Significance to Biomedical Research and the Program of the Institute: This research program is aimed at understanding, in detail, the mechanism of cell transformation by RNA tumor viruses, applying new information on viral carcinogenesis and on the molecular biology of human cells directly to the problems of human cancer. 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 therapy for viral diseases and cancer.

Proposed Course: The new DNA polymerase Cm will be purified and characterized, and its significance to transformation will be investigated. The relationship between this enzyme and particulate reverse transcriptase activity will be determined. Analysis of human normal and neoplastic tissue will be conducted for nucleic acid sequences specific for subhuman primate RNA tumor viruses.

Date Contract Initiated: June 1, 1976

SCRIPPS CLINIC AND RESEARCH FOUNDATION (NO1-CP7-1018) (Formerly CP4-3375)

Title: Immunologic Study of Type C RNA Tumor Virus

Contractor's Project Director: Dr. Frank J. Dixon

Project Officer (NCI): Dr. Robert J. Huebner

Objectives: This program is comprised of three integrated segments aimed at (1) identification of the various virion and non-virion-associated products of endogenous type C virus genomes in mice, (2) quantitation of the spontaneous immune responses of mice to the products of their endogenous type C virus genomes and any immunopathologic consequences thereof, and (3) modification of expression of endogenous type C virus genomes by immunization with virus or purified virus antigen vaccines or by immunosuppression.

Major Findings: Quantiative and biochemical studies were continued on the endogenously expressed murine leukemia virus (MuLV) gp70s in a number of murine strains. Expression of gp70 was found to be restricted to certain anatomical sites and cell types, among which lymphoid and epithelial cells were prominent. On a quantitative basis, the major site of gp70 expression was the male genital tract.

During fetal development, gp70 first appeared in the hematopoietic liver of 14-day old embryos and by day 18, it was already expressed at anatomical sites similar to those of the adult. In toto, these results showed that control of expression of the MuLV genome in adult and developing mice was linked to differentiation.

A number of gp70 proteins were isolated from purified virus as well as from sera and secretions of mice. A tryptic analysis of the peptides was done. In this way, the structure of a virion protein could be compared to its

endogenously expressed counterpart. The structure of the proteins from AKR and NZB mouse sera were compared to the Rauscher, SLV, AKR, NZB xenotropic, NIH xenotropic, BALB/c xenotropic, and NZW xenotropic viruses. It appeared that the endogenously expressed protein molecules resembled the gp70 of the xenotropic viruses, especially of the NZB type. More than one type of gp70 could be expressed in a single strain of mice depending on the anatomical site studied. This observation indicates that different endogenous viral genomes can be expressed at different sites of cell differentiation.

The immunopathologic consequences of oncornavirus expression were studied. An important advance was the characterization of two new strains of mice which develop a fatal lupus-like syndrome early in life. These mice become important in the search for common immunologic or virologic determinants of immunopathologic disease.

Human serum, but no serum from a number of other species, was observed to inactivate and lyse oncornaviruses from a number of different sources. Lysis occurred in the absence of antibody. A detailed analysis of the role of the human complement system in mediating this lytic process indicated that human C1q, in the absence of immunoglobin, interacts directly with oncornaviruses. Binding of C1 via C1q in this manner led to activation of C1r, C1s and thus of the classical complement pathway. Integrity of the classical pathway was an absolute requirement for lysis, although activation of the alternative pathway considerably amplