malignant morphology. These same lines are also being used to determine if there is a progression or increase of malignant potential after continued tissue culture passage. The transformed cell lines were inoculated at an early time (2-4 weeks) and again at a later time (6-8 months) after the original foci were picked. It appears that malignant potential does increase as evidenced by (1) a shorter latent period for tumors and (2) a greater percentage of cell lines causing tumors.

New contact-sensitive cell lines have been established from NZ^B and C57BL/6 mouse strains.

A wide variety of cell lines have been characterized for the production of MuLV. Characterization included: (1) immunofluorescence tests for MuLV-gs-1 antigen, (2) examination of tissue culture fluids for the release of polymerase containing particles, (3) mixed cytopathogenicity tests with XC indicator cells, and (4) XC plaque assays of tissue culture

fluids. All normal and transformed cell lines derived from C3H/10T1/2 were invariably negative for indicators of MuLV production. The AVP cell line, derived from ventral prostate cells of AKR, was positive for all viral parameters.

A wide variety of cell lines derived from normal and transformed C3H/10T1/2 cells were examined for inducibility of MuLV after treatment with IdU. Cells were incubated (when at approximately 50% confluence) with 50 μg/ml of IdU for 24 hours. Three days later the cells were trypsinized and replated on microscope slides for gs-1 immunofluorescence tests. Cells were grown for 24 hours on the microscope slides prior to fixation. Induction of MuLV gs-1 antigen was observed in several transformed lines of of C3H/10T1/2. In contrast, control 10T1/2 cells treated in a similar fashion showed only minimal degrees of expression of gs-1. In general, it appeared that with these conditions the transformed cells expressed gs-1 antigen to a greater extent than their normal counterparts. These experiments demonstrated that gs-1 antigen production could be induced in both normal and transformed cells, even though these cells normally repress the expression of this antigen.

Studies have been initiated to examine the relative roles of MuLV and metabolic activation of carcinogen in chemical oncogenesis. Mice from the reciprocal backcrosses (C3H x (C3H x AKR)F_1 and AKR x (C3H x AKR)F_1 have been typed by tail biopsy for the production of infectious MuLV and then inoculated subcutaneously with 250 μg 3-methylcholanthrene to test for susceptibility to chemical carcinogenesis. In subsequent months, as tumors occur in these mice, each animal will be typed for inducibility of aryl hydrocarbon hydroxylase.

Mice of the C57L x (C57L x AKR)F_1 backcross were examined for genetic segregation of infectious leukemia virus (MuLV), MuLV gs-1 antigen, and the MuLV-induced cell surface antigens G_{IX}, GCSA, G_L, and G_T. Segregation ratios indicated that (1) virus production was controlled by two non-linked dominant genes, (2) expression of gs-1 antigen was controlled by three non-linked dominant genes, (3) expression of G_{IX} and GCSA antigens was controlled by one dominant gene, and (4) expression of G_L and G_T antigen was controlled by two non-linked dominant genes. Linkage analysis between genes controlling virus production and expression of gs-1 antigen revealed three phenotypes--virus$^+$/gs-1$^+$, virus$^-$/gs-1$^-$, and virus$^-$/gs-1$^+$. These phenotypes segregated in ratios consistent with control of gs-1 by three unlinked genes, two of these genes being linked to virus production. Genes controlling the expression of G_{IX} and GCSA antigens were closely linked. Expression of G_{IX} antigen was not linked to expression of G_L or G_T antigens or to the production of MuLV. Genes controlling the expression of G_L and G_T antigens were loosely linked; expression of G_L and G_T antigens was loosely linked to virus production.

Significance to Biomedical Research and the Program of the Institute:

Carcinogenic chemicals can cause cancer in humans, mice and other animals.

An essential step toward the objective of the VCP is to determine what role, if any, viral genetic information might play in this process, as well as in spontaneous neoplasia. The work in mice under this contract will provide a guide to the solution of this problem in humans.

Proposed Course: Continuation to achieve the objectives described.

Date Contract Initiated: September 1, 1971

BAYLOR COLLEGE OF MEDICINE (NO1 CP 43355)

Title: Nonsense Suppressor Mutants in Mammalian Cells

Contractor's Project Director: Dr. Thomas Caskey

Project Officer (NCI): Dr. Edward Scolnick

Objectives: To develop suppressor mutants of mammalian cell lines and use these to investigate the genes involved in maintaining the transformed state in cell cultures.

Major Findings: It has been determined that single step selection of $HGPRT^-$ clones is a rare event. Using a variety of mutagen conditions and selection protocols, in excess of 10^{10} cells have been examined for the expression of this genetic defect. A number of variant clones have been isolated that are totally deficient in hypoxanthine-guanine phosphoribosyltransferase (HGPRT). Many of these clones are $HGPRT^-$, CRM^-, and can be reverted to $HGPRT^+$ and therefore, have properties commonly associated with nonsense mutations.

A large number of different Chinese hamster cells which are TK^- have been isolated. Also, (1) all mutants studied thus far yield revertants to the TK^+ state (with the aid of chemical mutagens) at frequencies of 10^{-6} to 10^{-5}, (2) the revertant TK^+ clones can attain 50% of wild type activity, and finally (3) the TK^+ revertant clones can then yield TK^- mutants with ease (10^{-5}). Since reversion is accomplished in a single step event, it is more likely that a clone with multiple and possible different TK mutations will only revert one locus. Thus isolation of enzyme negative clones from such TK^+ revertants can proceed in a single step fashion at high frequency since only a single gene need be mutated. In essence, a gene haploid strain is created by these isolations for study.

A simple and sensitive in vitro approach has been developed for detection of mammalian suppressor tRNAs; it utilizes RNA isolated from an amber coat mutant (sus3) of the bacteriophase f2. Extracts from a non-suppressing strain of E. coli supplemented with tRNA and aa-tRNA synthetases isolated from cultured Chinese hamster cells, are used for in vitro translation

of the mutant phage RNA. Addition of *E. coli* amber suppressor tRNA to these extracts stimulates $[^3H]$valine incorporation into protein. The sensitivity for detection of this suppressor tRNA is enhanced if extracts are pretreated with sheep immunoglobulin directed against the *E. coli* release factor, RF-1. Chinese hamster $[^3H]$Val-tRNA or *E. coli* $[^3H]$Val-tRNA were both found to be suitable precursors to radioactive protein when extracts were supplemented with *E. coli* suppressor tRNA indicating that Chinese hamster aa-tRNA participates in the synthesis of protein on *E. coli* ribosomes. This analytical procedure thus provides a simple and direct method for examining mammalian cells for amber suppressing tRNA.

To increase understanding of the mutational alteration of mammalian cells in culture, the isolation of variants of Chinese hamster lung cells that have a thermosensitive hypoxanthine-guanine phosphoribosyltransferase (HGPRT) was undertaken. Wild-type cells are sensitive to 6-thioguanine (6-TG) because HGPRT converts this analogue to a ribonucleotide, but they can grow in medium containing hypoxanthine, aminopterin, and thymidine (HAT) because the enzyme allows utilization of hypoxanthine when aminopterin inhibits purine biosynthesis. Cells were first selected that were able to form colonies at 39° in medium containing 6-TG. Those colonies that continued to grow when changed to HAT at 33° were isolated for further study. Some of the selected cell lines were sensitive to 6-TG and resistant to HAT at 33° but resistant to 6-TG and sensitive to HAT at 39°. They incorporated $[^{14}C]$ hypoxanthine into nucleic acids at 33°, but that incorporation was greatly reduced when the cells were maintained at 39°. HGPRT activity was detectable in extracts prepared from cells grown at 33°, but not from those grown at 39°. The enzyme from temperature-sensitive cells was precipitated by antiserum directed toward the wild-type enzyme. However, it could be distinguished from the wild-type enzyme *in vitro* by its increased heat lability. These data indicate that the thermosensitive variants carry a mutation in the gene for HGPRT that affects the stability of the enzyme.

Since definitive assignment of mutational type may require structural analysis of HGPRT, efforts have been initiated directed toward this goal. HGPRT was purified from crude cellular extracts >1000-fold by guanine-agarose affinity column chromatography.

Mutant stocks of Sindbis virus have now been prepared using nitrosoguanidine and U.V. mutagenesis. These stocks yield ~3 percent temperature sensitive mutants when tested against both Chinese hamster and 3T3 cells.

Significance to Biomedical Research and the Program of the Institute:

Understanding of the mechanism of action of tumor viruses will require an understanding of the specific genes of the viruses that are responsible for transforming a normal cell into a tumor cells. For these studies,

viral and cellular mutants will be essential. The system of suppressor mutants that Dr. Caskey proposes to develop has unique advantages over temperature sensitive mutants. They are simpler, less expensive, and produce "absolute, non-leaky" mutants.

Proposed Course: Work will proceed towards isolation of mammalian cells with SV^+ function.

Date Contract Initiated: January 15, 1972

HARVARD UNIVERSITY (NO1 CP 33390)

Title: Oncogenic Herpesviruses in Primates

Contractor's Project Director: Dr. Luis Melendez

Project Officer (NCI): Dr. Roy Kinard

Objectives: To further characterize two oncogenic herpesviruses, H. saimiri and H. ateles, known to be oncogenic in Aotus and Saguinus sp. and two new isolates, H. aotus and H. saguinus, and to determine if other new herpesviruses, isolated from primates indigenous to South and Central America, are oncogenic in the host or other species.

Major Findings: Spider monkeys have been shown to be latent carriers of several H. ateles strains. H. ateles strains can easily be obtained by co-culturing spleen derived lymphocytes with permissive cultures. More than 50% of the animals tested are carriers of H. ateles strains. Ten H. ateles strains have been obtained; all have been determined to be related to the prototype H. ateles, strain 810, by serum neutralization and fluorescent antibody techniques. HVA-73 (Chicago strain) is also oncogenic in cotton-top marmosets. Strain 16872, stock E460H, was inoculated in cotton-top marmosets; it did not induce malignant lymphoma nor did it protect the animals against the challenge with the H. ateles prototype, strain 810.

In studies in owl monkeys (Aotus trivirgatus), it was proved for the first time that H. saimiri lymphoma is a contagious disease, and not only an experimental infectious disease. Koch's postulates were proved with this natural H. saimiri lymphoma in owl monkeys. An epizootiological trip to the zone of Iquitos and Pucalpa, Peru indicated that the Aotus species is practically free of H. saimiri, and is most likely that this agent is transmitted horizontally by the Saimiri species. Several adeno-virus strains have been isolated from the Aotus species and they are being characterized.

H. saimiri has been inactivated with ultraviolet light, and the inactivated virus has been inoculated in cotton-top marmosets, (Saguinus oedipus).

H. saimiri has been passed serially more than 32 times in dog fetal lung (DFL) cultures. The serial passage has brought the titer of *H. saimiri* for OMK cells from 5.5/ml to ≤ 0.5/ml. Twelve *H. saimiri* antibody negative owl monkeys have been inoculated with a 22nd passage of *H. saimiri* in DFL cultures. The incubation period and incidence of malignant lymphoma in *Aotus species* inoculated with *DFL-H saimiri* passed 22 times in DFL cells is less than that observed with the wild parent virus.

Antibodies to the numerous isolates obtained from South American monkeys are being prepared.

Significance to Biomedical Research and the Program of the Institute:

The knowledge gained in the study of these herpesviruses will provide insight into malignant processes of man in which herpesviruses are considered likely candidates for etiology: Epstein-Barr virus (EBV) in Burkitt's lymphoma, and *Herpes simplex* type 2 in carcinoma of the cervix, and will provide well characterized antigens and other reagents for testing for presence of related viruses in humans.

Proposed Course: (1) To determine the oncogenic spectrum of herpesviruses in various species of primates from the Old and New World. (2) To determine if leukemia develops in any of the susceptible non-human primate species, as has been demonstrated earlier with *H. saimiri* in monkeys. (3) To determine susceptibility in small laboratory animals. This would allow the selection of animal species of lower cost than non-human primates for these studies. (4) To further characterize the viruses by biological procedures (plaquing, cell susceptibility and serology). (5) To investigate the epizootiology of *H. ateles*, *H. saimiri* and the new herpesvirus isolates in the natural environment of these South American monkey species. (6) To determine if herpesviruses attenuated by passage *in vitro* can be employed to prevent the malignant lymphoma induced by the parent virus in the susceptible animal species. (7) To determine if the oncogenic capacity of *H. saimiri* or *H. ateles* can be modified by U.V. or x-ray treatment of their DNA.

Date Contract Initiated: June 26, 1972

MELOY LABORATORIES (NO1 CP 43207)

Title: Spontaneous and Virus-induced Neoplastic Transformation

Contractor's Project Director: Dr. John E. Verna

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

Objectives: To study spontaneous and virus-induced neoplastic transformation, especially focusing on the extent of expression of endogenous type C viral information in normal and transformed cells and attempts to recover such complete endogenous type C viruses; and to use this information to find and characterize tumor virus information in human tumors, and to attempt to recover a human type C virus.

Major Findings: Type C viruses have been isolated from baboon tissues. The first isolate was obtained from a baboon placenta and the virus was found to grow well in a variety of heterologous cells, but especially in a dog thymus line. Several additional viruses were isolated from baboon spleen, lung, and testes. The DNA product of an endogenous reaction hybridized to normal baboon cell DNA and also to the cellular DNA of other primates (patas, African green, and two species of macaques--rhesus and stumptail). Viral expression (RNA, gs antigen) can be detected in normal baboon spleen, testes, and placenta. Eight to 12 DNA copies are found per diploid genome in all normal baboons tested. This is the first demonstration of endogenous type C virus in primates.

In contrast to the results with baboon type C virus, no nucleic acid sequences homologous to ^{3}H-DNA transcripts prepared from type C viruses isolated from tumor tissue of a woolly monkey and a gibbon ape could be detected in any primate tissue examined (including woolly monkey and gibbon ape). Thus, these viruses do not seem to be endogenous primate viruses. A partial nucleic acid homology was found between woolly monkey and gibbon type C viral ^{3}H-DNA and normal mouse cellular DNA.

There is partial sequence homology between the endogenous cat type C (RD-114/CCC) virus group and the endogenous primate virus group; the gs antigens and reverse transcriptases of viruses of these two groups share antigenic determinants with one another and their pseudotypes interfere with one another. The results indicate a closer evolutionary relationship between these two groups of viruses that would not have been expected based on the extensive genetic divergence between the species.

A reverse transcriptase has been purified from three cases of human acute myelogenous leukemia in collaboration with Dr. Robert Gallo's group. The enzymes have antigenic properties closely related to those of the type C viruses found in woolly monkeys and gibbon apes.

A new host range class of endogenous murine type C viruses has been identified which are unable to replicate in any mouse cell line tested so far, but replicate well in a variety of other mammalian cells, including rhesus monkey, dog, mink and rat cells. Because of the sensitivity of the rabbit cell line, SIRC, for detecting this group of viruses, they have been called "S-tropic" viruses. By host range properties and nucleic acid hybridization studies, this new class of viruses can be distinguished from both "N-" and "B-" tropic murine

type C viruses. The S-tropic virus is preferentially induced by halogenated pyrimidines from the Balb/3T3 line, although low levels of N-tropic type C virus are also produced. S-tropic virus has also been shown to be spontaneously released from certain Balb/3T3 derived cell lines. Balb/c splenocytes preferentially release S-tropic type C virus after induction and also after graft-versus-host or mixed splenocyte reactions. Spleens from older Balb/c animals generally contain S-tropic virus, whereas young animals do not. Normal weanling mice of C57BL, CBA, DBA, NZB, C58, and AKR strains have S-tropic virus. The S-tropic viruses from Balb/c cells and the S-tropic viruses from NIH Swiss cells are related but different.

A tissue culture system using an SV40-transformed human cell line has been developed to study the transforming effect of various sarcoma virus pseudotypes. This continuous line offers several advantages compared to diploid cells; transformed foci are recognized within five days, and the system lends itself to easy quantitation of transformation. Another transformation system using a mink cell line has proven to be useful for transformation assays since the S-tropic mouse viruses as well as both groups of cat viruses and both the infectious and endogenous primate viruses grow readily in these cells. Pseudotypes of mouse sarcoma, feline sarcoma, and woolly monkey sarcoma virus have been produced and the same mink cell has been transformed by sarcoma viruses from these three different species. Using these cells, it has been possible to generate feline sarcoma transformed nonproducer cells as well as mouse sarcoma virus transformed nonproducer cells. Each of these can be rescued by a variety of type C viruses enabling the study of pseudotypes with sarcoma and leukemia virus information derived from different species.

An endogenous type C virus has been isolated from a pig cell line. The reverse transcriptase and the major gs antigen have been purified and specific reagents have been prepared to them. Using these, it is possible to detect expression of type C virus information in pig tissues. This virus can be readily distinguished from all other mammalian type C viruses, and DNA copies of the viral information are found in normal pig tissue.

The spontaneous release of endogenous type C viruses from mammalian cell lines after hundreds of generations *in vitro* appears to be a relatively common phenomenon, and is not limited to undifferentiated cell lines or to cells derived from connective tissue or hematopoietic tissue.

Permanent cell lines were established using explants and trypsinization techniques from 13 tumors including carcinomas, sarcomas, melanomas and brain tumors.

Significance to Biomedical Research and the Program of the Institute:

Important findings regarding the mechanism of action of oncogenic viruses, their effects on cellular growth control mechanisms and their possible involvement in natural oncogenesis have been developed from a number of projects on this contract. A number of new type C viruses have been isolated and characterized. These have provided much information on the nature of type C virus-cell interactions. Hopefully, the techniques employed in inducing and growing these viruses may be used to isolate a complete human type C virus which would immeasurably aid in studies of the possible viral involvement in human cancer.

Proposed Course: Continuation to achieve the objectives described.

Date Contract Initiated: May 25, 1965

SUMMARY REPORT

8. PROGRAM RESOURCES AND LOGISTICS ADVISORY GROUP

The Viral Oncology Program Resources and Logistics Advisory Group was established by the Associate Division Director in 1972. This Group constitutes a standing intramural committee to provide support and make recommendations concerning resources and logistics matters, and to conduct appropriate reviews for those contracts administrated by the Office of Program Resources and Logistics. The Group is chaired by the Chief, Office of Program Resources and Logistics and is responsible to the Office. The membership includes three representatives from each of the three Branches and also representatives from the other major intramural areas of Viral Oncology. The current membership of the Group is listed in a previous section of this report concerning Program management personnel.

In addition to conducting discussions and making recommendations in an advisory capacity, the Group is responsible for formal review of resource and service type contracts. Such reviews consist primarily of an evaluation of relevance, priority, and need of proposed or ongoing activities in relation to the Program objective of investigating the potential viral etiology of human neoplasia. The types of contracts administrated by the Office of Program Resources and Logistics, and which are reviewed by this Advisory Group represent four general areas of activities. These include:

1. Contracts concerned with production and characterization of purified viruses and viral reagents.

2. Contracts concerned with acquisition, processing, storage, inventory, and distribution of normal and malignant human specimen material.

3. Contracts concerned with production, distribution, and maintenance of various species of experimental animals.

4. Contracts concerned with the provision of specialized testing services for the examination of experimental materials.

During fiscal year 1974 the Advisory Group was responsible for reviewing nine separate contracts directed toward virus and reagent production, eleven contracts involving human resource activities, nine contracts concerned with animal resource activities, and six contracts dealing with testing, service, or miscellaneous aspects. During this fiscal year the PR&L Advisory Group and the OPR&L concentrated a major portion of its effort on developing an integrated and comprehensive resource program, strengthening contract efforts of high priority and discontinuing unrelated or nonproductive activities. During the year, three additional new resource contracts were initiated, two appropriate Virus Cancer Program resource-type contracts were integrated within the OPR&L contract group, five efforts

were terminated, and two contracts were transferred to research segments within the VCP.

The percentage distribution of the total contract effort devoted to each of the above resource areas was:

Virus and reagent resources	26%
Human resources	31%
Animal resources	26%
Testing, service, and support	17%

The percent of the total resources funding effort necessary to support these four types of activities were distributed as follows:

Virus and reagent resources	55%
Human resources	7%
Animal resources	27%
Testing, service, and support	11%

The PR&L Advisory Group conducts the required reviews for all exclusively resource or service type contracts (Type III), and also performs the initial reviews necessary for research-resource efforts (Type I) and for extensively funded or comprehensive resource contracts (Type II). Because of this function, meetings are held at rather frequent intervals, usually every other month. In this respect the Advisory Group functions in a manner analagous to the Virus Cancer Program Joint Segment Chairmen and essentially reviews for relevance all resource contracts during the course of the calendar year. Type I and Type II research-resource efforts additionally receive a second review by the Viral Oncology Branch and Associate Branch Chiefs for scientific and technical excellence. In keeping with existing procedures, review of resources efforts is an intramural function.

OFFICE OF PROGRAM RESOURCES AND LOGISTICS

Dr. Jack Gruber, Chief, OPR&L, OADVO, DCCP, Chairman
Dr. David McB. Howell, OPR&L, OADVO, DCCP, Executive Secretary
Dr. Lea I. Sekely, OPR&L, OADVO, DCCP, Staff Scientist
Mrs. Wilma L. Varrato, OPR&L, OADVO, DCCP, Staff Member

CALIFORNIA, UNIVERSITY OF (NO1-CP-3-3237 and NO1-CP-4-0201)

Title: Development and Evaluation of Cell Substrates for the Study of Cancer Viruses

Contractor's Project Directors: Dr. Stewart Madin
Dr. Neylan Vedros
Dr. Adeline Hackett
Dr. Walter Nelson-Rees

Project Officers (NCI): Dr. James Duff
Dr. Jack Gruber

Objectives: The Cell Culture Laboratory (CCL) is physically located at the Naval Biomedical Research Laboratory (NBRL), in Oakland. The program of the CCL is funded by a contract (NO1-CP-3-3237) between the University of California and the NCI. In addition, maintenance and operating expenses generated by the CCL are repaid to NBRL by an interagency transfer of funds (NO1-CP-4-0201) between NCI and NBRL. This project includes the development and evaluation of cell substrates for the study of cancer viruses, development of large quantities of specific cell substrates, karyotyping of cell cultures, and performing biophysical, virological, and cytogenetic applied research.

Major Findings: The contractor has distributed a large variety of cell cultures derived from various tissues and from many different species to VCP investigators. From March 1, 1973 to January 31, 1974 913 cell culture seed stocks were provided to 139 recipients. Tissues are now procured not only from Los Angeles area institutions for processing in Oakland, but new sources in the San Francisco Bay Area have been established permitting emphasis on rapid initiation of cell culture within 1-2 hours after excision or biopsy.

The contractor has also received numerous samples of selected established normal and tumor cell cultures from various VCP investigators. Most cultures are monitored, preserved, and distributed following antibiotic-free cultivation, assurance of species and donor specificity, and freedom from microbial contamination.

Data now in the computer bank and the latest CCL catalogue (May, 1974) list human and other animal substrates available for distribution.

Rapid culturing plus techniques developed for selective cultivation of epithelial cells have resulted in a 24% success rate for establishing bonafide carcinoma cells and a 13% success for sarcomas. These cell strains are being extensively characterized for growth properties, morphology, and tumorgenicity in mice. The contract continues to characterize normal mouse epithelial cell strains developed in this laboratory as substrates for studies on viral induced carcinogenesis. Normal liver and mammary gland strains have been cloned and characterized. The clones vary in their capacity to be transformed by MSV or SV40.

Studies continue on the effect of DMB-rifamicin on cell transformation. A derivative of Balb/3T3 has been isolated which is resistant to high doses of the drug; however, MSV focus formation is still inhibited. Under these conditions SV40 transformation is unaffected, suggesting that the drug specifically interferes with oncornavirus replication.

The contractor continues to study RNA tumor virus interaction with other viral agents; transmission to other species; induction in tumor-bearing animals and the characterization of cell surface changes following productive and non-productive infection.

The karyology reference laboratory continues to function for the benefit of monitoring cell cultures within the contract as well as to study and record the karyotypes of cell cultures submitted by other VCP investigators.

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 VCP contract laboratories. The contract continues to develop techniques for the identification and study of tumor cells oriented toward a study of the fundamental biology of tumor cells, and the interaction between tumor cells and viruses of oncogenic importance.

Proposed Course: Continue to develop cell reagents as substrates for human carcinogenesis; attempt to isolate and characterize viral agents from human tumor cells; continue a reference laboratory for karyology of cells in culture; continue applied research in the biology of tumor viruses.

Date Contract Initiated: October 1, 1962

UNIVERSITY OF CALIFORNIA AT SAN DIEGO (N01-CP-02202)

Title: Development and Operation of a Breeding Colony of Domestic Cats

Contractor's Project Director: Dr. Alexis J. Kniazeff

Project Officer (NCI): Dr. Robert Holdenried

Objectives: To develop and operate a permanent breeding colony of cats which will supply offspring for cancer research.

Major Findings: The cat conditioning and breeding colony supplied pregnant animals to collaborating laboratories in the VCP, especially the Merck Institute for Therapeutic Research (Contract NO1-CP-1-2059). Cats purchased from pounds after selection for good health and other related criteria through physical examinations were held for a minimum of two months before breeding.

The Merck Institute's weekly requirements for pregnant cats varied throughout the previous year with regards both to number of animals and age of embryos. In order to meet this variable and occasionally unpredictable demand, at least ten cats were bred per week. The seasonal occurrence of natural oestrus in cats required artificial hormonal stimulation to provide gravid animals throughout the year. The contractor successfully developed a dependable system of artificial stimulation to provide gravid animals throughout the year.

Significance to Biomedical Research and the Program of the Institute: This colony provided the major source of pregnant cats used in the VCP for feline leukemia-sarcoma studies, and for viral vaccine developmental studies.

Proposed Course: Because of a decreased emphasis on animal model systems and a reduced need for pregnant cats, this contract was terminated June 25, 1974.

Date Contract Initiated: June 25, 1969

CHICAGO PARK DISTRICT, LINCOLN PARK ZOO (NO1-CP-3-3271)

Title: Marmoset Breeding Colony

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

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

Objectives: To provide marmosets in a quantity and quality sufficient for the needs of the research on tumor viruses conducted under Contract NO1-CP-3-3219 with Rush-Presbyterian-St. Luke's Medical Center, as well as for research conducted in other VCP laboratories.

Major Findings: The Lincoln Park Zoo has continued its mission of breeding marmosets for the purpose of furnishing live, healthy baby animals for cancer research. The medical research work is done at the Presbyterian-St. Luke's Hospital in Chicago.

At this time the breeding colony numbers 121 adults. There were 83 live and viable babies born with 29 dead on delivery. Total births during this report period number 112. There were 20 adult deaths during this period with primary cause being infectious disease problems.

Significance to Biomedical Research and the Program of the Institute: This contract is part of a program utilizing lower primates for testing selected laboratory specimens for oncogenic activity. The marmoset, a small, inexpensive primate, has been shown to be susceptible to several cancer viruses; newborn and young animals are in demand by VCP investigators.

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

Date Contract Initiated: June 28, 1965

THE CHILD RESEARCH CENTER OF MICHIGAN (NO1-CP-3-3333)

Title: Inter- and Intraspecies Identification of Cancer Cells In Vitro

Contractor's Project Director: C. S. Stulberg, Ph.D.

Project Officer (NCI): Dr. David McB. Howell

Objectives: The purpose of this project is to provide The Virus Cancer Program with a service that would rapidly establish or confirm inter- or intraspecies identity of *in vitro* systems.

Major Findings: The contractor uses species-specific immunofluorescent antisera, isoenzyme systems, and chromosomal methods as the basic procedures for determining: 1) species identity; 2) levels of "contamination" in allegedly pure cell populations; and 3) intraspecies differences, particularly in human cell systems.

During the first seven-month period of the contract, which was established in 1973, 56 cell strains were identified and examined for their inter- and intraspecies characteristics for contractors of the VCP. Detection of extraneous cells was accomplished with fluorescent-labeled species-specific antibodies, by genetically determined isozymes, by chromosomes and chromosomal banding, and by several or all of these procedures. Work on new cell identification procedures (especially lymphoid cell lines) was begun. Mixed or beginning contamination was determined by the ability to recognize one extraneous cell of another species in a large population of cells.

Work on new identifications employing T or B procedures on lymphoblastoid cell lines was initiated. It is important to note that 35.7% of the cell lines submitted have been found contaminated by cells of extraneous origins.

Significance to Biomedical Research and the Program of the Institute: In the search for oncogenic viruses, many cell cultures from the same or different species are used concurrently, which offer frequent opportunities for cross contamination. In multiple-species tumor transplantations, the species derivation of induced tumors sometimes comes into question. Generally, the significance of virus presence in tissue cells, the ability to grow virus, or the validity of virus isolator systems are all dependent upon assurance of the identity of the cell cultures used.

Proposed Course: The contractor will continue to provide service to The Virus Cancer Program and its contractors by rapidly determining interspecies and intraspecies identity of cell culture systems. Additionally, the contractor will extend and develop marker systems in accordance with needs for identification.

Date Contract Initiated: June 26, 1973

UNIVERSITY OF COLORADO MEDICAL CENTER (NO1-CP-3-3400)

Title: Collection of Neoplastic Tumor Specimens

Contractor's Project Director: Dr. William E. Hathaway

Project Officers (NCI): Dr. David McB. Howell
Dr. Lea I. Sekely

Objectives: To obtain tissues and serum specimens from patients with various types of malignancies for collaborative studies with the VCP.

Major Findings: During the past year, 1,235 tumor and control specimens and relevant patient information were obtained and sent to investigators within the VCP as directed by the Project Officer. Specimens were available from a wide variety of patients with diagnoses including leukemia, lymphoma, rhabdomyosarcoma, osteosarcoma, various carcinomas, neuroblastoma, and a variety of brain tumors. In addition, sera from close relatives of patients were collected whenever possible and a variety of normal tissues were made available to interested investigators.

Fresh and frozen tumor tissue (mostly malignant but some benign) from surgical procedures and autopsies were sent to the Flow Repository for storage and distribution to collaborating investigators. Additionally, large amounts of normal and patient sera were procured and sent to the VCP serum bank at Flow Laboratories.

Under Program direction, the contractor has placed new emphasis on procurement of large amounts of fresh, heparinized blood from new leukemia and Hodgkin's disease patients. Special requests from VCP investigators for

cord blood, body cavity fluids containing tumor cells, lymphocytes, and brain tumors (fresh tissue) were filled.

Significance to Biomedical Research and the Program of the Institute: This is a resource contract of major importance to the VCP, since it is a primary source of diverse cancer specimens for NCI and other VCP researchers on the East Coast. A continuing supply of such specimens is absolutely necessary to the pursuit of the viral etiology of human cancer.

Proposed Course: Continue to collect serum and tumor specimens as in the past. Materials will be provided to the VCP at the direction of the Project Officer for use in investigations by collaborating investigators.

Date Contract Initiated: June 18, 1969

UNIVERSITY OF CONNECTICUT (NO1-CP-33221)

Title: Development and Maintenance of a Specific Pathogen Free Flock of White Leghorn Chickens

Contractor's Project Director: Dr. Roy E. Luginbuhl

Project Officers (NCI): Dr. Robert Holdenried
Dr. Roy Kinard

Objectives: Establish and maintain a flock of chickens free of specified pathogens, including avian leukosis viruses, and to provide eggs for research use.

Major Findings: A specific pathogen free (SPF) flock of chickens was developed and maintained by this contractor. Approximately 1000 chickens ranging from 4 to 75 months of age were maintained free of, and monitored regularly for, *Mycoplasma gallisepticum* and *synoviae*, *Salmonella pullorum*, Newcastle disease, avian infectious bronchitis, avian adenovirus, infectious laryngotracheitis, fowl pox, avian encephalomyelitis, avian reovirus, infectious bursal agent and RSV (serotypes A & B).

Eggs and chicken embryo fibroblasts were supplied to various researchers, including Drs. F. Deinhardt, P. Sarma, H. Temin, M. Baluda, and H. Morgan.

Significance to Biomedical Research and the Program of the Institute: The availability of highly controlled and monitored flocks such as this one was of special importance to avian leukosis research.

Proposed Course: Because of the decreased need for the resources provided by this contractor, this effort terminated January 31, 1974.

Date Contract Initiated: June 18, 1962

CORNELL UNIVERSITY, NEW YORK STATE VETERINARY COLLEGE (NO1-CP-0-2224)

Title: Feline Tumor Viral Diagnostic Laboratory

Contractor's Project Director: Dr. James H. Gillespie

Project Officers (NCI): Dr. James T. Duff
Dr. David McB. Howell

Objectives: To produce and evaluate cat viral reagents; to monitor cat cell cultures and other materials associated with cat tumors for indigenous cat viruses and other microorganisms.

Major Findings: Since March 1, 1973 to the termination of this effort on December 24, 1973, approximately 650 feline tissue and swab samples were examined for the presence of indigenous or contaminating feline viral agents in cooperation with the NCI participating laboratory at Merck, Sharpe and Dohme. Samples processed included swabs taken from the eye, nose, throat and rectum of specific pathogen free cats in their colony as well as selected tissues taken both from monitor kittens and certain adult cats in the group. In no instance was a cytopathogenic viral agent isolated from any of these samples. The cats involved in the sampling were both caesarean-derived off-spring of conventional cats held in isolation since birth and naturally born kittens derived from this caesarean population. In long term monitoring of this colony there were only two instances when a kitten was found to harbor a cytopathogenic feline virus. Two kittens from two caesarean derived litters were found infected with the feline syncytium-forming virus. The two animals were the only kittens in their respective litters that were positive for the virus. It was concluded that congenital infection of the kitten with this agent is a rare event with an irregular distribution even within a litter.

In contrast to the absence of cytopathic-feline viruses isolated from this SPF population of cats the rates of isolation of various feline viruses from a conventional population of 100 cats (the queens from which the SPF colony was derived) were as follows: Panleukopenia virus, 0; Syncitium-forming virus, 20; Rhinotracheitis virus, 9; and Calicivirus, 15.

Numerous requests for the Crandell feline kidney cell line, as well as the feline tongue diploid cell line were filled during this report period.

Significance to Biomedical Research and the Program of the Institute:

This laboratory was a source of reagents and expertise concerning the feline tumor viruses, and made both available to scientists within the VCP. The contract provided a central laboratory where materials isolated from normal

cats and cats suffering from cancer could be sent to determine if they contained indigenous feline agents, as well as for viral identification.

Proposed Course: Because of the decreased need for the resources and services provided by this contractor, this effort terminated December 24, 1973.

Date Contract Initiated: June 25, 1970

ELECTRO-NUCLEONICS LABORATORIES, INC. (NO1-CP-3-3355)

Title: Development of Propagation Procedures, Purification, and Characterization of Viruses

Contractor's Project Director: Mr. John Lemp

Project Officers (NCI): Dr. George Todaro
Dr. Jack Gruber

Objectives: To develop propagation procedures to produce high virus yields from cell cultures, and to purify, determine particle count per ml., and otherwise characterize the produced virus.

Major Findings: A total of 30 cell lines, shedding C-type virus particles, were propagated and the viruses purified and characterized in the contractor's laboratory; all of these virus-yielding lines were new to the laboratory. The cell lines included murine (ATS-214-4B), feline (F679), gibbon ape (G-204), simian sarcoma (W-20), Mason-Pfizer (CM04) and many others.

The contractor has concentrated, partially purified, and characterized 4,299 liters of tissue culture-virus fluid in 173 runs which comprised double sucrose density gradient and pelletizing centrifugations.

Significance to Biomedical Research and the Program of the Institute:
The search for evidence of the viral etiology of human cancer must include studies on viruses present in cell cultures established from animal tumors as well as on those candidate human cancer viruses growing in either animal or human cell cultures. Large volumes of these well-characterized and concentrated viruses are essential for the preparation of specific antisera and for the biochemical, immunological, and epidemiological investigations necessary in cancer virus research.

Proposed Course: This contract will continue to provide new and improved tissue culture propagation procedures and optimize methods for providing the highest yield with consistantly high biological qualities. In addition, the contractor will continue to characterize the virus-yielding cultures by thin-section electron microscopy when they are received from the researcher, and during propagation.

Date Contract Initiated: May 28, 1971

ELECTRO-NUCLEONICS LABORATORIES, INC. (NO1-CP-2-3249)

Title: Large-Scale Production of Oncogenic Viruses

Contractor's Project Director: Mr. John Lemp

Project Officers (NCI): Dr. Jack Gruber
Dr. David McB. Howell

Objectives: To provide research and service related to the isolation, large-scale production, concentration, and assay of oncogenic viruses of animals and potentially oncogenic viruses of humans. Production and quality control involve tissue culture, electron microscopy, immunology, and various biochemical/biophysical techniques.

Major Findings: During the past year this contractor provided The Virus Cancer Program with approximately 8,292 liters of tissue culture-grown oncogenic and suspected oncogenic viruses which were concentrated and prepared for distribution. Agents which have been propagated in large quantities during this reporting period include Rauscher leukemia virus, AKR virus, and Gross leukemia viruses. Additionally, the Woolly monkey sarcoma virus (SSV-1) was propagated in both a rat cell line (NRK) and in the human lymphoblastoid line (NC37), and Moloney leukemia virus was produced in the Dense NIH-3T3 cell line.

These agents were distributed as directed by the Office of Program Resources and Logistics both to intramural investigators in Viral Oncology and to collaborating investigators within the Virus Cancer Program.

Significance to Biomedical Research and the Program of the Institute: In order to carry out important research on the biochemistry and biophysics of oncogenic animal viruses, it is imperative that large quantities of concentrated virus be available for analysis. This contract helps meet this need with oncogenic animal viruses that have been produced under rigidly controlled conditions, and also serves to find the best means of producing and concentrating large quantities of new candidate human cancer viruses as they are discovered.

Proposed Course: The contractor will continue to provide AKR, Gross, Moloney leukemia virus and special vaccine preparations, and will provide Woolly Monkey Sarcoma Virus and other viruses and special preparations as requested by the contract project officer. The contractor will continue to develop and optimize methods for producing the highest virus yield with consistantly high biological qualities with the flexibility to quickly accommodate shifts in Program requirements.

Date Contract Initiated: March 27, 1972

EMORY UNIVERSITY, YERKES PRIMATE CENTER (N01-CP-3-3343)

Title: Maintenance of a Colony of Irradiated, Aging Rhesus Monkeys

Contractor's Project Director: Dr. Harold McClure

Project Officer (NCI): Dr. Roy Kinard

Objectives: To determine the incidence of tumors in a unique group of irradiated, aging rhesus monkeys and to supply tissue from tumors to VCP collaborators for transplantation, tissue culture and virus isolation studies.

Major Findings: During the past year a group of 80 aging and/or irradiated rhesus monkeys and progeny were monitored by daily observations and triannual physical and hematologic evaluation. These examinations specifically concentrated on the detection of developing neoplasms. Thirteen of the animals were noted to be moderately emaciated despite an apparent adequate appetite. The remainder of the animals were in fair to good physical condition, with all animals having maintained essentially the same body weight throughout the year. Many of the animals continue to have dental abnormalities such as severely worn teeth, missing teeth and tooth decay. Two animals have prominent proliferative lesions of the gums.

Skin and or subcutaneous nodules or lesions were noted in 15 animals. These consisted of wart-like or papilloma-like lesions on the skin, and subcutaneous nodules or masses. These lesions appear to be about the same size as when first noted. One animal has a subcutaneous mass, approximately 6 x 8 cm., that appears to be slowly increasing in size, although a biopsy indicated that the lesion was a benign tumor of fibrous tissue origin. One animal shows constriction of the pupil of one eye, and another animal has a corneal opacity. Three animals have an enlarged, firm uterus.

During the most recent physical examination, two animals were found to have palpable masses in the lower abdominal cavity. A laparotomy was performed on each of these animals to determine the nature of these abdominal masses. In one animal the mass was found to be a cystic ovary with surrounding fibrous adhesions. In the other animal, a relatively large, firm, whitish mass was found in the mesentery, anterior to the uterus and ovaries. Microscopic examination of a biopsy specimen from this mass indicated that the lesion was an apparent case of endometriosis.

It is of interest to note that 3 of the 4 adult animals in this group that died during the past year had malignant tumors. Another point of interest is the extended length of time that two of these animals survived after they were found to have malignant tumors. One animal lived for more than 3 years

with an abdominal adenocarcinoma, the other survived for at least 4 years with a histologically malignant seminoma that did not metastisize during this period of time. During the past year, tumor specimens were shipped from the abdominal carcinoma and the testicular seminoma to Drs. Kawakami, Rabin, Wolfe, and Arnstein.

Significance to Biomedical Research and the Program of the Institute: The VCP conducts collaborative projects for the study of relationships between the etiologies of tumors of various primates. This project provided tumor tissues and other important specimens from aging non-human primates which have been subjected to irradiation for research within the VCP. At the same time the contractor conducts a screening operation for the appearance of virus-like particles or viral antigens in the monkeys. Malignant changes in these primates may provide useful information which might be applied to humans, who are also subjected to various forms of radiation as well as to the natural aging process.

Proposed Course: The entire group of monkeys will continue to be monitored for neoplasia by physical and hematologic examinations. All tumors which develop will be evaluated by the contractor by light and electron microscopy. Specimens of these tumors will be made available to VCP investigators. In addition, a breeding program is underway to evaluate the incidence of leukemia or other tumors in infants with aging and irradiated parents.

Date Contract Initiated: May 1, 1971

FLOW LABORATORIES, INC. (NO1-CP-3-3201)

Title: Maintenance of a Repository for Storage and Distribution of Reagents and Tissue Specimens

Contractor's Project Directors: Mr. Harry F. Adkins (Serum Repository)
Dr. Aloysius Kuo (Tissue Repository)

Project Officers (NCI): Dr. Jack Gruber
Dr. David McB. Howell

Objectives: To provide for the VCP a centrally located low temperature storage and distribution center for viral reagents and tissues.

Major Findings: The contractor made 466 shipments of viruses, viral reagents, sera, and tissues which comprised a total of 37,280 vials of material. It received 340 shipments of similar materials which comprised 26,051 vials. All incoming shipments were carefully checked for damage in transit and were cataloged before being placed in the low temperature repository.

During the past year the frozen tissue repository portion of the contract received 680 tissue and fluid specimens, most of which were from patients with neoplastic disease. All specimens were examined by Dr. Kuo, a pathologist, classified as to tumor or tissue type, and either cataloged and stored or sent to VCP investigators. During this period, 294 specimens were distributed to VCP investigators. Clinical data and pathologic diagnosis with microslides supplemented each specimen shipped. The repository currently has over 1,000 normal and neoplastic tissue specimens on deposit available for distribution.

Significance to Biomedical Research and the Program of the Institute: An efficient research program must have readily accessible adequate characterized resource materials. The laboratory, storage, and shipping facilities operated under this contract enable collaborating investigators to have access to a large inventory of special research materials without the burden of procurement, storage, inventory, and distribution.

Proposed Course: It is anticipated that the activities of this contract will continue to provide rapid and flexible support to changing needs of the VCP and its collaborating investigators.

Date Contract Initiated: June 22, 1965

FLOW LABORATORIES, INC. (NO1-CP-3-3391)

Title: Animal Holding Facility to Support Intramural Research on RNA Viruses and Breeding for Detection of Tumor Virus Information

Contractor's Project Director: Ms. J. Torgerson

Project Officers (NCI): Dr. John W. Pearson
Dr. Arnold Fowler

Objectives: The objective of this contract is to provide a small animal breeding and holding facility to support intramural research activities. These activities require large numbers of mice and rats as well as smaller numbers of hamsters and rabbits for genetic studies, and animals for inoculations with various agents to be monitored for experimentation and observation during the aging process.

Major Findings: The contractor receives and maintains mice, rats, hamsters, and other small animal species as required for the purpose of observation and experimentation during the aging process for the following research studies: (a) preventing and/or controlling the incidence of spontaneous neoplasia during the aging process in high incidence strains of mice, (b) the effect of various forms of therapy against several viral-associated transplantable lines in aged rats and guinea pigs which also requires long-term holding, monitoring, and observation, (c) to determine the role

and control mechanisms of viral information as it may pertain to human cancers, a study which requires breeding and holding facilities.

Approximately 3,000 animals have been held and aged for collaborative studies performed under this contract. Studies on the transplantable Nova leukemia maintained in aged Fischer rats that have shown a remission period of 10-12 days with cytoxan therapy have been continued. In addition, various types of therapeutic procedures were applied to study the prevention and/or control of spontaneous neoplasia in AKR and C3H/HL mice. The project on the enhancing effect of long term administration of interferon inducers on the autoimmune disease of NZB/NZW F_1 hybrid mice was discontinued in June, 1973. This project was replaced by the holding and breeding of selected strains of mice in order to characterize hormonally influenced viral gene expression.

In addition, a repository has also been established for frozen stocks of several rat leukemia and tumor lines as well as the guinea pig transplantable line.

Significance to Biomedical Research and the Program of the Institute: This contract provides a support service which helps provide experimental data useful for determining the association and influence of viruses on the development of the neoplastic process. Investigations of this type, utilizing chemo-immunotherapeutic and genetic approaches in both rat and mouse leukemia and tumor model systems, may result in information which will have considerable application in studies on similar diseases in man.

Proposed Course: The application of chemoimmunostimulation studies against the spontaneous AKR leukemia as well as the described rat leukemia systems will continue. Immunization studies involving the various rat leukemias cell lines will continue. Future work in this project will involve cross matings, backcross matings, and F_1 x F_1 matings to characterize hormonally influenced viral gene marker expression.

Date Contract Initiated: June 15, 1971

GEORGETOWN UNIVERSITY (NO1-CP-3-3404)

Title: Supply of Blood and Tissue Specimens from Patients with Malignancies

Contractor's Project Director: Dr. Richard A. Binder

Project Officer (NCI): Dr. David McB. Howell

Objectives: To collect fresh blood and tissue specimens from patients suffering from various neoplasias.

Major Findings: During the past year, a total of 176 tissue specimens, 346 sera, and 51 specimens of whole blood were collected. In addition, 11 whole units of blood, 1 defibrinated blood, and 1 gargle were obtained.

Arrangements were made to provide fresh breast tissue to the Electron Microscope Section, NCI. In addition, serial sera from a patient with Burkitt's lymphoma were obtained and provided to NCI investigators. All sterile fresh tissues were sent directly to the Resources Processing Laboratory at Litton Bionetics, Rockville, Maryland, for distribution. Frozen tissue specimens and sera were sent to the Flow Laboratories serum and tissue repository, and to specified investigators.

Significance to Biomedical Research and the Program of the Institute: It is vitally important that a continuing supply of specimens from patients suffering from neoplasias be available to researchers seeking the viral etiology of cancer. This contract is particularly advantageous because the contractor is located within a few miles of NCI. This proximity allows the formulation and alternation as necessary of particularly detailed protocols, and also allows for the availability of very fresh specimens to VCP researchers in the Washington area.

Proposed Course: It is anticipated that increasing numbers of fresh tissues will be procured and provided to The Virus Cancer Program.

Date Contract Initiated: June 28, 1972

GOODWIN INSTITUTE FOR CANCER RESEARCH (NO1-CP-2-3261)

Title: Resource for Germfree Animals

Contractor's Project Director: Dr. Joel Warren

Project Officer (NCI): Dr. David McB. Howell

Objectives: This contract was developed to provide germfree and specific pathogen-free animals in an environmentally controlled facility to support VCP and intramural research investigations requiring clean, well defined animals and viral reagents.

Major Findings: The contractor has provided the well defined animals and reagents indicated above. Germfree and specific pathogen-free Fischer 344 rats, Sprague-Dawley rats or Graffi strain hamsters were supplied to three laboratories during this period. Timed pregnant SPF rats were also supplied on a weekly basis to Drs. A. Bogden (Mason Laboratories) and J. Di Paolo (NCI). Germfree BALB/c mice were assigned to Mr. S. Poiley (NCI).

One small colony of cubicle-reared Sprague-Dawley rats were transferred to Life Sciences, Inc., in St. Petersburg, Florida, for propagation and future distribution to VCP investigators.

Significance to Biomedical Research and the Program of the Institute: This contract provided a support germfree animal resource of special importance for use in experiments on the role of viruses in animal cancers. Those studies required specialized animals free of interfering agents which might modify the course of study and interfere with the interpretation of results.

Proposed Course: Because of a greatly reduced need for the services provided by this contractor, the contract was terminated on October 31, 1973.

Date Contract Initiated: April 16, 1965

HEALTH RESEARCH INC. (RPMI) (NO1-CP-4-3392)

Title: Procurement of Leucocytes and Tissue Specimens from Patients with Malignancies for the VCP

Contractor's Project Director: Dr. Joseph Sokal

Project Officers (NCI): Dr. Lea I. Sekely
Dr. David McB. Howell

Objectives: To collect tissues and blood samples from adults suffering from neoplastic hematologic disorders, particularly Hodgkin's disease and leukemia, for use by researchers within the VCP.

Major Findings: Health Research, Inc. is located at the Roswell Park Memorial Institute in Buffalo, N. Y., one of the major centers for treatment of cancer in this country. The contractor shipped a total of 114 tissue specimens from 84 patients to the VCP tissue repository at Flow Laboratories during the past year. The aggregate weight of these specimens was 11.5 Kg. The most common diagnoses represented were carcinoma of the lung, carcinoma of the breast, gastrointestinal carcinoma, gynecologic malignancies, and leukemia. In addition, a total of 107 specimens of whole blood or leukocyte concentrates and 5 specimens of spleen were shipped to the processing laboratory at Litton Bionetics. Amounts of material shipped ranged from 10 ml. of whole blood to 15×10^{12} leukocytes, obtained by the NCI-IBM blood separator from a patient with chronic lymphocytic leukemia. Diagnoses represented were Hodgkin's disease, lymphocytic lymphoma, histiocytic lymphoma, giant follicle lymphoma, chronic lymphocytic leukemia, chronic myelocytic leukemia (including blastic phase), malignant melanoma, infectious mononucleosis and idiopathic thrombocytopenic purpura.

Significance to Biomedical Research and the Program of the Institute: A major goal of the VCP is to identify, isolate, propagate, and characterize candidate human cancer viruses. Of paramount importance in the efforts to reach this goal is the continued availability of clinical specimens and histories from patients suffering from cancer. This contractor, which sees a large number of these patients annually, is in an excellent position to help meet increasing program needs for large quantities of human neoplastic specimens.

Proposed Course: It is anticipated that the rate of shipment achieved during the past year will be increased.

Date Contract Initiated: June 22, 1972

HOSPITAL FOR SICK CHILDREN (NO1-CP-2-3266)

Title: Human Leukemic and Normal Tissue Collection and Preservation

Contractor's Project Director: Dr. Peter D. McClure

Project Officers (NCI): Dr. David McB. Howell
Dr. Charles Boone

Objectives: To obtain serum, plasma, and tumor specimens for a wide variety of research purposes from pediatric leukemics, relatives of such patients, and non-leukemic controls.

Major Findings: During the past year, the contractor continued to collect serum samples from new patients with leukemia and from patients on long-term follow-up for use by Virus Cancer Program investigators. Twenty-five frozen tissue samples and 71 frozen buffy coats from leukemics were shipped to the Litton Bionetics Resources Processing Laboratory for distribution to VCP investigators, while sera from 41 new leukemias, 35 follow-up leukemics, 40 new tumor cases, 21 follow-up tumor cases, 18 normal controls, 21 hematologic controls as well as 31 frozen tissue samples were shipped to the Flow Laboratories Repository for storage and later distribution. Arrangements have also been made to deliver fresh leukemic cells to NCI investigators within 24 hours of collection.

Significance to Biomedical Research and the Program of the Institute: As the largest pediatric hospital in North America, this contractor supplies many vital serum specimens to the VCP not readily available elsewhere. The contractor also provides an important service to Program by collecting numerous samples of tissue for uses in which the unavoidable delay in passage through customs is not critical. In addition, the contractor consistently refers patients to NCI for study whose cases are felt to be particularly relevant to the research needs of Program.

Proposed Course: The contractor will continue to collect solid tumor tissue, leukemic white cells and any other tissue specifically requested by NCI investigators.

Date Contract Initiated: February 3, 1965

HUNTINGDON RESEARCH CENTER (NO1-CP-3-3223)

Title: Development of Oncogenic Virus Diagnostic Reagents and Services

Contractor's Project Director: Dr. Roger E. Wilsnack

Project Officers (NCI): Dr. Robert Holdenried
Dr. Wallace Rowe
Dr. David McB. Howell

Objectives: To develop, produce, and characterize special diagnostic reagents for use in the VCP, 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 contractor during this reporting period produced and characterized antisera against a very wide array of antigens encountered in cancer research, and added a number of antisera to his inventory during the year. These included new antisera against Woolly Monkey Fibrosarcoma virus, RD-114 virus, RD-114 gs antigen, avian myeloblastosis virus, guanidine-purified Rauscher and feline leukemia viruses, Epstein-Barr virus, Gross leukemia virus, Gibbon Ape lymphoma virus, and rat leukemia virus. It also made large volumes of donkey antisera against species immunoglobulins for use in the double antibody radioimmunoassay system. These reagents were distributed to 60 investigators of The Virus Cancer Program.

Additionally, the contractor recently installed a computer terminal which permitted access to the NIH Computer Center. Data concerning the contractor's inventory of antisera were entered in the computer memory, thereby allowing the contractor to query the computer to determine the proper lot of antiserum to fit a large number of subtle biological parameters. Moreover, access to the computer has greatly facilitated inventory control.

Goats, swine, donkeys, rats, hamsters, and rabbits are currently being employed as hosts for antigens used in the continued production of oncogenic virus antisera.

Significance to Biomedical Research and the Program of the Institute: The reagents and test systems developed and produced by the contract are vital tools in cancer research. The project functions in close collaboration with VCP research projects and is of very significant usefulness to the needs of the program.

Proposed Course: To continue development, characterization, and production of antisera and serological test systems.

Date Contract Initiated: June 2, 1963

JEWISH HOSPITAL AND MEDICAL CENTER (NO1-CP-4-3215)

Title: Supply of Human Specimen Material from Patients with Chromosomal Abnormalities and Malignancies

Contractor's Project Director: Harvey Dosik, M. D.

Project Officers (NCI): Dr. George Todaro
Dr. Bernard Talbot

Objectives: To conduct systematic clinical, epidemiologic, and cytogenetic investigations of patients, and relatives of patients with chromosome abnormalities, increased risk of malignancy, and those on chemotherapy for malignancy, and to supply NCI investigators with cell cultures, sera, or other specimens from such patients.

Major Findings: By measuring the in vitro transformation frequency of human diploid fibroblasts by the oncogenic virus SV40, a number of highly susceptible groups of individuals were discovered. These included patients with Fanconi's anemia and Down's Syndrome, as well as patients undergoing radiation treatment. Besides increased SV40 transformation these groups have two other features in common: chromosome anomalies; and a high incidence of spontaneous neoplasms. Relatives of patients with Fanconi's anemia also had increased transformation as well as a high incidence of spontaneous neoplasms; in addition, there appeared to be an increase in minor chromosome anomalies.

During the past year 97 skin biopsies, and 136 serum samples and bloods for chromosome studies were obtained from controls and patients. Sixty sterile and 35 unsterile tumor specimens have been collected from biopsy and autopsy material. Skin specimens have been cultured at the Jewish Hospital and also sent to NCI investigators for culture and tumor viral studies. Serum samples have been frozen to provide a serum bank of normal and patient groups.

Significance to Biomedical Research and the Program of the Institute: These studies enable the VCP to determine, on a broader scale, the relationship between chromosome anomalies (particularly those which involve an excess of genetic material), susceptibility to cellular transformation by oncogenic agents, and an increased incidence of malignancy.

Proposed Course: Continue to supply and study viral transformability of normal and neoplastic tissues from individuals with chromosome abnormalities.

Date Contract Initiated: October 7, 1970

JOHNS HOPKINS UNIVERSITY (NO1-CP-3-3245)

Title: Pediatric Tumor Resource

Contractor's Project Director: Dr. Herbert Kaiser

Project Officers (NCI): Dr. Paul Peebles
Dr. Jack Gruber

Objectives: To provide fresh tumor specimens from pediatric patients to collaborating laboratories within the VCP for biochemical and virological investigations.

Major Findings: Since activation of the contract on 1 March 1973 all specimens have been sent directly to The Virus Cancer Program Resources Processing Laboratory at Litton-Bionetics for disbursement to investigators of the VCP. Over a period of one year, a total of 75 specimens from 37 different patients have been submitted. While it was only possible to provide one gram of solid tumor in a few instances, in most cases the amount of solid tumor or tissue sent ranged from 10-200 grams. In cases of tumor containing fluids (i.e. blood, ascites, etc.), sufficient volume to contain 10^7 to 10^9 tumor cells was usually submitted. In some instances it was possible to provide both tumor and normal tissue from the same patient. In all instances material was delivered within a few hours of the biopsy time.

While all cases submitted were of interest, tissues of special interest include a well documented case of Burkitt's lymphoma who subsequently had a leukemic relapse, a lymphoma with leukemic relapse, several leukemics with high peripheral white counts, a large Wilms' tumor with paired normal kidney tissue, a medulloblastoma whose cells grew extremely rapidly in culture and several neuroblastomas from patients who showed no evidence of increased catecholamine secretion.

Significance to Biomedical Research and the Program of the Institute: Detection, treatment, and prevention of human cancer require an accurate determination of its etiology. Viruses have been implicated as possible causative agents in human cancer. Unfortunately, a lack of sufficient tumor material from patients in the pediatric age group limits investigations for oncogenic viruses that may have been vertically transmitted from mother to child. Materials from this contract will help make possible these imperative human studies.

Proposed Course: It is anticipated that this contractor will continue to supply unique pediatric specimens to the Program.

Date Contract Initiated: March 1, 1973

LIFE SCIENCES, INC. (NO1-CP-3-3210)

Title: Production of Germfree and Reagent Grade Specific-Pathogen-Free Animals

Contractor's Project Director: Dr. Wendall M. Farrow

Project Officers (NCI): Mr. John P. Kvedar
Dr. David McB. Howell

Objectives: To produce both germfree and specific-pathogen-free (SPF) animals for research. SPF animals are maintained under environmentally controlled conditions which preclude intercurrent infection by pathogenic microorganisms or infestation by parasites and are referred to as "reagent grade" hosts.

Major Findings: The contractor's supply flock of SPF, leukosis-negative White Leghorn chickens, housed in a 2600-square-foot area protected by a shower lock and recirculated, filtered air, was producing about 600 fertile eggs per week, during this reporting period.

Selective breeding has produced a flock of barrier contained, reagent grade chickens with 75-80% fertility and 85-90% hatchability. The results of these efforts were reflected in the quality of the 4,000 eggs and 6,000 chicks from the production flock which were provided to numerous investigators within the VCP. All of the production flock were derived from phenotypically identifiable, pedigreed birds.

A production flock of Japanese quail consisting of 200 birds continued to supply fertile eggs to VCP users at a rate of about 9,150 eggs/year. In addition, it provided a steady input (600/year) of 21 to 28-day-old quail to Program. Representative sampling of aged quail in this production flock indicated that these birds were freed of the usual avian pathogens.

An inbred, pedigreed foundation colony of 190 adult BALB/c mice free of all laterally transmitted viruses tested for, continued to be maintained. Two random bred production colonies derived therefrom continued to supply certified SPF mice to certain VCP investigators. The demand for barrier sustained NIH Swiss mice has increased considerably. Three hundred time-bred mice were produced in addition to 275 young adult and weanling animals.

One colony of cubicle reared Sprague-Dawley rats has supplied 55 time-bred animals. This colony will be maintained at low bred maintenance (20 male and 60 female breeders) until demand increases.

Significance to Biomedical Research and the Program of the Institute: This contract serves as an essential supply of embryonated eggs and day-old chicks to contract NO1-CP-3-3205, which involves studies on Marek's disease as a model for herpesvirus-associated oncogenesis. It also provides other VCP investigators with genetically and microbiologically well-defined laboratory animals. The advantage of having such animals is that oncogenic and suspected oncogenic viruses can be administered to them with a minimal danger of interference from other contaminating, adventitious microorganisms. Therefore, research can be carried out upon animals with a known and controlled viral flora, and cell lines can be derived from these animals which share this same advantage.

Proposed Course: This service-type contract for the production of germfree and reagent grade SPF animals will be continued, with the flexibility of being reoriented as rapidly as possible to meet changing needs of VCP activities as they occur.

Date Contract Initiated: February 8, 1968

LIFE SCIENCES, INC. (NO1-CP-3-3291)

Title: Study and Production of Avian Leukosis Virus

Contractor's Project Director: Dr. Joseph W. Beard

Project Officers (NCI): Dr. Michael A. Chirigos
Dr. John W. Pearson

Objectives: The objectives of this project are: (1) to continue quantity and quality production of BAI strain A avian tumor virus; and (2) to continue investigations on RNA avian leukosis viruses. The contractor provides an average monthly production of approximately 60-80 gms. wet weight of plasma-derived AMV.

Major Findings: The current contract represents an extension of Contract Number NIH-71-2132 initiated April 19, 1971 at Duke University in Durham, N.C., but moved to Life Sciences Research Laboratories in St. Petersburg, Florida. Transfer of the program was begun in February and March of 1973, while work on the previous contract was still being carried on. During the past year, 750 grams of avian myeloblastosis virus was shipped to 73 investigators in this and other countries, including Russia, Israel, Germany, France, Italy, Sweden, Japan, Norway, England, South Africa, and Scotland. Most of the preparations were virus-containing plasma from leukemic chickens sent frozen. A smaller amount of virus was sent fresh from myeloblast tissue culture preparations. A total of 3,109 ml. of myeloblast cells from leukemic chickens was also shipped to recipients.

Significance to Biomedical Research and the Program of the Institute: One of the major objectives of the VCP is to explore fully all important animal model systems for the determination of the possible viral etiology of cancer in man. Avian tumor viruses induce a variety of diseases similar to those which occur in man (erythroblastosis, myeloblastosis, myelocytomatosis, reticuloendotheliosis, and sarcomas); the causative viruses have been isolated and the disease can be induced *in vivo* under controlled conditions which permit the study of the immunology, virology, biochemistry, and therapy of the tumor virus complex. Moreover, BAI Strain A avian tumor virus is the only RNA C-type virus which is at present available in large enough quantities to permit exhaustive investigation into the biochemical makeup and behavior of both the virus and its components. As such, it represents an important model for the C-type viruses of higher animals and is an essential tool in the search for cancer viruses in man. Future studies will depend upon large quantities of concentrated virus, which the contractor is uniquely in a position to supply.

Proposed Course: It is anticipated that the contractor will continue to meet requests for avian myeloblastosis virus and will continue the activities outlined above.

Date Contract Initiated: April 19, 1971

LITTON BIONETICS, INC. (NO1-CP-4-3224)

Title: Investigations of Viral Carcinogenesis in Primates

Contractor's Project Director: Dr. Harvey Rabin

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

Objectives: The objectives of this contract are threefold: the maintenance of monkey breeding colonies and laboratories necessary for inoculation, care, and monitoring of monkeys; the evaluation of long-term oncogenic effects of human and animal viral inocula in primates of various species; and collaborative research studies of primate virus isolates of demonstrated oncogenic potential which may be involved in the causation of human cancer.

Major Findings: At the end of January, 1974 the total inventory of primates in the Bionetics colony was over a thousand animals. Efforts to increase the number of animals and the diversity of species within the New World primates, and to establish breeding colonies, continued in this report period by the addition of a small colony of gibbons (*Hylobates lar*) to be utilized for breeding. Animals have been added to the New World breeding colonies which now have approximately 150 animals representing five species. Eighty-four simians were sent to other research centers from the Old World

breeding colony and the Primate Oncogenesis Section, which housed a total of 510 animals at the close of the report period. In order to promote breeding efficiency, efforts in semen evaluation, artificial insemination, and fertility enhancement were continued. One hundred fifty-six live births occurred during the report period from 170 recorded conceptions, and the Nursery received 154 newborn animals. During the report period, ninety-nine simians were transferred from the Nursery to the Primate Oncogenesis Section. The Hematology, Parasitology, Histopathology, Bacteriology, and Surgery Sections provided continued support to serve the objectives of the primate program.

A project was initiated with Dr. B. Lapin of the Sukhumi Primate Research Center, U.S.S.R., to examine the production of lymphomas in several species of nonhuman primates with virus-containing plasmas from the Sukhumi colony. A male baboon (Papio hamadryas) was received on December 3, 1973, from Dr. Lapin. The animal was maintained under quarantine and biohazard containment conditions for approximately one month. During that time, the animal experienced a gradual loss of condition despite antibiotic therapy and husbandry efforts. To preclude the loss of valuable material, the animal was euthanatized and a complete necropsy performed on January 3, 1974 with over 60 tissues taken for histologic examination. Tissues were also taken for virologic and cell culture studies. Collaborating investigators in this project include Drs. Parks, Goldberg, and Dalton of the NCI, Drs. Falk and Deinhardt of Rush-Presbyterian-St. Luke's Medical Center, and Dr. W. Nelson-Rees of the Naval Biological Laboratory. Attempts at establishing cultures from several tissues are underway in the contractor's laboratory.

With Drs. G. Pearson, Ablashi, Heine, and Adamson of the National Cancer Institute, collaborative studies on Herpesvirus saimiri (HVS) were continued in owl, capuchin, and white-lipped marmoset monkeys. In owl monkeys the research was directed at the development of techniques for the analysis of cell-mediated immunity and for confirmation and extension of biological parameters such as antibody patterns, immune dificiency, and virus rescue to disease. Examination of HVS-induced tumors for evidence of type-C virus has begun with Dr. Spiegelman's laboratory. In vitro studies were continued in attempts to raise virus and viral antigen production by HVS-cell lines primarily by means of nucleic acid base analogues and on detection of virus-induced proteins. Studies comparing surface properties of lymphoid tumor cells of various types including those related to HVS, EBV, and GLV were continued. A collaborative study on EBV inoculation of marmoset monkeys prior activation of B-cells was begun.

In a preliminary study designed to provide background for examination of coinfection of herpes and type-C viruses, SSV-1 of tissue culture origin was found to be oncogenic for a normal, adult, white-lipped marmoset monkey.

At the direction of the Project Officer, the following materials were supplied to the indicated investigators: Sixty-two rhesus monkeys (M. mulatta) were sent to Ms. Victoria Devanney, University of Nebraska Medical Center, Omaha, Nebraska; Ms. Barbara Fullerton, Boston University

School of Medicine, Boston, Massachusetts; Dr. Gene Bingham, University of Pittsburgh, Pittsburgh, Pennsylvania; Dr. W. London, National Institutes of Health, Bethesda, Maryland; Ms. D. Radcliffe, University of Waterloo, Ontario, Canada; Dr. A. Bogden, Mason Research Institute, Worchester, Massachusetts; and Dr. E. Taub, Institute of Behavioral Research, Silver Spring, Maryland. Two term placentae from M. mulatta were sent to Dr. M. Lieber, Meloy Laboratories, Springfield, Virginia and to Dr. M. Ahmed, Pfizer, Inc., Maywood, New Jersey. One cc. samples of serum from each of twenty M. fascicularis were sent to Dr. J. Hilgers, Netherlands Cancer Institute, Amsterdam, Holland; in addition, Dr. W. Voss, Baylor College of Medicine, Houston, Texas, was supplied with two baboons (papio cyanocephalis).

Significance to Biomedical Research and the Program of the Institute: Inasmuch as experimentation for the biological activity of candidate human viruses will not be carried out on humans, it is imperative that another system be developed for these determinations and subsequently for the evaluation of vaccines or other measures of control. The close phylogenetic relationship of the lower primates to man justifies utilization of these animals for these purposes.

Proposed Course: Additional numbers of New World animals will be obtained for experimentation and to increase the breeding effort. Work will continue on HVS-induced owl monkey lymphoma. Attempts will be made to determine the possible presence of type C virus in virus preparations and tumor tissues. Isolation and characterization of the antigens of the tumor cells as well as a series of studies in owl and capuchin monkeys and marmosets infected with HVS will be continued. Marmosets will also be inoculated with Epstein-Barr virus (EBV) and with Herpesvirus ateles (HVA). A spontaneous lymphoid disease in uninoculated owl monkeys as well as HVS-induced lymphoma in the same species will be studied. Attempts to produce in vitro transformation by EBV of the peripheral blood lymphocytes from several species of monkeys will continue.

Date Contract Initiated: February 12, 1962

LITTON BIONETICS, INC. (NO1-CP-4-3260)

Title: Acquisition of Fresh Human Specimens for Virus Cancer Program Investigators

Contractor's Project Director: Mrs. Shirley Norris

Project Officers (NCI): Dr. Jack Gruber
Dr. David McB. Howell
Dr. Lea I. Sekely

Objectives: The primary objective of this project is to coordinate collection, processing, and distribution of fresh human tissues from subjects with malignancies for Virus Cancer Program investigators under direction from NCI.

Major Findings: During this reporting period almost 1,000 specimens were received and distributed to 21 investigators and their associates on a rotating basis. These include 13 NCI researchers and 8 VCP contractors. As an additional activity, a serum bank containing serum from more than 3,000 donors is maintained by this contract for The Virus Cancer Program. Sera from 1,000 donors were aliquoted and added to the bank during the last 12 months. Sera were shipped on dry ice to investigators upon request from the Office of Program Resources and Logistics.

The Principal Investigator during a one-year period visited VCP human resources contractors in Baltimore, Washington, D.C., Denver, Tampa, Montreal, Toronto, and Buffalo to relate program needs and improve quality and quantity of tissues. Telephone communication and written correspondence were maintained on a frequent basis to keep contractors informed of continuing changes in priority for tissue collections.

Significance to Biomedical Research and the Program of the Institute: It is not sufficient merely that sources of human sera and tumor materials for cancer research exist; it is also necessary that the capabilities of these sources be coordinated with the needs of investigators who require specific, and often fresh, specimens. This contractor serves as a direct interface between user and supplier, occasionally sending personnel long distances to pick up specimens of unusual interest and perishability and to deliver them directly to the researcher, thereby avoiding the usual delays in shipment that might lead to the loss of an important factor or agent in the tissue.

Proposed Course: This project will remain responsive to changing program needs for fresh human tissues and attempt to increase amounts of important specimens collected.

Date Contract Initiated: November 1, 1973

UNIVERSITY OF LOUISVILLE (NO1-CP-6-0902)

Title: Preparation of Simian Foamy Virus Reagents and Antisera

Contractor's Project Director: Dr. Paul B. Johnston

Project Officers (NCI): Dr. Robert Holdenried
Dr. James Duff

Objectives: To prepare and test reference reagents (virus and corresponding antisera) for the simian foamy viruses, types 1-7, and foamy virus from other laboratory species.

Major Findings: The seven types of simian foamy viruses and antisera against them have been prepared, packaged, and tested for homogeneity, potency, and purity. During this reporting period, foamy viruses and antisera have been provided to a number of VCP investigators and to OPR&L for general use.

Special efforts were recently made to prepare new antisera to simian foamy viruses, types 5,6, and 7. Volumes of immune rabbit antisera obtained were 132 ml., 79 ml., and 135 ml., respectively, and serum titrations using the neutralization test are currently underway to characterize these preparations.

Significance to Biomedical Research and the Program of the Institute:
The simian foamy virus reagents are 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 are also useful in suppressing the growth of these adventitious viruses in primate tissue cultures.

Proposed Course: Because of the decreased need for the reagents provided by this laboratory, this contract is scheduled for termination on June 30, 1974.

Date Contract Initiated: June 13, 1966

MELOY LABORATORIES (NO1-CP-4-3263)

Title: Production of Murine Mammary Tumor Virus

Contractor's Project Director: Dr. John E. Verna

Project Officers (NCI): Dr. Wade Parks
Dr. David McB. Howell
Dr. Lea I. Sekely

Objectives: To propagate, concentrate, and distribute murine mammary tumor virus (MTV) for collaborating VCP investigators; to perform immunological and biological assays for the detection and quantitation of MTV; and to develop improved methods for the propagation and detection of MTV and MTV antigens.

Major Findings: The primary purpose of this contract is the production of quality reagents for the study of the mouse mammary tumor virus system as a model for the further examination of the human breast cancer problem.

The contractor is purifying MTV from the milk of RIII mice by the combination of rate zonal and isopycnic centrifugation. Purified virus is employed in the following ways: (a) as a source of supply to various VCP investigators; (b) as a reagent in the HA, HAI, CF, and ID tests; (c) employed to produce MTV antiserum in goats, swine, rabbits, and guinea pigs; and (d) used in cell culture experiments.

Purified virus and/or viral antisera and skim RIII mouse milk have been sent during the past year to the following investigators: Drs. S. Abraham, M. Ahmed, S. Aaronson, M. Rich, F. Dixon, W. Feller, J. Keydar, R. Gillette, V. Hollis, P. Kimble, R. Michalides, W. Parks, R. Huebner, J. Schlom, and others.

Some additional studies that are associated with the contract include the serological testing of human milk and sera as well as human and mouse tumor extracts and cloned cell culture lines that are received through the Project Officer or that are generated through the developmental phase of this contract. These samples are examined for the presence of MTV antigens or antibodies. Experiments are currently in progress which attempt to demonstrate the specificity of the reactions that have detected common or cross-reacting antigens or antibodies in human milk and sera. Complement fixation, protein analysis, and assay for reverse transcriptase have been added to the capabilities of the MTV assay laboratory and serve to supplement the present assays.

A developmental phase of the contract is concerned with the establishment of an in vitro cell culture source of MTV. Preliminary data indicate that MTV synthesis does occur in mammary tumor cell cultures. Current studies suggest that the regulation of MTV expression is very different from that previously described for other viruses of this type. Specifically, virus expression is associated with differentiated cell functions.

Significance to Biomedical Research and the Program of the Institute: Breast cancer is a leading cause of death from cancer among women. The finding of a virus, resembling a Type B RNA oncogenic virus of mice, in the milk of a significant number of women from high-risk breast cancer families strongly suggests a possible viral etiology for this disease. A major effort of The Virus Cancer Program is directed toward determining the relationship of viruses to human breast cancer. This contract was established for the purpose of obtaining correlative information on the detection, isolation, and propagation of a murine mammary tumor virus, because this is the only available animal model system in which approaches to the study of viruses as a cause of breast cancer in humans may be developed.

Proposed Course: Purified MTV, viral reagents, and mouse milk will continue to be supplied as needed by VCP investigators. The contractor will also attempt to establish possible recipient epithelial cultures from normal mouse mammary glands as substrates for in vitro mammary tumor virus infection.

Date Contract Initiated: December 30, 1965

MEMORIAL HOSPITAL FOR CANCER AND ALLIED DISEASES (NO1-CP-4-3335)

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

Contractor's Project Director: Dr. Yashar Hirshaut

Project Officer (NCI): Dr. Jack Gruber

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

Major Findings: The contractor operates within one of the largest hospital centers in the nation devoted solely to the care of the patient with cancer. During the reporting period, over 1340 individual sera specimens were collected from both normal subjects and from patients with malignancies. Additionally, over 500 surgical tumor specimens were obtained. These materials were either distributed directly to cooperating VCP research laboratories or frozen and stored at their own regional repository for future Program distribution as directed by NCI. Clinical information for each serum and tissue specimen is periodically entered into the NIH computer bank, under direction from the OPR&L, and a computer printout of the contractor's entire inventory of frozen specimens is prepared on a monthly basis.

Numerous samples of a wide variety of materials have been distributed to collaborating VCP investigators, including Drs. Hanafusa, Huebner, Spiegelman, Herberman, Schlom, Girardi, Yohn, Pagano, Gallo, and others. The VCP Litton-Bionetics resources processing laboratory (4-3260) and the VCP Flow Laboratories tissue repository (3-3201) also received materials for distribution and storage.

Significance to Biomedical Research and the Program of the Institute: In the last ten years, rapid progress has been made in the study of oncogenic animal viruses. Unfortunately, human studies have frequently been limited by the lack of suitable materials to be used in virus isolation and detection attempts. The procurement program at Memorial Hospital for Cancer and Allied Diseases in New York City provides cooperating investigators with sufficient numbers of specimens from tumor-bearing patients to permit them to undertake intensive studies of the possible viral etiology of human cancer.

Proposed Course: It is anticipated that this contractor will maintain the present high level of material supplied to the Program.

Date Contract Initiated: March 1, 1971

UNIVERSITY OF MICHIGAN (NO1-CP-3-3224)

Title: Collection of Leukemia-Lymphoma Specimens

Contractor's Project Director: Dr. Chris J. D. Zarafonetis

Project Officers (NCI): Dr. David McB. Howell
Dr. Charles Boone

Objectives: To collect and distribute specimens and information from patients with leukemia or lymphoma.

Major Findings: The major efforts of the contractor have been divided between procurement of specimens and evaluation of special clinical situations. During the past year, the contractor added over 4,116 sera including multiple vials from 200 normal males for whom a complete medical evaluation is available, 141 plasma specimens and 900 bone marrow aspirates, bringing the total number of sera to 23,429. Ninety-two (92) sera from patients with breast cancer and 164 tissues were supplied to Flow Laboratories, Litton Bionetics, and other investigators. The collaborative study involving HLS testing in Hodgkin's disease has been completed.

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 VCP, i.e., to identify rescue, characterize, and propagate a candidate human cancer virus. The sera which this contractor has provided to the Program have provided useful information in studies on the immunological response to cancer and in the search for antibodies against antigens associated with viruses which may be involved in human cancer.

Proposed Course: With the completion of scheduled activities, this contract was terminated on February 28, 1974. Sera collected will continue to be available through other VCP repositories.

Date Contract Initiated: June 21, 1965

MICROBIOLOGICAL ASSOCIATES, INC. (NO1-CP-6-0914)

Title: Establish and Operate a BALB/c Mouse Colony

Contractor's Project Director: Mr. Wilbur Athey

Project Officers (NCI): Mr. Samuel Poiley
Dr. Michael Chirigos
Mr. Clarence Reeder

Objectives: To provide BALB/c mice for laboratory investigations supported by the VCP, primarily for virus bioassays on Contract NO1-CP-3-3248.

Major Findings: A pedigreed foundation colony was maintained to produce replacement breeders for the pedigreed foundation, pedigreed expansion, and the production colonies. All the weanlings from the production colony and the surplus weanlings from the pedigreed foundation and the pedigreed expansion colonies have been offered to NCI for issue. From July, 1973 to January, 1974, the contractor produced 31,874 weanlings and issued 13,807 weanlings, 1191 pregnants, 385 mothers with litters, and 254 retired breeders. The balance of the production that was not used for replacement breeders were destroyed as instructed by the Project Officer.

The fecal testing for Salmonella and Pseudomonas and the serologic testing for a battery of murine viruses were scheduled as directed by the Project Officer. Tests for murine viruses indicated that the colony is negative for all viruses except MHV, which showed a positive test on 2 out of 50 samples.

Significance to Biomedical Research and the Program of the Institute: The murine tumor viruses are being extensively studied as models for human cancer viruses. The availability of high quality BALB/c mice is important for assay of these viruses as well as for other studies in viral oncogenesis.

Proposed Course: With the present wide availability of BALB/c mice, the need for this specific contract effort was diminished, and contract activities were terminated on January 31, 1974.

Date Contract Initiated: June 16, 1966

MICROBIOLOGICAL ASSOCIATES, INC. (NO1-CP-3-3288)

Title: Development of Laboratory Animal Virus Diagnostic Reagents and Services

Contractor's Project Director: Dr. John C. Parker

Project Officers: Dr. Robert Holdenried (NCI)
Dr. Wallace P. Rowe (NIAID)
Dr. David McB. Howell (NCI)

Objectives: To develop reagents and tests for the detection of murine and other laboratory rodent and cat viruses; to apply these and other tools in the determination of the importance of the indigenous viruses in experimental systems; to study means for elimination of viruses from laboratory populations; and to assist in the characterization of the gene-dependent expression of murine leukemia.

Major Findings: During the past year, 6,701 sera were submitted to the serodiagnostic laboratory where 40,114 tests were performed. The following types of specimens were received and tested: 4,061 mouse sera (17,645 tests), 183 rat sera (1,632 tests), 582 hamster sera (2,316 tests), 108 guinea pig sera (648 tests), 1,496 MAP sera (17,616 tests), and 271 coded sera and antigens (287 tests). The virus diagnostic laboratory received 87 requests for virus testing by antibody induction tests (MAP, RAP). Twenty-one of these specimens were found to be contaminated by one or more viruses. Viruses found as contaminants in decreasing order of prevalence were: minute virus of mice, lactic dehydrogenase virus, polyoma virus, reovirus, and mouse hepatitis virus. In addition to the diagnostic testing services, the contract prepared where necessary and maintained an inventory of monotypic, certified viral antigens and antisera for 20 murine and 17 feline viruses. Antigens are available to investigators in either the infectious or inactivated form.

The full potential of Sendai virus as an epizootiological agent was realized when approximately 4,000 rats and mice in an experimental animal colony became infected with Sendai virus.

In a collaborative study with Dr. M. Gardner and his associates (NO1-CP-8-1030) at the University of Southern California, a spontaneous lower motor neuron disease in a wild mouse population which is apparently caused by an indigenous type-C RNA virus has been studied and described. Additionally, many aspects of the biology and epizootiology of mouse thymic virus have been studied and elucidated.

Numerous service and research projects were completed or are in progress. Some of these projects are directed towards unraveling the genetic complexities of MLV infection in AKR mice, others involve elimination of virus contaminants from reference reagent pools of The Virus Cancer Program and still others deal with epizootiological investigation in laboratory animal colonies.

Significance to Biomedical Research and the Program of the Institute: The virus diagnostic capabilities provide the NCI with the ability to monitor laboratory rodent and cat colonies and laboratory animal-produced viral reagents and tumors which have resulted in the production of highly characterized systems for cancer research. This contract provides assistance and guidance of particular importance for the detection of LCM in rodent systems. LCM virus, in addition to being infectious for humans, is difficult to detect. Significant contributions are being made to the knowledge of the natural history of several indigenous viruses of laboratory animals.

Proposed Course: To continue the serodiagnostic services outlined above and to improve the sensitivity of the tests. To apply the information developed to reduce and control viral infections in laboratory animal colonies and materials derived from animals.

Date Contract Initiated: April 10, 1961

MONTREAL CHILDREN'S HOSPITAL (NO1-CP-3-3377)

Title: Procurement of Normal and Leukemic Sera from Children

Contractor's Project Director: Dr. Ronald L. Denton

Project Officers (NCI): Dr. David McB. Howell
Dr. Charles Boone

Objectives: To obtain sera from a variety of pediatric oncology patients, family members, and controls for virologic study, to identify special cases for more extended workup.

Major Findings: During this reporting period 383 specimens of serum and other special procurements were made which involved 32 new acute leukemia and solid tumor patients. Added effort was also made to obtain more surgical and post-mortem specimens; 55 tissue preparations were collected as compared to 30 in the previous year.

Periodic shipping of frozen specimens to the NCI Flow Laboratories repository as well as special specimens to the Litton Bionetics resources processing laboratory was done for storage and distribution to NCI investigators.

Significance to Biomedical Research and the Program of the Institute: This contract is one of the Program's primary sources of serum from leukemic children and from suitable, normal controls. The increasing need within the VCP for samples of this kind makes it essential that the supply be continued to satisfy research requirements.

Proposed Course: Continue to collect serum specimens for distribution to collaborating investigators within the VCP.

Date Contract Initiated: September 24, 1965

UNIVERSITY OF PADUA (NO1-CP-3-3359)

Title: Collection of Human Tissue Specimens

Contractor's Project Director: Professor Giovanni Dogo

Project Officers (NCI): Dr. Robert H. Depue, Jr.
Dr. Charles W. Boone

Objectives: To establish fibroblast cultures from skin biopsies taken from inbred and isolated human donors and to provide these cultures to NCI for use in research programs.

Major Findings: Skin biopsies have been obtained from inbred and isolated populations in Sappada and Sauris, two isolated communities in the Dolomite Mountains of Italy, whose inhabitants are highly inbred. Sauris is particularly interesting, since the frequency of both cancer and non-cancer related suicides is very high. Moreover, these frequencies still affect a group of former Sauris inhabitants who descended into a plain community near Tolmezzo at the beginning of this century.

A sample of each skin biopsy has been sent to NCI; in addition, blood samples have been procured from each donor and genealogies are being established for donors from Sauris. Family trees have already been worked out for the Sappada population. The distribution of stomach cancer was uneven between the families from Sappada. The SV40 transformation rates of the skin biopsy material in tissue culture was high but these high rates did not correspond to the indicence of stomach cancer.

Cell lines derived from lymphomas, sarcomas and rhabdomyosarcomas have been established and are available for distribution.

Significance to Biomedical Research and the Program of the Institute: The cell lines established from these skin biopsies will be used in a project to detect human oncogenic viruses *in vitro* and to determine the significance of the transformation test to oncogenesis.

Proposed Course: To collect and culture human skin biopsies and blood samples as before, and to establish genealogies of the Sauris population.

Date Contract Initiated: October 27, 1964

PFIZER, INC. (NO1-CP-3-3234)

Title: Large-scale Tissue Culture Virus Production

Contractor's Project Director: Dr. Sami Mayyasi

Project Officer (NCI): Dr. Jack Gruber

Objectives: To provide a service facility for the production of large volumes of selected oncogenic and suspected oncogenic viruses, cellular antigens, tissue culture cell lines, and specific antisera to various oncogenic viruses. Production of these materials is supported by appropriate laboratory groups whose activities include process improvement, product standardization, quality control testing, and applied developmental research.

Major Findings: During the past year, the contractor processed over 30,000 liters of harvest fluids from a wide variety of tissue culture systems and distributed purified virus concentrates or cells to over 100 different laboratories throughout the world. This year there was an increase in the production of Mason-Pfizer monkey virus (M-PMV), Gibbon ape lymphoma (GALV) virus, and Woolly monkey sarcoma (SSV-1) virus. New production and process improvement measures studied by the contractor included the use of roller bottle apparatus, new cores for zonal centrifuges, and UV monitoring of gradients. Other virus agents also produced for utilization by VCP participating investigators included Rauscher leukemia virus from the JLSV-9 and HEK cell lines, Epstein-Barr virus from the P3 and HR1K cell lines, RD-114B virus, Feline leukemia virus, the Stewart Gomez and Worley agents in special systems, and the AO and J96 cell cultures obtained by NCI from the U.S.S.R. as part of the effort to increase international cooperation in cancer research.

A major effort was directed toward the development of production methods with the new simian agents. The SSV-1 is now in production (200 l/week) in two cell lines: a marmoset line (HF-Deinhardt) and a human lymphoblastoid line (NC-37) infected in the contractor's laboratories. Both lines yield abundant virus which has similar biological and biochemical properties. The infectivity of SSV-1 preparations is monitored on normal rat kidney cells (RNK). The Gibbon ape lymphoma virus is produced in the original tumor line (Kawakami) at low yield, but human lymphoblastoid cells (NC-37) infected with this agent yield 100 times as much virus. The GALV-NC-37 is in production at 100 l/week, and the GALV (Kawakami) is produced on request.

Quality control was improved by the development of a rapid and sensitive method for analysis of viral RNA by gel electrophoresis of detergent-disrupted virus and spectrophotometric scanning of the gel. The RNA from as few as 10^9 virus particles can be detected by this assay, which is 10-20 times more sensitive than sucrose gradient procedures.

The development of a prototype sub-micron particle analyzer continues in collaboration with General Electric Co. Efforts are being made to increase the sensitivity (currently at 10^8 particles/ml.) and reproducibility of the instrument and to compare the results with those obtained by conventional electron microscopic enumerations. Additionally, studies are in progress to utilize a critical-point drying procedure and a specially modified transmission electron microscope to develop a highly accurate, quantitative, and computerized virus particle counting procedure.

The contractor is also in the process of initiating a specially equipped and isolated containment-type quarantine laboratory. This laboratory will function as a receiving station for viruses, cell lines, or other materials received from foreign sources which might contain material of economic significance.

Significance to Biomedical Research and the Program of the Institute: Since its inception, this contract has been an invaluable back-up to intramural and collaborating investigators involved in virus-cancer research.

The staff and facilities have been consistently responsive to changing needs of the NCI Virus Cancer Program. They have provided support to a wide variety of collaborating investigators making possible studies on viruses in cancer that could not otherwise be conducted.

Recent research developments to determine the association of viruses with human neoplasia have involved activities concerned with the molecular biology, biochemistry and immunology of oncogenic viruses. These types of investigations may provide clues to the mechanism whereby viruses mediate the transformation process. Such knowledge could indicate methods by which neoplastic transformation can be averted or inhibited and provide appropriate control measures applicable to the human cancer situation. Studies of this type have created a substantial demand for large quantities of concentrated and purified oncogenic and suspected oncogenic viruses. This contractor has both the capability to help meet such needs and the flexibility to quickly accommodate shifts in Program requirements.

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

Date Contract Initiated: November 6, 1961

ST. JOSEPH'S HOSPITAL (NO1-CP-3-3393)

Title: Study of Human Sarcomas and Their Possible Viral Etiology

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

Project Officers (NCI): Dr. Albert J. Dalton
Dr. David McB. Howell

Objectives: To supply fresh human sarcomas or other tumors which contain EM evidence of virus particles, to attempt to establish cell cultures from these tumors, and to detect virus expression by immunologic and biochemical techniques in those tissues.

Major Findings: During the past year, the contractor continued collection of human sarcomas. Sixty-two continuous tissue cultures were grown successfully from the 73 tissues collected. Electron microscope screening yielded one tumor, a Hodgkin's sarcoma with herpes-type virus. Six tissue cultures were derived from various tumors. These first grew in monolayer culture and then developed cells in suspension. Five of these cultures were found positive for EB virus antigen by immunofluorescence. The sixth, derived from a primary squamous cell carcinoma of the vagina, was negative for EB virus as well as for Herpes type 2 antigens.

Induction experiments with IUdR and BUdR of both short and very long duration were conducted on 3 cell lines growing in suspension. EBV was found in line R 271 and the EM findings were reported.

Frozen sections of the accessioned tumor tissues were screened for EB virus antigens and with autologous serum for tumor specific antigens, so far with negative results.

Homogenates from 17 malignant tumors were inoculated into pathogen-free chick embryo fibroblasts, WI-38, and Vero cells. No cytopathogenic effect was observed.

Human placentas of term pregnancies and fetal tissues from the first trimester were screened for C-type particles. Eighteen placentas and 8 cultures derived from fetal or placental tissues were screened by EM with negative results.

A total of 209 human tumor specimens were collected. One hundred eighty-four of these were shipped to various NCI investigators, primarily through the Flow Laboratories Tissue Repository or the Litton Bionetics Resources Processing Laboratory. Tissue cultures of human sarcomas, cultures of placental cytotrophoblasts, blocks embedded for EM were also supplied to NCI investigators.

Significance to Biomedical Research and the Program of the Institute: This is an important project concerned with the search for viruses in human tumors. Extensive and careful examination, by electron microscopy of a large number of human tumors and cell cultures established from these tumors is essential in determining the viral etiology of cancer.

Proposed Course: Collection of specimens, shipping to assigned laboratories and screening of cultures for EB Virus by EM and by indirect immunofluorescent techniques will continue. The contractor will screen human sarcomas by EM and will continue co-cultivation and chemical induction experiments on sarcoma cultures. Biochemical studies of isoenzymes of nucleic acid metabolism and attempts to isolate virus from induction experiments will continue.

Date Contract Initiated: June 24, 1969

SOUTHWEST FOUNDATION FOR RESEARCH AND EDUCATION (NO1-CP-3-3340)

Title: Housing and Maintenance of a Chimpanzee Colony

Contractor's Project Director: Dr. Seymour S. Kalter

Project Officer (NCI): Dr. Roy Kinard

Objectives: To supply young chimpanzees to VCP investigators.

Major Findings: The chimpanzees on this program continue in a breeding situation. One famale delivered during January. Two additional young females are approaching maturity and should breed in the near future. Five animals died during this period. Physical examinations have been performed on the remaining animals and all were found to be healthy. The animals now number 11.

Type-C virus particles, recovered from baboon placentas and cocultivated in a line of dog thymus cells, were inoculated into newborn chimpanzees by various routes.

Significance to Biomedical Research and the Program of the Institute: The chimpanzee now appears to be the laboratory animal most similar to humans, biochemically and immunologically. Newborn chimpanzees are particularly useful in determining susceptibility to suspected human cancer viruses because their resistance to virus infection is very low. This is the only source of newborn chimpanzees for the VCP.

Proposed Course: The chimpanzee colony will be maintained as before and newborn animals will continue to be supplied to investigators within the VCP.

Date Contract Initiated: April 25, 1969

UNIVERSITY LABORATORIES, INC. (NO1-CP-3-3222)

Title: Production of Oncogenic Viruses and Antisera

Contractor's Project Director: Dr. Eugene H. Bernstein

Project Officers (NCI): Dr. Robert Holdenried
Dr. Jack Gruber

Objectives: Production of leukemia and sarcoma viruses and antisera.

Major Findings: The production of Rous sarcoma virus (Prague strain) in tissue culture roller bottles continued at a very high level. The virus preparations were highly infective to chickens, inducing wing-web tumors in 72-96 hours. The average preparation contained in excess of 9 logs of viral particles per ml. Tissue culture assay revealed more than 7 log P.F.U./ml. NCI investigators used these virus preparations in reverse transcriptase, hybridization and RNA studies. The current level of production is 60 liters per week or a total of 3,100 liters (3,120,000 ml.) annually.

The production in tissue culture roller bottles of Feline leukemia virus using cat thymus cells, was maintained at the high level of 7,200 ml. of 10 fold concentrated virus during the past year. The preparations contained almost 11 logs of viral particles per ml. Of the concentrated FeLV produced, over 5,000 ml. were shipped to designated recipients, and the balance was stored at the Flow Laboratories Repository. The major recipients were Dr. G. Todaro, Dr. R. Gallo, Dr. P. Fischinger, of NCI., and Dr. R. Wilsnack, Huntingdon Research Center. This program was recently terminated and replaced by the production of Moloney Sarcoma Virus obtained from BALB/c mouse tumors.

The production of Rauscher leukemia virus (RLV) from BALB/c mouse plasma continued uninterrupted during the past year. Mouse whole blood was diluted 1:1 with sodium citrate anticoagulant and the resultant plasma virus preparations proved to be of high infectivity, and very useful in reverse transcriptase, hybridization and RNA studies. During the past year, in excess of 10,000 ml. of Rauscher leukemia virus, plasma 1:1, have been produced. The virus preparations average well in excess of 9 logs of viral particles per ml. In assay, they average 4.5-4.9 log F.F.U./ml. on S+L- cells.

The production of MSV from BALB/c mouse tumors commenced early February, 1974. This virus was produced because of a sizable number of requests to NCI for this agent. It is anticipated that 50-100 ml. of the virus will be produced weekly, and shipped to the Flow Repository for dispensing.

RSV (Prague) - approximately 300 ml. of moderate titer antisera to the PR-3 has been produced in chickens. The antisera neutralizes in excess of 2 log F.F.U./ml. of concentrated Prague virus.

University Laboratories, Inc. also assumed responsibility for the existing NIH-NCI-E-73-3222 subcontract program with Dr. K. Maramorosch, Boyce Thompson Institute, Yonkers, N. Y., August, 1973. On March 1, 1974, Dr. Maramorosch relocated to Rutgers University Institute of Microbiology, New Brunswick, N. J. This effort is entitled Electron Microscopic (EM) Studies on the Products of Sarcoma Viruses and Helper Viruses, and its function is three fold: (1) The EM evaluation of virus products for Dr. E. H. Bernstein of University Laboratories, Inc., (2) EM detection of virus particles associated with human breast cancer in collaboration with Dr. M. A. Rich of Michigan Cancer Foundation, and (3) other collaborative studies as requested by the Contract Officer and Dr. E. H. Bernstein.

Significance to Biomedical Research and the Program of the Institute: The supply of highly standardized oncogenic viruses and antisera produced by this contractor has been extensively used by VCP researchers and is essential to the continuation of many important research projects presently being carried out in the Program.

Proposed Course: Production of needed strains of oncogenic viruses and their antisera will continue in volumes necessary to meet VCP needs.

Date Contract Initiated: June 4, 1962

WOLF RESEARCH AND DEVELOPMENT CORP. (NO1-CP-4-3351)

Title: Computer Services in Support of Cancer Research

Contractor's Project Director: Mr. Edwin Lisiecki

Project Officer (NCI): Ms. Wilma Varrato
Dr. David McB. Howell
Dr. Lea I. Sekely

Objectives: The major objective of this contract is the implementation of a computerized central inventory system for the various resources of the VCP.

Major Findings: The system now being installed embraces a number of institutional repository subsystems contributing to the central base currently in the NCI Office of Program Resources & Logistics (OPR&L). Each computerized subsystem is used at a given resource repository to inventory the items produced or stored at that location. Since the types of resources at the various resource banks differ in kind, the subsystems all differ to some extent. They are, however, all designed to contribute compatible information to the central system, thus forming the base for a program-wide central inventory and control.

The resource bank subsystems are also used at storage sites for controlling materials in functions unique to each institution, and this contributes to the detailed differences. Some of these secondary functions include catalog production, local inventory, and retrieval of clinical information.

During the past year, the contractor extended the central inventory system for VCP resources to make optimum use of the private disk obtained by OPR&L, and of direct-access disk programming techniques. The extended system is now in full operation, and is used for all regular maintenance and production runs of the inventory system. Catalog and management report programming was completed.

The contractor has continued support of the resource bank subsystems in the collection of data, reporting of the central system, and in their own computer and manual inventory management. Continued support for data conversion was supplied to Memorial Hospital, and the Naval Biomedical Research Laboratory. In addition, data from Montreal Children's Hospital and the Hospital for Sick Children in Toronto were coded and merged into the central inventory system.

The interactive antisera retrieval system was installed at Huntingdon Research Center, and is operating successfully. An interactive update system for HRC was completed.

Significance to Biomedical Research and the Program of the Institute: The necessary expansion of the inventory of viruses, sera, tissue cultures, human specimens, and other materials used in cancer research makes it vital that close control be exercised over these resources. Computerization of the inventory will eventually make it possible for the NCI Office of Program Resources and Logistics to rapidly obtain information necessary to determine availability, location, quantity, etc. of all resources within its jurisdiction, thereby permitting rapid response to the needs of the Program while avoiding resource excesses or shortages.

Proposed Course: The contractor will continue to extend and refine the central inventory system. Additional management reports will be generated for VCP resources control. Additional work on implemented subsystems will include: regular collection of new data; maintenance of automated subsystems; addition of interactive updates of test and bleeding data in Huntingdon Research Center's production of antisera. Work on new subsystems will be scheduled by OPR&L as needed.

Date Contract Initiated: May 3, 1971

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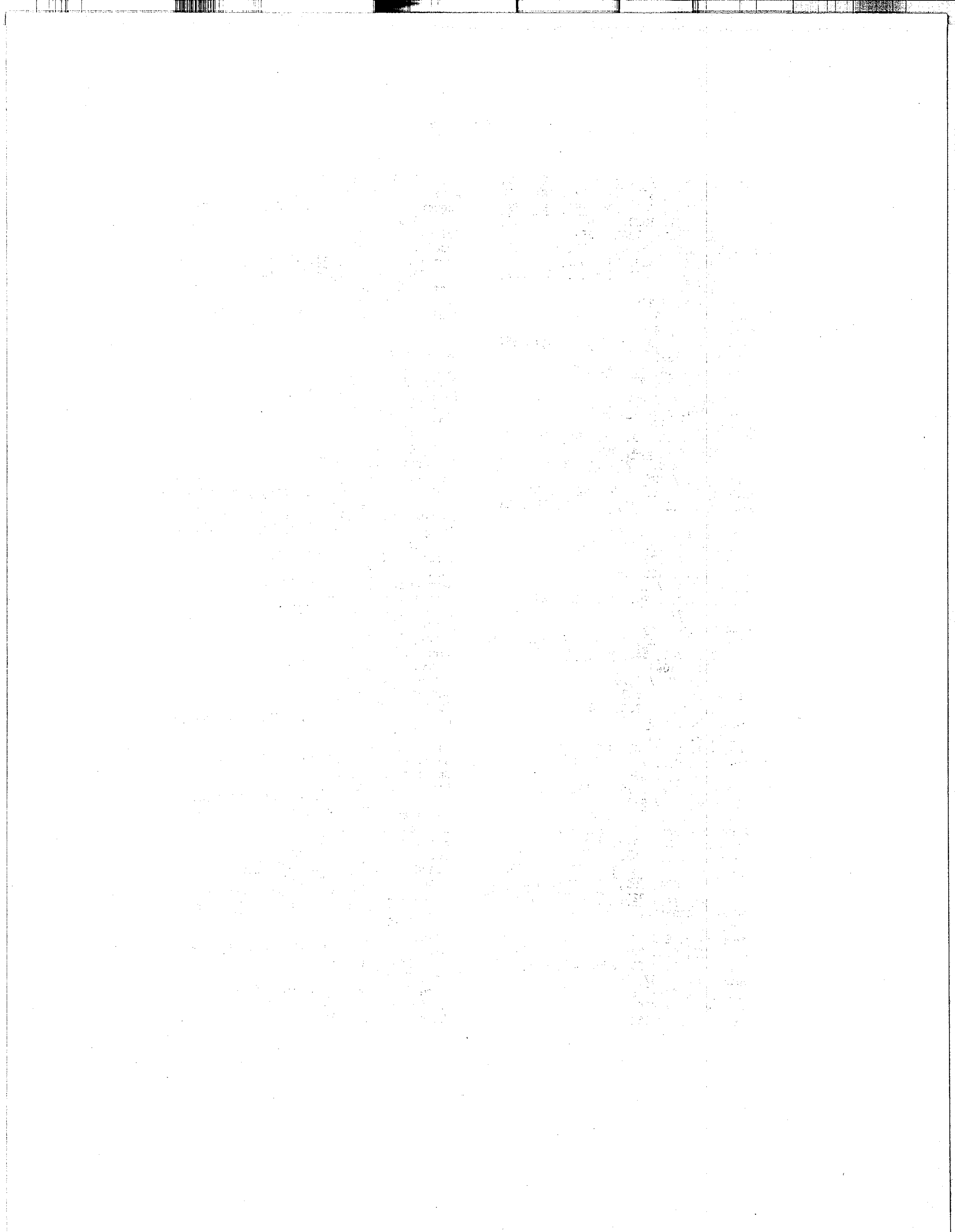

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Albert Einstein College of Medicine	(CP 3-3311)
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Mt. Sinai School of Medicine	(CP 4-3225)
Naples, University of	(CP 3-3314)
Naval Biomedical Research Laboratory	(CP 4-0200)
Naval Biomedical Research Laboratory	(CP 4-0201)
Netherlands Cancer Institute	(CP 3-3368)
New York Medical College	(CP 3-3398)
New York State Dept. of Health	(CP 2-3247)
North Carolina, University of	(CP 3-3336)
North Dakota, University of	(CP 6-0008)
Ohio State Research Foundation	(CP 4-3217)
Pennsylvania State University	(CP 0-2024)
Pfizer, Inc.	(CP 3-3234)
Pfizer, Inc.	(CP 3-3239)
Radiobiological Institute	(CP 4-3328)
Rockefeller University	(CP 3-3306)
Rush-Presbyterian-St. Luke's Medical Center	(CP 3-3219)
Salk Institute for Biological Studies	(CP 4-3243)
Scripps Clinic and Research Foundation	(CP 3-3204)
Scripps Clinic and Research Foundation	(CP 4-3375)

Southern California, University of	(CP 4-3242)
Southern California, University of	(CP 8-1030)
Southwest Foundation for Research and Education	(CP 3-3340)
Southwest Foundation for Research and Education	(CP 4-3214)
St. Joseph's Hospital	(CP 3-3393)
St. Louis University	(CP 4-3359)
Stanford University	(CP 4-3244)
Tel Aviv University	(CP 2-3237)
Texas, University of	(CP 1-3135)
Texas, University of	(CP 3-3292)
Texas, University of	(CP 3-3301)
Texas, University of	(CP 3-3304)
Tulane Univ.-Delta Reg. Primate Ctr.	(CP 3-3396)
University Laboratories, Inc.	(CP 3-3222)
Washington, University of	(CP 3-3372)
Washington, University of	(CP 3-3236)
Weizmann Institute of Science	(CP 4-3241)
Wisconsin, University of	(CP 2-2022)
Wistar Institute of Anatomy and Biology	(CP 3-3250)
Wolf Research and Development Corp.	(CP 4-3351)

CONTRACTOR : Aichi Cancer Center (CP 3-3290)
ADDRESS : Research Institute, Aichi Cancer Center, Tashiro-cho, Chigusa-ku, Nagoya, Japan
CNTRCT TITLE: Immunologic and Epideminologic Studies on Cancer Patients in Japan
PRINC INVEST: Dr. Yohei Ito, Laboratory of Viral Oncology
PHONE : AC-052, 762-6111, x-731
PROJ OFFICER: Dr. Jack Gruber, Bldg. 37, Rm. 1A19, x-61718,
SEGMENT : Immunology-Epidemiology

CONTRACTOR : Albert Einstein College of Medicine (CP 3-3349)
ADDRESS : Yeshiva Univ., 1300 Morris Park Avenue, Bronx, New York 10461
CNTRCT TITLE: Genetic and Immunologic Factors in Viral Leukemogenesis
PRINC INVEST: Dr. Frank Lilly
PHONE : AC-212, 430-2826
PROJ OFFICER: Dr. Robert J. Huebner, Bldg. 37, Rm. 2D24, x-63301,
SEGMENT : Solid Tumor Virus

CONTRACTOR : Albert Einstein College of Medicine (CP 3-3311)
ADDRESS : Yeshiva Univ., 1300 Morris Park Avenue, Bronx, New York 10461
PRINC INVEST: Dr. J. Thomas August, Dept. of Molecular Biology
PHONE : AC-212, 430-2000
PROJ OFFICER: Dr. Robert Manaker, Bldg 37, Rm. 1B14, x-63323,
SEGMENT : Developmental Research

CONTRACTOR : Atomic Energy Commission (CP 2-0208)
ADDRESS : Oak Ridge National Laboratory, P. O. Box Y
Oak Ridge, Tennessee 37830
CNTRCT TITLE: Studies in Viral Chemical CoCarcinogenesis
PRINC INVEST: Dr. Francis T. Kenney, Biology Division
PHONE : AC-615, 483-8611
PROJ OFFICER: Dr. Bernard Talbot, Bldg. 37, Rm. 1B22, x-66135,
SEGMENT : Tumor Virus Detection

CONTRACTOR : Atomic Energy Commission (CP 4-3210)
ADDRESS : Univ. of Tennessee
Hesler Biology Bldg., Room 419
Knoxville, Tennessee 37916
CNTRCT TITLE: Studies on the Relationship of Fetal Antigens to the Etiology and Control of Cancer
PRINC INVEST: Dr. Joseph Coggin, Jr., Dept. of Microbiology
PHONE : AC-615, 974-2356
PROJ OFFICER: Dr. Gary Pearson, Bldg. 37, Rm. 1B05, x-62600,
SEGMENT : Immunology-Epidemiology

CONTRACTOR : Baylor University College of Medicine (CP 3-3257)
ADDRESS : Texas Medical Center, Houston, Texas 77025
CNTRCT TITLE: Studies on Viruses Related to Cancer
PRINC INVEST: Dr. Joseph L. Melnick
PHONE : AC-713, 529-4951, x-403
PROJ OFFICER: Dr. Robert Manaker, Bldg 37, Rm. 1B14, x-63323,
Dr. Paul Gerber, Bldg. 29A, Rm. 3B03, x-62696
Dr. Michael Chirigos, Bldg. 37, Rm. 1D19, x-61478
SEGMENT : Developmental Research

CONTRACTOR : Baylor University College of Medicine (CP 4-3355)
ADDRESS : 1200 Moursund Ave., Texas Medical Center
Houston, Texas 77025
CNTRCT TITLE: Nonsense Suppressor Mutants for 3T3 Cells
PRINC INVEST: Dr. C. Thomas Caskey, Department of Medicine and Biochemistry
PHONE : AC-713, 5129-4951
PROJ OFFICER: Dr. Edward M. Scolnick, Bldg. 37, Rm. 1B22, x-66135,
SEGMENT : Tumor Virus Detection

CONTRACTOR : Biolabs, Inc. (CP 2-2068)
ADDRESS : 2910 MacArthur Boulevard
Northbrook, Illinois 60062
CNTRCT TITLE: Development and Evaluation of Methods for Large Scale Preparation of Purified Oncogenic Herpesviruses
PRINC INVEST: Dr. Clyde R. Goodheart
PHONE : AC-312, 498-6020
PROJ OFFICER: Dr. Dharam Ablashi, FCRC, Bldg. 538, Rm. 205B, 393-1839, x-2181,
SEGMENT : Tumor Virus Detection

CONTRACTOR : California, Univ. of / at Berkeley (CP 4-3212)
ADDRESS : 118 California Hall, Berkeley, California 94720
CNTRCT TITLE: Studies on the Structure and Replication of Viruses and Mechanisms of Regulation
PRINC INVEST: Dr. Peter Duesberg
PHONE : AC-415, 642-0942
PROJ OFFICER: Dr. George Todaro, Bldg. 37, Rm. 1B22, x-66135,
SEGMENT : Tumor Virus Detection

CONTRACTOR : California, Univ. of / at Davis (CP 3-3242)
ADDRESS : Davis, California 95616
CNTRCT TITLE: Comparative Leukemia and Sarcoma Viral Studies
PRINC INVEST: Dr. Thomas G. Kawakami
PHONE : AC-916, 752-1944
PROJ OFFICER: Dr. Wade P. Parks, Bldg. 37, Rm. 1B17, x-65968,
Dr. James T. Duff, Bldg. 37, Rm. 1B17, x-65968
SEGMENT : Solid Tumor Virus

CONTRACTOR : California, Univ. of / at Davis (CP 3-3253)
ADDRESS : Davis, California 95616
CNTRCT TITLE: In Vitro Cultivation of Human and Mouse Mammary Tumor Virus
PRINC INVEST: Dr. Robert D. Cardiff
PHONE : AC-916-752-3179 (office) 752-3358 (lab)
PROJ OFFICER: Dr. Robert Bassin, Landow Bldg., Rm. C308, x-64533
Dr. Jeffrey Schlom, Landow Bldg., Rm. C308, x-64533
SEGMENT : Breast Cancer Virus

CONTRACTOR : California, Univ. of / at L. A. (CP 3-3283)
ADDRESS : Center for Health Sciences,
Los Angeles, California 90024
CNTRCT TITLE: Search for Viral DNA in Tissue from Cancer Patients
PRINC INVEST: Dr. Marcel Baluda
PHONE :
PROJ OFFICER: Dr. George Todaro, Bldg. 37, Rm. 1B22, x-66135,
Dr. Bernard Talbot, Bldg. 37, Rm. 1B22, x-66135
SEGMENT : Tumor Virus Detection

CONTRACTOR : California, Univ. of / at L. A. (CP 4-3211)
ADDRESS : 405 Hilgard Avenue, Los Angeles, California 90024
CNTRCT TITLE: Studies on the Interrelationship of Viruses, Genetics and Immunity in the Etiology and Control of Cancer
PRINC INVEST: Dr. Paul I. Terasaki, Department of Surgery
PHONE : AC-213, 825-7651
PROJ OFFICER: Dr. Paul Levine, Landow Bldg., Rm. C306, x-66085,
Dr. Ernest J. Plata, Landow Bldg., Rm. C308, x-64534
Dr. Gary Pearson, Bldg. 37, Rm. 1B05, x-62600
SEGMENT : Immunology-Epidemiology

CONTRACTOR : California, Univ. of / La Jolla (CP 4-3298)
ADDRESS : P. O. Box 109
La Jolla, California 92037
CNTRCT TITLE: The Study of Regulatory Proteins in Oncogenic Viruses
Dr. Theodore Friedmann
PHONE : AC-714, 453-2000, x-2323
PROJ OFFICER: Dr. Edward M. Scolnick, Bldg. 37, Rm. 1B22, x-66135
SEGMENT : Tumor Virus Detection

CONTRACTOR : California, Univ. of / at Oakland (CP 3-3237)
ADDRESS : Naval Biological Research Laboratory, Naval Supply Center
Oakland, California 94625
CNTRCT TITLE: Development and Evaluation of Cell Substrates for the Study of Cancer Viruses
PRINC INVEST: Dr. Stewart H. Madin
PHONE : AC-415, 642-1055
PROJ OFFICER: Dr. James T. Duff, Bldg. 37, Rm. 1B17, x-65968,
SEGMENT : Program Resources and Logistics

CONTRACTOR : California, Univ. of/ at San Francisco (CP 3-3293)
ADDRESS : San Francisco Medical Center
San Francisco, California 94122
CNTRCT TITLE: Studies on the Role of Virion-Associated DNA Polymerases in Malignant Transformation by Avian Tumor Viruses
PRINC INVEST: Dr. J. Michael Bishop
AC-415,666-9000
PROJ OFFICER: Dr. Edward M. Scolnick, Bldg. 37, Rm. 1B22, x-66135
SEGMENT : Solid Tumor Virus

CONTRACTOR : California, Univ. of / at San Francisco (CP 3-3332)
ADDRESS : San Francisco, California 94112
CNTRCT TITLE: Hormonal Control of Gene Expression in Tumor Viruses
PRINC INVEST: Dr. Gordon Tomkins
PHONE : AC-415, 666-4132
PROJ OFFICER: Dr. Edward M. Scolnick, Bldg. 37, Rm. 1B22, x-66135
Dr. James T. Duff, Bldg. 37, Rm. 1B17, x-65968
SEGMENT : Tumor Virus Detection

CONTRACTOR : California Institute of Technology (CP 4-3306)
ADDRESS : 1201 East California Blvd.
Pasadena, California 91109
CNTRCT TITLE: Electron Microscope Studies of Tumor Virus Nucleic Acids
PRINC INVEST: Dr. Norman Davidson
PHONE : AC-213, 795-6841/795-6811, x-2055
PROJ OFFICER: Dr. Edward M. Scolnick, Bldg. 37, Rm. 1B22, x-66135
SEGMENT : Solid Tumor Virus

CONTRACTOR : California State Dept. of Public Health (CP 4-3209)
ADDRESS : 2151 Berkeley Way, Berkeley, California 94704
CNTRCT TITLE: Role of Oncogenic Viruses in the Causation of Cancer in Man
PRINC INVEST: Dr. Edwin Lennette, Viral and Rickettsial Disease Laboratory
PHONE : AC-415, 843-7900, 514
PROJ OFFICER: Dr. Paul Arnstein, Bldg. 37, Rm. 1B17, x-65968
Dr. Padman Sarma, Bldg. 37, Rm. 2D23, x-63301
SEGMENT : Solid Tumor Virus

CONTRACTOR : Center for Disease Control (CP 4-0202)
ADDRESS : 1600 Clifton Road, N. E.,
Atlanta, Georgia 30322
CNTRCT TITLE: Epidemiologic Studies of Leukemia and Related Diseases
PRINC INVEST: Dr. Clark W. Heath, Jr., Epidemiology Program
PHONE : AC-404, 633-3311, x-3961
PROJ OFFICER: Mr. Roger Connelly, Landow Bldg., Rm. A506, x-65251
SEGMENT : Immunology-Epidemiology

CONTRACTOR : Chicago Park District (Lincoln Park Zoo)
ADDRESS : 100 West Webster, Chicago, Illinois 60614
CNTRCT TITLE: Maintenance of a Marmoset Breeding Colony
PRINC INVEST: Dr. Lester Fisher
PHONE : AC-312-294-4660
PROJ OFFICER: Dr. Lea Sekely, Bldg. 37, Rm. 1D21, x-61718
Dr. David McB. Howell, Bldg. 37, Rm. 1D21, x-61718
SEGMENT : Program Resources and Logistics

CONTRACTOR : Child Research Center of Michigan (CP 3-3333)
ADDRESS : 3901 Beaubein Boulevard
Detroit, Michigan
CNTRCT TITLE: Inter-Intraspecies Identification of Cancer Cell in Vitro
PRINC INVEST: Dr. Cyril A. Stulberg
PHONE : AC-313, 494-5569
PROJ OFFICER: Dr. David McB. Howell, Bldg. 37, Rm. 1D21, x-61718
Dr. Robert Bassin, Landow Bldg., Rm. C308, x-64533
SEGMENT : Program Resources and Logistics

CONTRACTOR : Children's Cancer Research Fdn., Inc. (CP 3-3333)
ADDRESS : 35 Binney Street
Boston, Massachusetts 02115
CNTRCT TITLE: Isolating Type C Virus Cultured Human Leukemic Cells
PRINC INVEST: Dr. George E. Foley
PHONE : AC-617, 734-6000, x-3173
PROJ OFFICER: Dr. Bernard Talbot, Bldg. 37, Rm. 1B22, x-66135
SEGMENT : Tumor Virus Detection

CONTRACTOR : Children's Hospital of Philadelphia (CP 3-3272)
ADDRESS : 1740 Bainbridge Street
Philadelphia, Pennsylvania 19146
CNTRCT TITLE: Propagation and Seroepidemiology of EB Virus
PRINC INVEST: Dr. Gertrude Henle, Research Department
PHONE : AC-215, 546-2700
PROJ OFFICER: Dr. Paul Levine, Landow Bldg., Rm. C306, x-66085
Dr. George Burton, Landow Bldg., Rm. C309, x-66085
SEGMENT : Immunology-Epidemiology

CONTRACTOR : Colorado, University of (CP 3-3400)
ADDRESS : Medical Center, 4200 East 9th Avenue
Denver, Colorado 80220
CNTRCT TITLE: Collection of Pediatric Tumor Specimens
PRINC INVEST: Dr. William E. Hathaway, Dept. of Pediatrics
PHONE : AC-303, 394-8471
PROJ OFFICER: Dr. David McB. Howell, Bldg. 37, Rm. 1D21, x-61718
Dr. Lea Sekely, Bldg. 37, Rm. 1D21, x-61718
SEGMENT : Program Resources and Logistics

CONTRACTOR : Columbia, University of (CP 3-3258)
ADDRESS : Institute for Cancer Research, 99 Fort Washington Ave.,
New York, New York 10031
CNTRCT TITLE: Replication of Oncogenic RNA Viruses and Its Relation to Human Cancer
PRINC INVEST: Dr. Sol Spiegelman
PHONE : AC-212, 579-8582
PROJ OFFICER: Dr. Robert Manaker, Bldg 37, Rm. 1B14, x-63323
Dr. Maurice L. Guss, Bldg. 47, Rm. 1B14, x-63323.
SEGMENT : Developmental Research

CONTRACTOR : Dow Chemical Company (CP 3-3243)
ADDRESS : Ocean Science Bldg., 3 Choke Cherry Road
Rockville, Maryland 20850
CNTRCT TITLE: Research and Development of Biohazards Containment Facilities
PRINC INVEST: Mr. Cyril B. Henke, Environmental Bio-Engineering
PHONE : AC-301, 869-2700
PROJ OFFICER: Dr. W. Emmett Barkley, Bldg. 41, Rm. A115, x-66981
Dr. Alfred Hellman, Bldg. 41, Rm. A103, x-66758
SEGMENT : Program Resources and Logistics

CONTRACTOR : Duke University (CP 3-3308)
ADDRESS : Durham, North Carolina 27706
CNTRCT TITLE: Studies on the Expression of the RNA Tumor Virus Genome in Animal
and Human Malignant Cells
PRINC INVEST: Dr. Dani P. Bolognesi
PHONE : AC-919, 684-3541
PROJ OFFICER: Dr. Peter J. Fischinger, Bldg. 41, Rm. A117, x-66588
Dr. Maurice L. Guss, Bldg. 47, Rm. 1B14, x-63323
SEGMENT : Developmental Research

CONTRACTOR : Electro-Nucleonics Laboratories, Inc. (CP 2-3249)
ADDRESS : 12059 Tech Road, Montgomery Industrial Park
Silver Spring, Maryland 20904
CNTRCT TITLE: Production and Purification of Oncogenic or Potentially Oncogenic
Viruses
PRINC INVEST: Mr. John Lemp, Jr., Biological Engineering Laboratory
PHONE : Ac-301, 652-4923, x-42
PROJ OFFICER: Dr. Jack Gruber, Bldg. 37, Rm. 1A19, x-61718
Dr. David McB. Howell, Bldg. 37, Rm. 1D21, x-61718
SEGMENT : Program Resources and Logistics

CONTRACTOR : Electro-Nucleonics Laboratories, Inc. (CP 3-3355)
ADDRESS : 12050 Tech Road, Montgomery Industrial Park
Silver Spring, Maryland 20904
CNTRCT TITLE: Development of Propagation Procedures, Purification and
Characterization of Viruses
PRINC INVEST: Mr. John Lemp, Jr., Biological Engineering Laboratory
PROJ OFFICER: Dr. George Todaro, Bldg. 37, Rm. 1B22, x-66135
Dr. Jack Gruber, Bldg. 37, Rm. 1A19, x-61718
SEGMENT : Program Resources and Logistics

CONTRACTOR : Emory University (CP 2-2301)
ADDRESS : School of Medicine, 46 Armstrong St.
Atlanta, Georgia 30303
CNTRCT TITLE: Collaboratorive Project on the Oncogenic Potential of Herpes Viruses in Primates
PRINC INVEST: Dr. Andre Nahmias, Dept. of Pediatrics
PHONE : AC-404, 659-1212
PROJ OFFICER: Dr. Robert Manaker, Bldg 37, Rm. 1B14, x-63323
Dr. Gary Pearson, Bldg. 37, Rm. 1B05, x-62600
SEGMENT : Developmental Research

CONTRACTOR : Emory University (CP 3-3343)
ADDRESS : Yerkes Regional Primate Research Center
Atlanta, Georgia 30322
CNTRCT TITLE: Maintenance of a Colony of Irradiated Rhesus Monkeys
PRINC INVEST: Dr. Harald M. McClure
PHONE : AC-404, 377-2411
Dr. David McB. Howell, Bldg. 37, Rm. 1D21, x-61718
SEGMENT : Program Resources and Logistics

CONTRACTOR : Flow Laboratories, Inc. (CP 3-3201)
ADDRESS : 1710 Chapman Avenue
Rockville, Maryland 20852
CNTRCT TITLE: Maintenance of a Low Temperature Repository for Storage and Distribution of Serum and Tissue Specimens
PRINC INVEST: Dr. Harry F. Adkins (Respository)
Dr. Fang-tsum Kuo (Laboratory)
PHONE : AC-301, 881-2900
Dr. Jack Gruber, Bldg. 37, Rm. 1A19, x-61718
SEGMENT : Program Resources and Logistics

CONTRACTOR : Flow Laboratories, Inc. (CP 3-3247)
ADDRESS : 1710 Chapman Avenue
Rockville, Maryland 20852
CNTRCT TITLE: Studies of Herpes Viruses and C-Type Viruses in Relations to Oncogenic Potential
PRINC INVEST: Dr. Raymond V. Gilden
PHONE : AC-301, 881-2900
PROJ OFFICER: Dr. Robert J. Huebner, Bldg. 37, Rm. 2D24, x-63301
Dr. James T. Duff, Bldg. 37, Rm. 1B17, x-65968
Dr. Berge Hampar, Bldg. 37, Rm. 1B17, x-65967
SEGMENT : Solid Tumor Virus

CONTRACTOR : Flow Laboratories, Inc. (CP 3-3247)
ADDRESS : 1710 Chapman Avenue
Rockville, Maryland 20852
CNTRCT TITLE: Maintenance of a Repository for Storage and Distribution of Reagents and Tissue Specimens
PRINC INVEST: Dr. Raymond V. Gilden
PHONE : AC-301, 881-2900
PROJ OFFICER: Dr. Robert J. Huebner, Bldg. 37, Rm. 2D24, x-63301
SEGMENT : Solid Tumor Virus

CONTRACTOR : Flow Laboratories, Inc. (CP 3-3391)
ADDRESS : 1710 Chapman Avenue
Rockville, Maryland 20852
CNTRCT TITLE: Animal Holding and Breeding for Detection of Tumor Virus Information
PRINC INVEST: Ms. Judy Torgersen
PHONE : AC-301, 881-2900
PROJ OFFICER: Dr. John Pearson, Bldg. 37, Rm. 1D16, x-61478
Dr. Adi Gazdar, Bldg. 41, Rm. 200, x-61200
SEGMENT : Program Resources and Logistics

CONTRACTOR : Frederick Cancer Res. Center (Litton Bionetics) (CP 2-0207)
ADDRESS : Fort Detrick, Frederick, Maryland 21701
CNTRCT TITLE: Interagency Agreement for Support Servies for the Frederick Cancer Research Center
PRINC INVEST: Colonel Bost
PHONE : AC-301, 663-7309/ NIH dial 9, 393-1839
PROJ OFFICER: Dr. William Payne, FCRC, 9/393-1839
Dr. Henry Hearn, FCRC, 9/393-1839
SEGMENT : Program Management

CONTRACTOR : George Washington University (CP 2-3251)
ADDRESS : 2300 K Street, N. W.
Washington, D. C. 20006
CNTRCT TITLE: In Vivo and In Vitro Studies of the Immune Response to EBV-Associated Antigens in Lymphoma Patients and Controls
PRINC INVEST: Dr. T. Crandall Alford
PHONE : AC-202, 676-6562
PROJ OFFICER: Dr. Ronald Herberman, Bldg. 10, Rm. 5B49, x-61366
Dr. Paul Levine, Landow Bldg., Rm. C306, x-66085
SEGMENT : Immunology-Epidemiology

CONTRACTOR : Georgetown University (CP 3-3404)
ADDRESS : 3800 Reservoir Road, N. W.
Washington, D. C. 20007
CNTRCT TITLE: Supply of Blood Tissue Specimens from Patients with Malignancies
PRINC INVEST: Dr. Richard A. Binder
PHONE : AC-202, 625-7488
PROJ OFFICER: Dr. David McB. Howell, Bldg. 37, Rm. 1D21, x-61718
SEGMENT : Program Resources and Logistics

CONTRACTOR : Georgetown University (CP 5-0053)
ADDRESS : 3800 Reservoir Road, N. W.
Washington, D. C. 20007
CNTRCT TITLE: Human Breast Cancer Virus Studies
PRINC INVEST: Dr. William F. Feller
PHONE : AC-202, 625-7368
PROJ OFFICER: Dr. Ernest J. Plata, Landow Bldg., Rm. C308, x-64534
Dr. Takis S. Papas, Bldg. 37, Rm. 1B17, x-65967
SEGMENT : Breast Cancer Virus

CONTRACTOR : Harvard College (CP 3-3265)
ADDRESS : Thorndike Memorial Laboratory, Boston City Hospital
Boston, Massachusetts 02118
CNTRCT TITLE: Primary Structure and Synthesis of Avian Leukosis Virus Protein
PRINC INVEST: Dr. David W. Allen, Hematology Division
PHONE : p AC-617, 424-4252
PROJ OFFICER: Dr. Padman S. Sarma, Bldg. 37, Rm. 2D23, x-63301
Dr. Bernard Talbot, Bldg. 37, Rm. 1B22, x-66135
SEGMENT : Solid Tumor Virus

CONTRACTOR : Harvard College (CP 3-3390)
ADDRESS : New England Regional Primate Center, 1 Pine Hill Drive
Southborough, Massachusetts 01772
CNTRCT TITLE: Isolation and Characterization of DNA Viruses Associated with Primate Tumors
PRINC INVEST: Dr. Luis Melendez
PHONE : AC-617, 481-0400
PROJ OFFICER: Dr. Gary Pearson, Bldg. 37, Rm. 1B05, x-62600
SEGMENT : Tumor Virus Detection

CONTRACTOR : Harvard College (CP 4-3299)
ADDRESS : School of Medicine, 25 Shattuck St.
Boston, Massachusetts 02115
CNTRCT TITLE: Studies of Relationship Between Cells Transformed by DNA and RNA Viruses and Genetic and Biochemical Studies of MLV and MSV
PRINC INVEST: Dr. Thomas Benjamin
PHONE : AC-617, 734-3300
PROJ OFFICER: Dr. George Todaro, Bldg. 37, Rm. 1B22, x-66135
SEGMENT : Tumor Virus Detection

CONTRACTOR : Harvard School of Public Health (CP 4-3218)
ADDRESS : 55 Shattuck Street
Boston, Massachusetts 02115
CNTRCT TITLE: Pilot Study of Cancer and Chronic Diseases of Virologic and Laboratory Workers
PRINC INVEST: Dr. Thomas M. Mack
PHONE : AC-617, 734-3300, x-595
PROJ OFFICER: Dr. Alfred Hellman, Bldg. 41, Rm. A103, x-66758
SEGMENT : Program Management

CONTRACTOR : Hazleton Laboratories, Inc. (CP 3-3212)
ADDRESS : 9200 Leesburg Pike
Vienna, Virginia 22180
CNTRCT TITLE: The Roles of Viruses and Experimental Oncogenesis and Human Cancer
PRINC INVEST: Dr. Robert C. Good
PHONE : AC-703, 893-5400
PROJ OFFICER: Dr. Stuart Aaronson, Bldg. 37, Room 1B22, x-66135
Dr. John R. Stephenson
SEGMENT : Solid Tumor Virus

CONTRACTOR : Hebrew University (CP 3-3210)
ADDRESS : Hadassah Medical School
Jerusalem Israel
CNTRCT TITLE: Studies on Herpes Virus (EBV) and Its Role in Human Cancer
PRINC INVEST: Dr. Yechiel Becker, Department of Virology
PHONE : 4111, x027
PROJ OFFICER: Dr. Berge Hampar, Bldg. 37, Rm. 1B17, x-65967
Dr. Bernard Talbot, Bldg. 37, Rm. 1B22, x-66135
SEGMENT : Tumor Virus Detection

CONTRACTOR : Hebrew University (CP 3-3342)
ADDRESS : Hadassah Medical School
Jerusalem, Israel
CNTRCT TITLE: A Multidisciplinary Study of Hodgkin's Disease in Israel
PRINC INVEST: Dr. Natan Goldblum
PHONE :
PROJ OFFICER: Dr. Paul Levine, Landow Bldg., Rm. C306, x-66085
Mr. Roger Connelly, Landow Bldg., Rm. A506, x-65251
Dr. George Burton, Landow Bldg., Rm. C309, x-66085
SEGMENT : Immunology-Epidemiology

CONTRACTOR : Hopital St. Louis (CP 3-3365)
ADDRESS : Institut de Recherches sur les Maladies du Sang
2, Place du Docteur-Fournier
Paris X, France
CNTRCT TITLE: Molecular Virology Studies on Human Leukemia
PRINC INVEST: Dr. Michel Boiron
PHONE : Cen 9054
PROJ OFFICER: Dr. George Todaro, Bldg. 37, Rm. 1B22, x-66135
Dr. Robert Manaker, Bldg 37, Rm. 1B14, x-63323
SEGMENT : Developmental Research

CONTRACTOR : Hospital for Sick Children (CP 2-3266)
ADDRESS : 555 University Avenue
Toronto 2, Ontario, Canada
CNTRCT TITLE: Collection of Specimens from Human Pediatric Leukemia Patients As Non-Leukemia Controls
PRINC INVEST: Dr. Peter D. McClure
PHONE : AC-416, 366-7242
PROJ OFFICER: Dr. David McB. Howell, Bldg. 37, Rm. 1D21, x-61718
Dr. Charles Boone, Bldg. 37, Rm. 1C08, x-65141
SEGMENT : Program Resources and Logistics

CONTRACTOR : Howard University (CP 4-3287)
ADDRESS : College of Medicine, Freedman's Hosp. Cancer Res. Center
6th & Bryant Street, N. W., Washington, D. C. 20001
CNTRCT TITLE: Correlation of Molecular Virology Studies to Diagnosis of Breast Cancer
PRINC INVEST: Dr. William W. Funderburk
PHONE : AC-202, 462-2120
PROJ OFFICER:
SEGMENT : Breast Cancer Virus

CONTRACTOR : Huntingdon Research Center (CP 3-3223)
ADDRESS : Div. of Becton -Dickenson & Co, P. O. Box 6857
Baltimore, Maryland 21204
CNTRCT TITLE: Preparation, Characterization and Distribution of Antisera to Oncogenic Viral Antigens
PRINC INVEST: Dr. Roger Wilsnack
PHONE : AC-301, 825-3484
PROJ OFFICER: Dr. Robert Holdenried, Bldg. 41, Rm. A102, x-64333
Dr. Wallace Rowe, Bldg. 7, Rm. 304, x-62613
Dr. David McB. Howell, Bldg. 37, Rm. 1D21, x-61718
SEGMENT : Program Resources and Logistics

CONTRACTOR : Illinois University of (CP 4-3318)
ADDRESS : P. O. 6998
Chicago, Illinois 60680
CNTRCT TITLE: Studies of the Molecular Mechanisms of Carcinogenesis by Oncogenic Viruses
PRINC INVEST: Dr. Giampiero di Mayorca, Dept. of Medical Microbiology
PHONE : AC-312, 996-7473
PROJ OFFICER: Dr. Bernard Talbot, Bldg. 37, Rm. 1B22, x-66135
Dr. George Todaro, Bldg. 37, Rm. 1B22, x-66135
Dr. Robert Bassin, Landow Bldg., Rm. C308, x-64533
SEGMENT : Tumor Virus Detection

CONTRACTOR : Institut du Radium (CP 4-3219)
ADDRESS : 26 Rue d'Ulm
Paris Ve, France
CNTRCT TITLE: Molecular and Genetic Studies of Rous Sarcoma Virus
PRINC INVEST: Dr. Roman Latarjet
PHONE : 907-6467
PROJ OFFICER: Dr. Robert Bassin, Landow Bldg., Rm. C308, x-64533
Dr. Bernard Talbot, Bldg. 37, Rm. 1B22, x-66135
SEGMENT : Tumor Virus Detection

CONTRACTOR : Institute for Medical Research (CP 3-3339)
ADDRESS : Sheridan and Copewood Streets
Camden, New Jersey 08103
CNTRCT TITLE: Studies of Human Milk and Mammary Tumors
PRINC INVEST: Dr. Dan Moore
PHONE : AC-609, 966-7377
PROJ OFFICER: Dr. Jeffrey Schlom, Landow Bldg., Rm. C308, x-64533
Dr. Wade P. Parks, Bldg. 37, Rm. 1B17, x-65968
Dr. Takis S. Papas, Bldg. 37, Rm. 1B17, x-65967
SEGMENT : Breast Cancer Virus

CONTRACTOR : International Agency for Research on Cancer (CP 4-3296)
ADDRESS : 150 Cours Albert Thomas
69008 Lyon, France
CNTRCT TITLE: Sero-epidemiological Laboratory Studies on Naso-pharyngeal Carcinoma and Burkitt's Lymphoma
PRINC INVEST: Dr. Guy de The
PHONE : AC-78, 52-32-40
PROJ OFFICER: Dr. Paul Levine, Landow Bldg., Rm. C306, x-66085
Dr. George Burton, Landow Bldg., Rm. C309, x-66085
SEGMENT : Immunology-Epidemiology

CONTRACTOR : International Union Against Cancer (CP 4-3292)
ADDRESS : P. O. Box 400
1211, Geneva 2, Switzerland
CNTRCT TITLE: International Investigation on the Epidemiology of Lymphomas
PRINC INVEST: Dr. Jean F. Delafresnaye
PHONE :
PROJ OFFICER: Mr. Roger Connelly, Landow Bldg., Rm. A506, x-65251
SEGMENT : Immunology-Epidemiology

CONTRACTOR : Jackson Memorial Laboratory (CP 3-3255)
ADDRESS : Bar Harbor, Maine 04609
CNTRCT TITLE: Murine Leukemia-Sarcoma Complex: Natural Occurence of Leukemia Virus and the Sarcoma Genome in Mice
PRINC INVEST: Dr. Hans Meier
PHONE : AC-207, 288-3373
PROJ OFFICER: Dr. Robert J. Huebner, Bldg. 37, Rm. 2D24, x-63301
Dr. Wade P. Parks, Bldg. 37, Rm. 1B17, x-65968
SEGMENT : Solid Tumor Virus

CONTRACTOR : Jewish Hosp. and Medical Ctr. of Brooklyn (CP 4-3251)
ADDRESS : 555 Prospect Place
Brooklyn, New York 11238
CNTRCT TITLE: Study of Viral Transformation and Chromosome Abnormalities in Human Tumors
PRINC INVEST: Dr. Harvey Dosik
PHONE : AC-212, 240-1211,(office) 240-1427 (lab)
PROJ OFFICER: Dr. George Todaro, Bldg. 37, Rm. 1B22, x-66135
Dr. Bernard Talbot, Bldg. 37, Rm. 1B22, x-66135
SEGMENT : Program Resources and Logistics

CONTRACTOR : Johns Hopkins University (CP 3-3245)
ADDRESS : 601 North Broadway
Baltimore, Maryland 21205
CNTRCT TITLE: Pediatric Tumor Resource
PRINC INVEST: Dr. Herbert Kaizer
PHONE : AC-301, 366-3300
PROJ OFFICER: Dr. Paul T. Peebles, Bldg. 41, Rm. 400, x-66588
Dr. Jack Gruber, Bldg. 37, Rm. 1A19, x-61718
SEGMENT : Program Resources and Logistics

CONTRACTOR : Johns Hopkins University (CP 3-3337)
ADDRESS : Charles and 34th Streets
Baltimore, Maryland 21218
CNTRCT TITLE: Anti-tumor Reactivity in Patients with Leukemia and Lymphoma
PRINC INVEST: Dr. George W. Santos, Dept. of Medicine
PHONE : AC-301, 955-3300
PROJ OFFICER: Dr. Paul Levine, Landow Bldg., Rm. C306, x-66085
Dr. John Pearson, Bldg. 37, Rm. 1D16, x-61478
Dr. Ronald Herberman, Bldg. 10, Rm. 5B49, x-61366
SEGMENT : Immunology-Epidemiology

CONTRACTOR : Johns Hopkins University (CP 3-3345)
ADDRESS : Charles and Fourth Streets
Baltimore, Maryland 21218
CNTRCT TITLE: Studies on Herpes Virus Antigens and Virions in Neoplastic Cells from Cervical Carcinoma
PRINC INVEST: Dr. Laure Aurelian, Div. of Laboratory Animal Medicine
PHONE : AC-301, 366-3300
PROJ OFFICER: Dr. Gary Pearson, Bldg. 37, Rm. 1B05, x-62600
Dr. Charles Boone, Bldg. 37, Rm. 1C08, x-65141
SEGMENT : Developmental Research

CONTRACTOR : Johns Hopkins University (CP 4-3266)
ADDRESS : School of Medicine
Baltimore, Maryland 21205
CNTRCT TITLE: Studies of New Papoviruses Isolated from Man
PRINC INVEST: Dr. Richard T. Johnson
PHONE : AC-301, 955-3726
PROJ OFFICER: Dr. Bernard Talbot, Bldg. 37, Rm. 1B22, x-66135
SEGMENT : Tumor Virus Detection

CONTRACTOR : Karolinska Institutet (CP 3-3316)
ADDRESS : Torsplan 7, S-104 01
Stockholm 60, Sweden
CNTRCT TITLE: Studies on the Significance of Herpes-Type and RNA Viruses in the Etiology of Some Human Cancer
PRINC INVEST: Dr. George Klein, Department of Tumor Biology
PHONE : 235-480
PROJ OFFICER: Dr. Charles Boone, Bldg. 37, Rm. 1C08, x-65141
Dr. Gary Pearson, Bldg. 37, Rm. 1B05, x-62600
SEGMENT : Developmental Research

CONTRACTOR : Life Sciences, Inc. (CP 3-3205)
ADDRESS : 2900 72nd Street, North
St. Petersburg, Florida 33710
CNTRCT TITLE: Studies on Marek's Disease as a Model for Herpesvirus-Associated Oncogenesis
PRINC INVEST: Dr. Jack W. Frankel and Dr. Vincent Groupe
PHONE : AC-813, 345-9371
PROJ OFFICER: Dr. Charles Boone, Bldg. 37, Rm. 1C08, x-65141
Dr. Gary Pearson, Bldg. 37, Rm. 1B05, x-62600
Dr. Michael Chirigos, Bldg. 37, Rm. 1D19, x-61478
SEGMENT : Developmental Research

CONTRACTOR : Life Sciences, Inc. (CP 3-3210)
ADDRESS : 2900 72nd Street North
St. Petersburg, Florida 33710
CNTRCT TITLE: Production and Maintenance of Germfree and Selected Reagent Grade SPF Animals
PRINC INVEST: Dr. Wendall Farrow
PROJ OFFICER: Mr. John Kvedar, Bldg. 41, VTPL, x-65341
Dr. David McB. Howell, Bldg. 37, Rm. 1D21, x-61718
SEGMENT : Program Resources and Logistics

CONTRACTOR : Life Sciences, Inc. (CP 3-3291)
ADDRESS : 2950 72nd Street
St. Petersburg, Florida 33710
CNTRCT TITLE: Studies of Avian MC-29 Tumor; AMV Production
PRINC INVEST: Dr. Joseph W. Beard
PHONE : AC-813, 347-6191
PROJ OFFICER: Dr. Michael Chirigos, Bldg. 37, Rm. 1D19, x-61478
Dr. Jack Gruber, Bldg. 37, Rm. 1A19, x-61718
SEGMENT : Program Resources and Logistics

CONTRACTOR : Litton Bionetics, Inc. (CP 2-3294)
ADDRESS : Frederick Cancer Research Center, Fort Detrick
Frederick, Maryland 21701
CNTRCT TITLE: Operation of the Frederick Cancer Research Center
PRINC INVEST: Dr. Robert Stevenson
PROJ OFFICER: Dr. William Payne, FCRC, 9/393-1839
Dr. Henry Hearn, FCRC, 9/393-1839
SEGMENT : Program Management

CONTRACTOR : Litton Bionetics, Inc. (CP 3-3211)
ADDRESS : 7300 Pearl Street
Bethesda, Maryland 20014
CNTRCT TITLE: Studies on Molecular Events Leading to Transformation by RNA Oncogenic Viruses
PRINC INVEST: Dr. Alan M. Wu
PHONE : AC-301, 652-6616
PROJ OFFICER: Dr. Robert Gallo, Bldg. 10, Rm 6B18, x-66007
SEGMENT : Developmental Research

CONTRACTOR : Litton Bionetics, Inc. (CP 4-3224)
ADDRESS : 5510 Nicholson Lane
Kensington, Maryland 20795
CNTRCT TITLE: Investigation of the Carcinogenic Activity of Selected Virus Preparation in the Newborn Monkey
PRINC INVEST: Dr. Harvey Rabin and Dr. David P. Martin
PROJ OFFICER: Dr. Jack Gruber, Bldg. 37, Rm. 1A19, x-61718
Dr. Gary Pearson, Bldg. 37, Rm. 1B05, x-62600
SEGMENT : Program Resources and Logistics

CONTRACTOR : Litton Bionetics, Inc. (CP 4-3249)
ADDRESS : 7315 Wisconsin Avenue
Bethesda, Maryland 20014
CNTRCT TITLE: Application of Animal Virus Model Systems to Human Neoplasia
PRINC INVEST: Dr. Alan Rein
PHONE : AC-301, 881-5600
PROJ OFFICER: Dr. Robert Bassin, Landow Bldg., Rm. C308, x-64533
Dr. Brenda Gerwin, Bldg. 41, Rm. 300, x-66178
Dr. Bernard Talbot, Bldg. 37, Rm. 1B22, x-66135
SEGMENT : Solid Tumor Virus

CONTRACTOR : Litton Bionetics, Inc. (CP 4-3252)
ADDRESS : 7315 Wisconsin Avenue
Bethesda, Maryland 20014
CNTRCT TITLE: Application of Immunologic Techniques to Studies on the Viral Etiology of Human Cancer
PRINC INVEST: Dr. Maneh Gravell
PHONE : AC-301, 881-5600
PROJ OFFICER: Dr. Paul Levine, Landow Bldg., Rm. C306, x-66085
SEGMENT : Immunology-Epidemiology

CONTRACTOR : Litton Bionetics, Inc. (CP 4-3260)
ADDRESS : 7315 Wisconsin Avenue
Bethesda, Maryland 20014
CNTRCT TITLE: Acquisition of Fresh Human Specimens
PRINC INVEST: Ms. Shirley Norris
PHONE : AC-301, 881-5600
PROJ OFFICER: Dr. Jack Gruber, Bldg. 37, Rm. 1A19, x-61718
Dr. David McB. Howell, Bldg. 37, Rm. 1D21, x-61718
Dr. Lea Sekely, Bldg. 37, Rm. 1D21, x-61718
SEGMENT : Program Resources and Logistics

CONTRACTOR : Louisville, University of (CP 6-0902)
ADDRESS : School of Medicine, Preston and Walnut Streets
Louisville, Kentucky 40201
CNTRCT TITLE: Studies on Foamy Virus Reagents and Antisera
PRINC INVEST: Dr. Paul B. Johnston, Dept. of Microbiology
PHONE : AC-502, 582-2211, x-335
PROJ OFFICER: Dr. Robert Holdenried, Bldg. 41, Rm. A102, x-64333
Dr. James T. Duff, Bldg. 37, Rm. 1B17, x-65968
SEGMENT : Program Resources and Logistics

CONTRACTOR : Mason Research Institute (CP 3-3358)
ADDRESS : Harvard Street
Worcester, Massachusetts 01608
CNTRCT TITLE: Study on the Role of Hormonal Factors on Induction of Breast Cancer in M-PMV and R33 MTV Infected Animals
PRINC INVEST: Dr. Arthur E. Bogden
PHONE : AC-617, 752-4601
PROJ OFFICER: Dr. Jeffrey Schlom, Landow Bldg., Rm. C308, x-64533
Dr. Ernest J. Plata, Landow Bldg., Rm. C308, x-64534
Dr. Arnold K. Fowler, Bldg. 41, Rm. A103, x-66578
SEGMENT : Breast Cancer Virus

CONTRACTOR : Massachusetts General Hospital (CP 3-3366)
ADDRESS : John Collins Warren Laboratory
Boston, Massachusetts 02114
CNTRCT TITLE: Transfer RNA Studies
PRINC INVEST: Dr. Paul C. Zamecnik
PHONE : AC-617, 726-3651
PROJ OFFICER: Dr. George Vande Woude, Bldg. 41, Rm. 100, x-66738
Dr. Maurice L. Guss, Bldg. 47, Rm. 1B14, x-63323
SEGMENT : Developmental Research

CONTRACTOR : Massachusetts General Hospital (CP 4-3222)
ADDRESS : Fruit Street
Boston, Massachusetts 02114
CNTRCT TITLE: Activation of Oncogenic Viruses and Induction of Cancer by Immunologic and Non-Immunologic Methods
PRINC INVEST: Dr. Paul H. Black, Infectious Disease Unit
PHONE : AC-617, 726-3813
PROJ OFFICER: Dr. Michael Chirigos, Bldg. 37, Rm. 1D19, x-61478
Dr. Adi Gazdar, Bldg. 41, Rm. 200, x-61200
SEGMENT : Developmental Research

CONTRACTOR : Massachusetts Institute of Technology (CP 3-3348)
ADDRESS : Cambridge, Massachusetts 02139
CNTRCT TITLE: Studies of Leukemia Virus DNA Polymerases
PRINC INVEST: Dr. David Baltimore, Division of Sponsored Research
PHONE : AC-617, 253-1000, x-4725
PROJ OFFICER: Dr. Edward M. Scolnick, Bldg. 37, Rm. 1B22, x-66135
Dr. Bernard Talbot, Bldg. 37, Rm. 1B22, x-66135
SEGMENT : Tumor Virus Detection

CONTRACTOR : Medical College of Pennsylvania (CP 4-3319)
ADDRESS : 3300 Henry Avenue
Philadelphia, Pennsylvania 19129
CNTRCT TITLE: Immunological Characterization of EBV Antigens
PRINC INVEST: Dr. B. A. Zajac
PHONE :
PROJ OFFICER: Dr. Gary Pearson, Bldg. 37, Rm. 1B05, x-62600
SEGMENT : Immunology-Epidemiology

CONTRACTOR : Meloy Laboratories, Inc. (CP 2-2020)
ADDRESS : 6715 Electronic Drive
Springfield, Virginia 22151
CNTRCT TITLE: Studies of Influenza Virus Enhanced Tumor Immunity and Services for the Cell Biology Section VBB, NCI
PRINC INVEST: Dr. Kenneth Blackman
PHONE : AC-703, 354-2600, x-282
PROJ OFFICER: Dr. Charles Boone, Bldg. 37, Rm. 1C08, x-65141
SEGMENT : Developmental Research

CONTRACTOR : Meloy Laboratories, Inc. (CP 2-2306)
ADDRESS : 6715 Electronic Drive
Springfield, Virginia 22151
CNTRCT TITLE: Collaborative Project on the Oncogenic Potential of Herpes Viruses in Primates
PRINC INVEST: Dr. John Verna
PHONE : AC-703, 354-2600, x-202 and 206
PROJ OFFICER: Dr. Robert Manaker, Bldg 37, Rm. 1B14, x-63323
Dr. Wm. T. London, Bldg. 36, Rm. 5C19, x-62093
SEGMENT : Developmental Research

CONTRACTOR : Meloy Laboratories, Inc. (CP 4-3207)
ADDRESS : 6715 Electronic Drive
Springfield, Virginia 22151
CNTRCT TITLE: Spontaneous and Virus Induced Neoplastic Transformation
PRINC INVEST: Dr. John Verna
PHONE : AC-703, 354-2600
PROJ OFFICER: Dr. George Todaro, Bldg. 37, Rm. 1B22, x-66135
Dr. Bernard Talbot, Bldg. 37, Rm. 1B22, x-66135
SEGMENT : Tumor Virus Detection

CONTRACTOR : Meloy Laboratories, Inc. (CP 4-3223)
ADDRESS : 6715 Electronic Drive
Springfield, Virginia 22151
CNTRCT TITLE: Molecular Studies of Human and Animal Cancer with Emphasis on Breast Carcinoma
PRINC INVEST: Dr. John Verna
PHONE : AC-703, 354-2600, x-202 and 206
PROJ OFFICER: Dr. Jeffrey Schlom, Landow Bldg., Rm. C308, x-64533
SEGMENT : Breast Cancer Virus

CONTRACTOR : Meloy Laboratories, Inc. (CP 4-3236)
ADDRESS : 6715 Electronic Drive
Springfield, Virginia 22151
CNTRCT TITLE: Study of MTV Expression in Mice Relative to the Development of Mammary Tumors
PRINC INVEST: Dr. John Verna
PHONE : AC-703, 354-2600, x-202 and 206 / Rockville AC-301, 948-9565
PROJ OFFICER: Dr. Wade P. Parks, Bldg. 37, Rm. 1B17, x-65968
Dr. David McB. Howell, Bldg. 37, Rm. 1D21, x-61718
Dr. Lea Sekely, Bldg. 37, Rm. 1D21, x-61718
SEGMENT : Tumor Virus Detection

CONTRACTOR : Meloy Laboratories, Inc. (CP 4-3263)
ADDRESS : 6715 Electronic Drive
Springfield, Virginia 22151
CNTRCT TITLE: Mouse Mammary Tumor Virus Production Facility
PRINC INVEST: Dr. John Verna
PHONE : AC-703, 354-2600, x-202 and 206
PROJ OFFICER: Dr. Wade P. Parks, Bldg. 37, Rm. 1B17, x-65968
Dr. David McB. Howell, Bldg. 37, Rm. 1D21, x-61718
Dr. Lea Sekely, Bldg. 37, Rm. 1D21, x-61718
SEGMENT : Program Resources and Logistics

CONTRACTOR : Memorial Hosp. for Cancer & Allied Diseases (CP 4-3208)
ADDRESS : 494 East 68th Street
New York, New York 10021
CNTRCT TITLE: Collection of Human Sera Specimens from Defined Populations
PRINC INVEST: Dr. Herbert F. Oettgen
PHONE : AC-212, 879-3000
PROJ OFFICER: Dr. Ernest J. Plata, Landow Bldg., Rm. C308, x-64534
Dr. Jeffrey Schlom, Landow Bldg., Rm. C308, x-64533
SEGMENT : Breast Cancer Virus

CONTRACTOR : Memorial Hosp. for Cancer & Allied Diseases (CP 4-3335)
ADDRESS : 494 East 68th Street
New York, New York 10021
CNTRCT TITLE: Acquisition of Human Materials to Be Used in the Search for Transmissible Agents in Human Tumors
PRINC INVEST: Dr. Yashar Hirshaut, Division of Cell Biology
PHONE : AC-212, 879-3000
PROJ OFFICER: Dr. Jack Gruber, Bldg. 37, Rm. 1A19, x-61718
SEGMENT : Program Resources and Logistics

CONTRACTOR : Merck and Company, Inc. (CP 1-2059)
ADDRESS : West Point, Pennsylvania 19486
CNTRCT TITLE: Oncogenic Virus Research and Vaccine Development
PRINC INVEST: Dr. Maurice R. Hilleman, Virus and Cell Biology Research
PHONE : AC-215, 699-5311, x-5532
PROJ OFFICER: Dr. Robert Manaker, Bldg 37, Rm. 1B14, x-63323
Dr. Michael Chirigos, Bldg. 37, Rm. 1D19, x-61478
SEGMENT : Developmental Research

CONTRACTOR : Miami, University of (CP 4-3358)
ADDRESS : School of Medicine, P. O. Box 7278
Miami, Florida 33155
CNTRCT TITLE: Immunological Studies on Animal Breast Carcinoma
PRINC INVEST: Dr. Michael M. Sigel and Dr. Diana M. Lopez
PHONE : AC-305, 350-6567
PROJ OFFICER: Dr. Paul Levine, Landow Bldg., Rm. C306, x-66085
SEGMENT : Immunology-Epidemiology

CONTRACTOR : Michigan Cancer Foundation (CP 3-3347)
ADDRESS : 4811 John R Street
Detroit, Michigan 48201
CNTRCT TITLE: Studies on Milk in High-Risk Breast Cancer Families
PRINC INVEST: Dr. Marvin Rich
PHONE : AC-313, 833-0710
PROJ OFFICER: Dr. Jeffrey Schlom, Landow Bldg., Rm. C308, x-64533
Dr. Ernest J. Plata, Landow Bldg., Rm. C308, x-64534
SEGMENT : Breast Cancer Virus

CONTRACTOR : Microbiological Associates, Inc. (CP 3-3288)
ADDRESS : 4733 Bethesda Avenue
Bethesda, Maryland 20014
CNTRCT TITLE: Mouse Virus Typeing and Diagnostic Reagents
PRINC INVEST: Dr. John C. Parker, II
PHONE : AC-301, 654-3400
PROJ OFFICER: Dr. Robert Holdenried, Bldg. 41, Rm. A102, x-64333
Dr. Wallace Rowe, Bldg. 7, Rm. 304, x-62613
Dr. David McB. Howell, Bldg. 37, Rm. 1D21, x-61718
SEGMENT : Program Resources and Logistics

CONTRACTOR : Microbiological Associates, Inc. (CP 4-3240)
ADDRESS : 4733 Bethesda Avenue
Bethesda, Maryland 20014
CNTRCT TITLE: Roles of Viruses and Chemicals in the Etiology of Cancer
PRINC INVEST: Dr. Riley Housewright
PHONE : AC-301, 654-3400
PROJ OFFICER: Dr. Robert J. Huebner, Bldg. 37, Rm. 2D24, x-63301
SEGMENT : Solid Tumor Virus

CONTRACTOR : Microbiological Associates, Inc. (CP 4-3254)
ADDRESS : 4733 Bethesda Avenue
Bethesda, Maryland 20014
CNTRCT TITLE: Studies of Type C RNA Tumor Viruses
PRINC INVEST: Dr. Damodar Deshmukh
PHONE : AC-301, 654-3400
PROJ OFFICER: Dr. Padman Sarma, Bldg. 37, Rm. 2D23, x-63301
Dr. Robert J. Huebner, Bldg. 37, Rm. 2D24, x-63301
SEGMENT : Solid Tumor Virus

CONTRACTOR : Microbiological Associates, Inc. (CP 6-0914)
ADDRESS : 4733 Bethesda Avenue
Bethesda, Maryland 20014
CNTRCT TITLE: Operation of a BALB/c Mouse Colony
PRINC INVEST: Mr. Wilbur Athey
PHONE : AC-301, 654-3400, x-300
PROJ OFFICER: Mr. Samuel Poiley, Bldg. 37, Rm. 5E10, x-61323
Dr. Michael Chirigos, Bldg. 37, Rm. 1D19, x-61478
Mr. Clarence Reeder, Bldg. 37, Rm. 5E12A, x-61323
SEGMENT : Program Resources and Logistics

CONTRACTOR : Minnesota, University of (CP 3-3357)
ADDRESS : College of Medical Sciences, 315 Morrill Hall
Minneapolis, Minnesota 55455
CNTRCT TITLE: The Search for Tumor Virus Related Information in Human Immunodeficiency in Patients with Cancer
PRINC INVEST: Dr. John H. Kersey
PHONE : AC-612 373-2793
PROJ OFFICER: Dr. George Todaro, Bldg. 37, Rm. 1B22, x-66135
Dr. Bernard Talbot, Bldg. 37, Rm. 1B22, x-66135
Dr. Wade P. Parks, Bldg. 37, Rm. 1B17, x-65968
SEGMENT : Tumor Virus Detection

CONTRACTOR : Minnesota, University of (CP-4-3285)
ADDRESS : School of Public Health
Minneapolis, Minnesota 55455
CNTRCT TITLE: Development of Short Courses on the Principles of Biohazard and Injury Control for the Biomedical Laboratory
PRINC INVEST: Dr. George Michaelson, Div. of Enironmental Health
PHONE : AC-612, 373-3167
PROJ OFFICER: Dr. W. Emmett Barkley, Bldg. 41, Rm. A115, x-66981
SEGMENT :

CONTRACTOR : Montreal Children's Hospital (CP 3-3377)
ADDRESS : 2300 Tupper Street
Montreal 108, Quebec, Canada
CNTRCT TITLE: Supply of Leukemia and Normal Blood Plasma Specimens
PRINC INVEST: Dr. Ronald L. Denton, Department of Hematology
PHONE : AC-514, 937-8511
PROJ OFFICER: Dr. Davic McB. Howell, Bldg. 37, Rm. 1D21, x-61718
Dr. Charles Boone, Bldg. 37, Rm. 1C08, x-65141
SEGMENT : Program Resources and Logistics

CONTRACTOR : Mt. Sinai School of Medicine (CP 4-3225)
ADDRESS : Fifth Avenue at 100th Street
New York, New York 10029
CNTRCT TITLE: Stimulation of Immunity to Virus Associated and Tumor Associated Antigens in AKR and BALB/c Mice
PRINC INVEST: Dr. James F. Holland and Dr. J. George Bekesi
PHONE : AC-212, 876-1000, x-6384
PROJ OFFICER: Dr. Clarice Gaylord, Landow Bldg., Rm. C306, x-66086
Dr. Michael R. Blaese, Bldg. 10, Rm. 4N108, x-66781
Dr. John Pearson, Bldg. 37, Rm. 1D16, x-61478
SEGMENT : Immunology-Epidemiology

CONTRACTOR : Naples, University of (CP 3-3314)
ADDRESS : Instituto di Clinica Medica Generale
Policlinico - Piazza Miraglia
80138 Napoli, Italy
CNTRCT TITLE: Studies on the Role of Herpes Simplex Virus (HSV), Types I and II, in Human Malignant Neoplasias.
PRINC INVEST: Dr. Guilio Tarro, Institute of Pathology
PHONE : 218-254 (PBX)
PROJ OFFICER: Dr. Charles Boone, Bldg. 37, Rm. 1C08, x-65141
Dr. Michael Chirigos, Bldg. 37, Rm. 1D19, x-61478
SEGMENT : Developmental Research

CONTRACTOR : Naval Biomedical Research Laboratory (CP 4-0200)
ADDRESS : U. S. Naval Supply Center
Oakland, California 94625
CNTRCT TITLE: Studies of Environmental and Physiological Factors Influencing Virus-Houst Interactions
PRINC INVEST: Dr. M. A. Vedros
PHONE : AC-415, 832-5217
PROJ OFFICER: Dr. Alfred Hellman, Bldg. 41, Rm. A103, x-66758
Dr. Arnold Fowler, Bldg. 41, Rm. A103, x-66578
Dr. W. Emmett Barkley, Bldg. 41, Rm. A115, x-66981
SEGMENT :

CONTRACTOR : Naval Biomedical Research Laboratory (CP 4-0201)
ADDRESS : U. S. Naval Supply Center
Oakland, California 94625
CNTRCT TITLE: Facility Support for Cell Culture Research and Propagation
PRINC INVEST: Capt. James F. Pribnow, MSC, USN
PHONE : AC-415, 832-5217
PROJ OFFICER: Dr. James T. Duff, Bldg. 37, Rm. 1B17, x-65968
Dr. Jack Gruber, Bldg. 37, Rm. 1A19, x-61718
SEGMENT : Program Resources and Logistics

CONTRACTOR : Netherlands Cancer Institute (CP 3-3368)
ADDRESS : Sarphatistraat 108
Amsterdam C., The Netherlands
CNTRCT TITLE: Immunogenetic Studies on Breast Cancer and Leukemia
PRINC INVEST: Dr. Lourens M. Boot and J. H. M. Hilgers
PHONE : AC-020, 943-434
PROJ OFFICER: Dr. Walter E. Heston, Bldg. 37, Room 2E24, x-61018
Dr. Ernest J. Plata, Landow Bldg., Rm. C308, x-64534
SEGMENT : Breast Cancer Virus

CONTRACTOR : New York Medical College (CP 3-3398)
ADDRESS : Flower and 5th Avenue Hospital, 5th Ave. at 106th Street
New York, New York 10029
CNTRCT TITLE: Immunological Responses of Breast Cancer Patients Against Autologous Breast Cancer Tissues
PRINC INVEST: Dr. Maurice Black
PHONE : AC 212, 876-5500, x-238
PROJ OFFICER: Dr. Clarice Gaylord, Landow Bldg., Rm. C306, x-66086
Dr. Michael Blaese, Bldg. 10, Rm. 4N108, x-66095
Dr. Paul Levine, Landow Bldg., Rm. C306, x-66085
SEGMENT : Immunology-Epidemiology

CONTRACTOR : New York State Dept. of Health (CP 2-3247)
Roswell Park Institute, 666 Elm Street
Buffalo, New York 14203
CNTRCT TITLE: Supply of Blood and Tissue Specimens from Patients with Malignancies
PRINC INVEST: Dr. Joseph Sokal
PHONE : AC-716, 845-3010
PROJ OFFICER: Dr. Lea Sekely, Bldg. 37, Rm. 1D21, x-61718
Dr. David McB. Howell, Bldg. 37, Rm. 1D21, x-61718
SEGMENT : Program Resources and Logistics

CONTRACTOR : North Carolina, University of (CP 3-3336)
ADDRESS : School of Medicine, 503 Swing Bldg.
Chapel Hill, North Carolina 25514
CNTRCT TITLE: Molecular Studies on Herpes-Type Viruses of Potential Oncogenicity
PRINC INVEST: Dr. Joseph S. Pagano
PHONE : AC-919, 966-1183
PROJ OFFICER: Dr. Maurice L. Guss, Bldg. 47, Rm. 1B14, x-63323
Dr. Robert Manaker, Bldg 37, Rm. 1B14, x-63323
SEGMENT : Developmental Research

CONTRACTOR : North Dakota, University of (CP 6-0008)
ADDRESS : School of Medicine
Grand Forks, North Dakota 58201
CNTRCT TITLE: Quantitative Studies on the Transmission of Feline Oncogenic RNA Viruses and Selected Herpesviruses by Certain Bloodsucking Anthropods
PRINC INVEST: Dr. Robert Fischer, Dept. of Microbiology
PHONE : AC-701, 777-2411
PROJ OFFICER: Dr. George Burton, Landow Bldg., Rm. C309, x-66085
SEGMENT : Program Management

CONTRACTOR : Ohio State Research Foundation (CP 4-3217)
ADDRESS : 1314 Kinnear Road
Columbus, Ohio 43212
CNTRCT TITLE: Biohazards Control and Containment in Oncogenic Virus Research
PRINC INVEST: Dr. David S. Yohn
PHONE : AC-614, 422-5661
PROJ OFFICER: Dr. Alfred Hellman, Bldg. 41, Rm. A103, x-66758
Dr. Phyllis Kind, Bldg. 41, Rm. A109, x-66758, Bldg. 41, Rm. A109, s-66758
SEGMENT : Developmental Research

CONTRACTOR : Pennsylvania State University (CP 0-2024)
ADDRESS : Milton S. Hershey Medical Center
Hershey, Pennsylvania 17033
CNTRCT TITLE: Studies on the Oncogenic Potential of Defective Human Viruses
PRINC INVEST: Dr. Fred Rapp, Department of Microbiology
PHONE : AC-717, 534-8254
PROJ OFFICER: Dr. Robert Manaker, Bldg 37, Rm. 1B14, x-63323
Dr. Michael Chirigos, Bldg. 37, Rm. 1D19, x-61478
SEGMENT : Developmental Research

CONTRACTOR : Pfizer, Inc. (CP 3-3234)
ADDRESS : John L. Smith Memorial for Cancer Research, 199 Maywood Avenue
Maywood, New Jersey 07607
CNTRCT TITLE: Development of Virus-Cancer Test System; Virus Production; Production of Human Virus-Cancer Cell Lines; Immunology
PRINC INVEST: Dr. Sami A. Mayyasi
PHONE : AC-201, 845-5665
PROJ OFFICER: Dr. Jack Gruber, Bldg. 37, Rm. 1A19, x-61718
Dr. David McB. Howell, Bldg. 37, Rm. 1D21, x-61718
SEGMENT : Program Resources and Logistics

CONTRACTOR : Pfizer, Inc. (CP 3-3239)
ADDRESS : John L. Smith Memorial for Cancer Research, 199 Maywood Avenue
Maywood, New Jersey 07607
CNTRCT TITLE: Viral Studies of Human and Animal Breast Cancer
PRINC INVEST: Dr. M. Ahmed and Mr. George Schidlovsky
PHONE : AC-201, 845-5665
PROJ OFFICER: Dr. Robert Bassin, Landow Bldg., Rm. C308, x-64533
Dr. Jeffrey Schlom, Landow Bldg., Rm. C308, x-64533
Dr. Brenda Gerwin, Bldg. 41, Rm. 300, x-66178
SEGMENT : Breast Cancer Virus

CONTRACTOR : Radiobiological Institute (CP 4-3328)
ADDRESS : Organization for Health Research, TNO
151 Lange Kleiweg
Rijwsijk (Z. H.), The Netherlands
CNTRCT TITLE: The Study of the Release of RNA Tumor Viruses and Its Genetic Control
PRINC INVEST: Dr. Peter Bentvelzen
PROJ OFFICER: Dr. Jeffrey Schlom, Landow Bldg., Rm. C308, x-64533
SEGMENT : Breast Cancer Virus

CONTRACTOR : Rockefeller University (CP 3-3306)
ADDRESS : 66th St. & York Avenue
New York, New York 10021
CNTRCT TITLE: Evaluation of Methods for Isolation of Viruses from Human Neoplasia
PRINC INVEST: Dr. Hidesaburo Hanafusa
PHONE : AC-212, 360-1782
PROJ OFFICER: Dr. Charles Boone, Bldg. 37, Rm. 1C08, x-65141
Dr. Robert Manaker, Bldg 37, Rm. 1B14, x-63323
SEGMENT : Developmental Research

CONTRACTOR : Rush-Presbyterian-St. Luke's Medical Center (CP 3-3219)
ADDRESS : 1753 West Congress Parkway
Chicago, Illinois 60612
CNTRCT TITLE: Studies of Tumor Viruses in Non-Human Primates
PRINC INVEST: Dr. Friedrich Deinhardt, Dept. of Microbiology
PHONE : AC-312, 942-5442
PROJ OFFICER: Dr. Robert Manaker, Bldg 37, Rm. 1B14, x-63323
Dr. Michael Chirigos, Bldg. 37, Rm. 1D19, x-61478
SEGMENT : Developmental Research

CONTRACTOR : Salk Institute for Biological Studies (CP 4-3243)
ADDRESS : P. O. Box 1809
San Diego, California 92112
CNTRCT TITLE: Characterization of Temperature-Sensitive Mutants of Polyoma Virus and Interaction Between Polyoma Virus and C-Type RNA Viruses
PRINC INVEST: Dr. Walter Eckhart
PHONE : AC-714, 453-4100
PROJ OFFICER: Dr. Stuart Aaronson, Bldg. 37, Room 1B22, x-66135
Dr. James T. Duff, Bldg. 37, Rm. 1B17, x-65968
SEGMENT : Solid Tumor Virus

CONTRACTOR : Scripps Clinic and Research Foundation (CP 3-3204)
ADDRESS : 476 Prospect Street
La Jolla, California 92037
CNTRCT TITLE: Immunopathologic Study of Leukemia
PRINC INVEST: Dr. Michael B. A. Oldstone
PHONE : AC-714, 459-2390, x-466 (office) x-472 (lab)
PROJ OFFICER: Dr. Tadao Aoki, Bldg. 41, Rm. 300, x-66178
Dr. Vincent Hollis, Bldg. 41, Rm. A110, x-67178
SEGMENT : Immunology-Epidemiology

CONTRACTOR : Scripps Clinic and Research Foundation (CP 4-3375)
ADDRESS : 476 Prospect Street
La Jolla, California 92037
CNTRCT TITLE: Immunologic Study of RNA Tumor (Type C) Viruses
PRINC INVEST: Dr. Frank Dixon
PHONE : AC-714, 459-2390, x301
PROJ OFFICER: Dr. Robert J. Huebner, Bldg. 37, Rm. 2D24, x-63301
Dr. Wade P. Parks, Bldg. 37, Rm. 1B17, x-65968
SEGMENT : Solid Tumor Virus

CONTRACTOR : Southern California, University Of (CP 4-3242)
ADDRESS : School of Medicine, 2035 Zonal Avenue
Los Angeles, California 90033
CNTRCT TITLE: Conditioned Lethal Mutants of RNA Tumor Viruses (RSV)
PRINC INVEST: Dr. Peter K. Vogt
PHONE : AC-213, 225-1511
PROJ OFFICER: Dr. Gary J. Kelloff, Bldg. 37, Room 2C07, x-61320
Dr. Stuart Aaronson, Bldg. 37, Room 1B22, x-66135
SEGMENT : Solid Tumor Virus

CONTRACTOR : Southern California, University of (CP 8-1030)
ADDRESS : Cancer Research Building, 1200 North State Street
Los Angeles, California 90033
CNTRCT TITLE: A Comprehensive Field and Laboratory Research Program on the Etiology and Epidemiology of Human Cancer
PRINC INVEST: Dr. Murray Gardner, Dept. of Pathology, and Dr. Robert McAllister
PHONE : AC-213, 225-1511, x-384
PROJ OFFICER: Dr. Robert J. Huebner, Bldg. 37, Rm. 2D24, x-63301
Dr. Wade Parks, Bldg. 37, Room 1B17, x-65968
SEGMENT : Solid Tumor Virus

CONTRACTOR : Southwest Foundation for Research and Education (CP 3-3340)
ADDRESS : P. O. Box 28147, 7480 West Commerce Street
San Antonio, Texas 78284
CNTRCT TITLE: Housing and Maintenance of a Chimpanzee Breeding Colony
PRINC INVEST: Dr. S. S. Kalter, Department of Virology
PHONE : AC-512, 674-1410
PROJ OFFICER: Dr. David McB. Howell, Bldg. 37, Rm. 1D21, x-61718
Dr. Lea Sekely, Bldg. 37, Rm. 1D21, x-61718
SEGMENT : Program Resources and Logistics

CONTRACTOR : Southwest Foundation for Research and Education (CP 4-3214)
ADDRESS : P. O. Box 28147, 7480 West Commerce Street
San Antonio, Texas 78284
CNTRCT TITLE: Study of Latent Virus Infection and Transmission Significance of C-Type Particles
PRINC INVEST: Dr. R. L. Heberling, Department of Virology
PHONE : AC-512, 674-1410
PROJ OFFICER: Dr. James Strickland, Bldg. 41, Rm. 600, x65329
Dr. Arnold Fowler, Bldg. 41, Rm. A103, x-66578
SEGMENT :

CONTRACTOR : St. Joseph's Hospital (CP 3-3393)
ADDRESS : 3001 W. Buffalo Avenue
Tampa, Florida 33607
CNTRCT TITLE: Study on Human Sarcomas and Their Possible Viral Etiology
PRINC INVEST: Dr. Jeno E. Szakacs
PHONE : AC-813, 877-8161, x-246
PROJ OFFICER: Dr. Albert Dalton, Bldg. 37, Room 1C15, x-64311
SEGMENT : Program Resources and Logistics

CONTRACTOR : St. Louis University (CP 4-3359)
ADDRESS : 2681 Park Avenue
St. Louis, Missouri 63110
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
PRINC INVEST: Dr. Maurice Green, Inst. for Molecular Virology
PHONE : AC-314, 664-9800, x-545
PROJ OFFICER: Dr. Robert J. Huebner, Bldg. 37, Rm. 2D24, x-63301
Dr. James T. Duff, Bldg. 37, Rm. 1B17, x-65968
SEGMENT : Solid Tumor Virus

CONTRACTOR : Stanford University (CP 4-3244)
ADDRESS : Stanford, California 94305
CNTRCT TITLE: Procurement, Processing, Storage, Distribution and Study of Human Cell Cultures and Operation of a Central Mycoplasma Diagnostic Laboratory; Studies on Hodgkin's Disease and Other Malignant Lymphomas
PRINC INVEST: Dr. Leonard Hayflick, Dept. of Medical Microbiology
PROJ OFFICER: Dr. James T. Duff, Bldg. 37, Rm. 1B17, x-65968
Dr. Robert J. Huebner, Bldg. 37, Rm. 2D24, x-63301
SEGMENT : Solid Tumor Virus

CONTRACTOR : Tel Aviv University (CP 2-3237)
ADDRESS : 155 Herzl Street
Tel Aviv, Abur-Kabir, Israel
CNTRCT TITLE: Isolation, Purification and Propagation of B-Type Particles from Human Milk in Israel
PRINC INVEST: Dr. Jafa Keydar
PROJ OFFICER: Dr. Wade P. Parks, Bldg. 37, Rm. 1B17, x-65968
Dr. Ernest J. Plata, Landow Bldg., Rm. C308, x-64534
SEGMENT : Breast Cancer Virus

CONTRACTOR : Texas, University of (CP 1-3135)
ADDRESS : Southwestern Medical School, 5323 Harry Hines Blvd.
Dallas, Texas 75235
CNTRCT TITLE: Biohazards Information Gathering Center: Surveillance of Laboratory -Acquired Infection and Accidents
PRINC INVEST: Dr. Robert M. Pike and Dr. Jonathan W. Uhr
PHONE : AC-214, 631-3220
PROJ OFFICER: Dr. Alfred Hellman, Bldg. 41, Rm. A103, x-66758
Dr. W. Emmett Barkley, Bldg. 41, Rm. A115, x-66981
SEGMENT :

CONTRACTOR : Texas, University of (CP 3-3292)
ADDRESS : M. D. Anderson Hosp. and Tumor Inst.
6723 Bertner Drive
Houston, Texas 77025
CNTRCT TITLE: Immunity to Sarcoma-Related Antigens in Patients and Controls
PRINC INVEST: Dr. Joseph G. Sinkovics and Dr. David Kay
PROJ OFFICER: Dr. Gary Pearson, Bldg. 37, Rm. 1B05, x-62600
Dr. Paul Levine, Landow Bldg., Rm. C306, x-66085
SEGMENT : Immunology-Epidemiology

CONTRACTOR : Texas, University of (CP 3-3301)
ADDRESS : M. D. Anderson Hosp. and Tumor Inst.
6723 Bertner Drive
Houston, Texas 77025
CNTRCT TITLE: Human Immunity and Immune Response to the Rauscher Leukemia Virus
PRINC INVEST: Dr. Evan Hersh
PHONE : AC-713, 526-5411, x-546
PROJ OFFICER: Dr. Paul Levine, Landow Bldg., Rm. C306, x-66085
Dr. Janet Hartley, Bldg. 7, Rm. 304, x-62613
Dr. Ronald Herberman, Bldg. 10, Rm. 5B49, x-61366
SEGMENT : Immunology-Epidemiology

CONTRACTOR : Texas, University of (CP 3-3304)
ADDRESS : M. D. Anderson Hosp. and Tumor Inst.
6723 Bertner Drive
Houston, Texas 77025
CNTRCT TITLE: Studies on the Relationships of Viruses to Human Leukemia, Lymphomas and Solid Tumors
PRINC INVEST: Dr. Leon Dmochowski
PROJ OFFICER: Dr. Gary Pearson, Bldg. 37, Rm. 1B05, x-62600
Dr. Robert Manaker, Bldg 37, Rm. 1B14, x-63323
SEGMENT : Developmental Research

CONTRACTOR : Tulane Univ.-Delta Reg. Primate Ctr. (CP 3-3396)
ADDRESS : Tulane University
Covington, Louisiana 70433
CNTRCT TITLE: Role of Humoral and Cellular Immunity in Determining the Outcome of Herpes Saimiri (HSV) Infection in Squirrel Monkeys
PRINC INVEST: Dr. William P. Allen
PROJ OFFICER: Dr. Dharam Ablashi, FCRC, Bldg. 538, Rm. 205B, 393-1839, x-2181
Dr. Gary Pearson, Bldg. 37, Rm. 1B05, x-62600
Dr. George Burton, Landow Bldg., Rm. C309, x-66085
SEGMENT : Immunology-Epidemiology

CONTRACTOR : University Laboratories, Inc. (CP 3-3222)
ADDRESS : 810 North Second Avenue
Highland Park, New Jersey 08904
CNTRCT TITLE: The Production of Sarcoma Viruses, Helper Viruses and Antisera to These Viruses
PRINC INVEST: Dr. Eugene Bernstein
PHONE : AC-201, 246-1146
PROJ OFFICER: Dr. Robert Holdenried, Bldg. 41, Rm. A102, x-64333
Dr. Jack Gruber, Bldg. 37, Rm. 1A19, x-61718
SEGMENT : Program Resources and Logistics

CONTRACTOR : Washington, University of (CP 3-3372)
ADDRESS : Medical School
Seattle, Washington 98105
CNTRCT TITLE: Studies on Tumor Specific Transplantation Antigen
PRINC INVEST: Dr. Karl E. Hellstrom, Dept. of Pathology, and Dr. Ingegerd Hellstrom
PHONE : AC-206, 543-1448
PROJ OFFICER: Dr. Robert J. Huebner, Bldg. 37, Rm. 2D24, x-63301
Dr. James T. Duff, Bldg. 37, Rm. 1B17, x-65968
SEGMENT : Solid Tumor Virus

CONTRACTOR : Washington, University of (CP 3-3236)
ADDRESS : U. S. P. H. S. Hospital, 1131 14th Avenue, South
Seattle, Washington 98144
CNTRCT TITLE: Immunological and Transplantation Studies on Dogs with Cancer for Detection of an Oncogenic Virus-Carrier
PRINC INVEST: Dr. Rainer Storb, Dept. of Medicine
PHONE : AC-206, 543-4043
PROJ OFFICER: Dr. Gary Pearson, Bldg. 37, Rm. 1B05, x-62600
Dr. Thomas Cameron, Landow Bldg., Rm. C325, x-64875
Dr. Dharam Ablashi, FCRC, Bldg. 538, Rm. 205B, 393-1839, x-2181
SEGMENT : Immunology-Epidemiology

CONTRACTOR :	Weizmann Institute of Science (CP 4-3241)
ADDRESS :	Section of Genetics Rehovot, Israel
CNTRCT TITLE:	Study on Virus-Induced Tumor Specific Transplantation Antigen
PRINC INVEST:	Dr. Leo Sachs
PHONE :	
PROJ OFFICER:	Dr. Robert J. Huebner, Bldg. 37, Rm. 2D24, x-63301 Dr. James T. Duff, Bldg. 37, Rm. 1B17, x-65968
SEGMENT :	Solid Tumor Virus

CONTRACTOR :	Wisconsin, University of (CP 2-2022)
ADDRESS :	McArdle Laboratory for Cancer Research 450 Randall Avenue Madison, Wisconsin 53706
CNTRCT TITLE:	Studies on Induction of Neoplasia In Vivo and In Vitro Using Known Carcinogenetic Chemical Methods
PRINC INVEST:	Dr. Robert Nowinski
PHONE :	AC-608, 262-2177
PROJ OFFICER:	Dr. George Todaro, Bldg. 37, Rm. 1B22, x-66135 Dr. Peter J. Fischinger, Bldg. 41, Rm. A117, x-66588 Dr. Bernard Talbot, Bldg. 37, Rm. 1B22, x-66135
SEGMENT :	Tumor Virus Detection

CONTRACTOR :	Wistar Institute of Anatomy and Biology (CP 3-3250)
ADDRESS :	36th Street at Spruce Philadelphia, Pennsylvania 19104
CNTRCT TITLE:	Extraction and Characterization of Virus-Induced Transplantation Antigens and Rescue of Virus from Sarcomas and Leukemias
PRINC INVEST:	Dr. Anthony Girardi
PHONE :	AC-215, 222-6700, x-226
PROJ OFFICER:	Dr. James T. Duff, Bldg. 37, Rm. 1B17, x-65968 Dr. Robert J. Huebner, Bldg. 37, Rm. 2D24, x-63301
SEGMENT :	Solid Tumor Virus

CONTRACTOR :	Wolf Research and Development Corp. (CP 4-3351)
ADDRESS :	1530 East Jefferson Street Rockville, Maryland 20852
CNTRCT TITLE:	Data Processing Support for Biomathematical and Biomedical Research
PRINC INVEST:	Mr. Edwin J. Lisiecki
PHONE :	AC-301, 770-5500
PROJ OFFICER:	Mrs. Wilma Varrato, Landow Bldg., Rm. C-308, x-61951 Dr. David McB. Howell, Bldg. 37, Rm. 1D21, x-61718 Dr. Lea Sekely, Bldg. ~~37,~~ Rm. 1D21, x-61718
SEGMENT :	Program Resources and Logistics

ADDENDUM

Recently Negotiated Contracts

CONTRACTOR : Alabama, University of (CP 4-3394)
ADDRESS : University Station
Birmingham, Alabama 35294
CNTRCT TITLE: Immunological Studies on the Relationship of Embryonic Antigen to Virus Induced Tumor Antigens
PRINC INVEST: Dr. Eddie W. Lamon
PHONE : AC-205, 934-4138
PROJ OFFICER: Dr. Gary Pearson, Bldg. 37, Rm. 1B05, x-62600
SEGMENT : Immunology-Epidemiology

CONTRACTOR : Albert Einstein College of Medicine (CP 4-3380)
ADDRESS : 1300 Morris Park Avenue
Bronx, New York 10461
CNTRCT TITLE: Study of the Molecular Mechanism of the Murine FV-L Gene in Restricting Replication of Strains of Friend Leukemia Virus
PRINC INVEST: Dr. Ruy Soeiro and Dr. Bernard Fields
PHONE : AC-212, 430-2159
PROJ OFFICER: Dr. Bernard Talbot, Bldg. 37, Rm. 1B22, x-66135
SEGMENT : Tumor Virus Detection

CONTRACTOR : Baylor University (CP 4-3385)
ADDRESS : College of Medicine, 1200 Moursund Avenue
Houston, Texas 77025
CNTRCT TITLE: Regulation of Gene Expression in Mouse Mammary Cancer
PRINC INVEST: Dr. Susan Socher, Dept. of Cell Biology, and Dr. J. Rosen
PHONE : AC-713, 529-4951
PROJ OFFICER: Dr. Wade Parks, Bldg. 37, Rm. 1B17, x-65968
SEGMENT : Breast Cancer Virus

CONTRACTOR : Biotech Research Laboratories (CP 4-3365)
ADDRESS : 12601 Twinbrook Parkway
ADDRESS : Rockville, Maryland 20852
CNTRCT TITLE: Transformation of Fibroblast Cells Derived from Patients with Genetic Diseases
PRINC INVEST: Dr. C. Y. Ting
PHONE : AC-301, 770-2740
PROJ OFFICER: Dr. Adi Gazdar, Bldg. 41, Rm. 200, x-61200
SEGMENT : Immunology-Epidemiology

CONTRACTOR : Boston Hospital for Women (CP 4-3379)
ADDRESS : Lying-in Division
221 Longwood Avenue
Boston, Massachusetts 02115
CNTRCT TITLE: Retrospective Cohort Study of Cervical Neoplasia and Herpes Simplex Virus, Type II
PRINC INVEST: Dr. Lorna D. Johnson
PHONE : AC-617, 734-5300
PROJ OFFICER: Dr. Robert Manaker, Bldg. 37, Rm. 1B14, x-63323
SEGMENT : Developmental Research

CONTRACTOR : California, Univ. of / at San Francisco (CP 4-3381)
ADDRESS : School of Medicine, Cancer Research Inst.
San Francisco, California 94143
CNTRCT TITLE: Isolation of Human Xenotropic Viruses
PRINC INVEST: Dr. Jay A. Levy
PHONE : AC-415, 666-4071
PROJ OFFICER: Dr. Robert J. Huebner, Bldg. 37, Rm. 2D24, x-63301
SEGMENT : Solid Tumor Virus

CONTRACTOR : Cornell Univ., N.Y.State Veterinary Coll. (CP 3-3346)
ADDRESS : Ithaca, New York 14850
CNTRCT TITLE: Application of Feline Virus Systems in the Study of Viral Relationships to Human Neoplasia
PRINC INVEST: Dr. Charles Rickard, Dept. of Pathology
PHONE : AC-607, 256-3215
PROJ OFFICER: Dr. Robert Manaker, Bldg. 37, Rm. 1B14, x-63323
SEGMENT : Developmental Research

CONTRACTOR : Department of Agriculture (CP 4-0214)
ADDRESS : Animal Physiology and Genetics Institute, Bldg. 262
Agricultural Research Center, Beltsville, Md. 20705
CNTRCT TITLE: Studies on Inheritance and Oncogenicity of Endogenous RNA Tumor Viruses
PRINC INVEST: Dr. Lyman G. Crittenden, Chief, Avian Physiology Lab.
PHONE : AC-301, 344-2545
PROJ OFFICER: Dr. Padman Sarma, Bldg. 37, Rm. 2D23, x-63301
SEGMENT : Solid Tumor Virus

CONTRACTOR : Duke University (CP 4-3395)
ADDRESS : Medical Center
Durham, North Carolina 27706
CNTRCT TITLE: Immunological Studies on the Relationship of Embryonic Antigen to Virus Induced Tumor Antigens
PRINC INVEST: Dr. Samuel A. Wells, Jr. and Dr. John P. Grant
PHONE : AC-919, 684-5989
PROJ OFFICER: Dr. Gary Pearson, Bldg. 37, Rm. 1B05, x-62600
SEGMENT : Immunology-Epidemiology

CONTRACTOR : Electro-Nucleonics Laboratories, Inc. (CP 4-3334)
ADDRESS : 12050 Tech Road, Montgomery Industrial Park
Silver Spring, Maryland 20904
CNTRCT TITLE: Virus Production and Processing Facility
PRINC INVEST: Mr. John F. Lemp, Jr.
PHONE : AC-301, 652-4923, x-42
PROJ OFFICER: Dr. George Vande Woude, Bldg. 41, Rm. 100, x-66788
Dr. David McB. Howell, Bldg. 37, Rm. 1D21, x-61718
SEGMENT : Program Resources and Logistics

CONTRACTOR : Emory University (CP 4-3393)
ADDRESS : School of Medicine, 46 Armstrong St., N. E.
Atlanta, Ga. 30303
CNTRCT TITLE: Cellular Immunity Studies to Herpes Simplex Associated Antigens Cancer Patients and Controls
PRINC INVEST: Dr. Andre Nahmias, Dept. of Pediatrics
PHONE : AC-404, 659-1212, x-226
PROJ OFFICER: Dr. Paul Levine, Landow Bldg., Rm. C306, x-66085
SEGMENT : Immunology-Epidemiology

CONTRACTOR : Flow Laboratories, Inc. (CP 4-3387)*
ADDRESS : 12420 Parklawn Drive
Rockville, Maryland 20852
CNTRCT TITLE: Virus and Reagent Production and Purification
PRINC INVEST: Dr. Raymond V. Gilden
PHONE : AC-301, 881-2900
PROJ OFFICER: Dr. Robert J. Huebner
SEGMENT : Solid Tumor Virus

*Formerly part of Flow 3-3247

CONTRACTOR : Flow Laboratories, Inc. (CP 4-3388)*
ADDRESS : 12420 Parklawn Drive
Rockville, Maryland 20852
CNTRCT TITLE: Studies on Type C Viruses in Relation to Oncogenic Potential
PRINC INVEST: Dr. Raymond V. Gilden
PHONE : AC-301, 881-2900
PROJ OFFICER: Dr. Robert J. Huebner
SEGMENT : Solid Tumor Virus

CONTRACTOR : Flow Laboratories, Inc. (CP 4-3389)*
ADDRESS : 12420 Parklawn Drive
Rockville, Maryland 20852
CNTRCT TITLE: Studies on Herpesviruses
PRINC INVEST: Dr. Raymond V. Gilden
PHONE : AC-301, 881-2900
PROJ OFFICER: Dr. Berge Hampar, Bldg. 37, Rm 1B17, x-65967
SEGMENT : Solid Tumor Virus

CONTRACTOR : Hoektoen Inst. for Medical Research (CP 4-3344)
ADDRESS : 627 South Wood Street
Chicago, Illinois 60612
CNTRCT TITLE: Supply of Fresh Human Materials Obtained from Patients with Neoplastic Diseases
PRINC INVEST: Dr. Paul B. Szanto
PHONE : AC-312, 738-3100
PROJ OFFICER: Dr. Lea Sekely, Bldg. 37, Rm. 1B17, x-65967
SEGMENT : Program Resources and Logistics

CONTRACTOR : Illinois, University of (CP 4-3345)
ADDRESS : Medical Center
P. O. Box 6998
Chicago, Illinois 60680
CNTRCT TITLE: Human Materials from Patients with Neoplastic Diseases
PRINC INVEST: Dr. Tapas K. Das Gupta
PHONE : AC-312, 996-6667
PROJ OFFICER: Dr. Lea Sekely, Bldg. 37, Rm. 1B17, x-65967
SEGMENT : Program Resources and Logistics

CONTRACTOR : Johns Hopkins University (CP 4-3330)
ADDRESS : School of Medicine,
725 N. Wolfe Street., Baltimore, Maryland 21205
CNTRCT TITLE: Cellular Immunity Studies to Herpes Simplex Associated Antigens in Cancer Patients and Controls
PRINC INVEST: Dr. Laure Aurelian, Dept. of Microbiology & Lab. Animal Medicine
PHONE : AC-301, 955-3273
PROJ OFFICER: Dr. Paul Levine, Landow Bldg., Rm. C306, x-66085
SEGMENT : Immunology-Epidemiology

CONTRACTOR : Litton Bionetics, Inc. (CP 4-3333)
ADDRESS : 5516 Nicholson Lane
Kensington, Maryland 20795
CNTRCT TITLE: Immunological Assays for DNA and RNA Viruses
PRINC INVEST: Dr. Bruce Maurer
PHONE : AC-301, 881-5600, x-556
PROJ OFFICER: Dr. Paul Levine, Landow Bldg., Rm. C306, x-66085
SEGMENT : Immunology-Epidemiology

*Formerly part of Flow 3-3247

IOIAU04662

CONTRACTOR : Michigan Cancer Foundation (CP 4-3391)
ADDRESS : 4811 John R. Street
Detroit, Michigan 48201
CNTRCT TITLE: Collection of Large Quantities of Human Milk
PRINC INVEST: Dr. Samuel Albert
PHONE : AC-313, 833-0710, x-333
PROJ OFFICER: Dr. Ernest Plata, Landow Bldg., Rm. C308, x-64534
SEGMENT : Program Resources and Logistics

CONTRACTOR : Tennessee, University of (CP 4-3325)
ADDRESS : Hesler Biology Bldg., Room 419
Knoxville, Tennessee 37916
CNTRCT TITLE: Immunological Studies on the Relationship of Embryonic Antigen to Virus Induced Tumor Antigen
PRINC INVEST: Dr. Joseph Coggin, Jr., Dept. of Microbiology
PHONE : AC-615, 974-2356
PROJ OFFICER: Dr. Gary Pearson, Bldg. 37, Rm. 1B05, x-62600
SEGMENT : Immunology-Epidemiology

CONTRACTOR : Texas, University of (CP 4-3370)
ADDRESS : M. D. Anderson Hospital & Tumor Inst.
Houston, Texas 77025
CNTRCT TITLE: Immunlogical Studies on Human Breast Carcinoma
PRINC INVEST: Dr. James M. Bowen and Dr. K. Maruyama
PHONE : AC-713, 792-3330
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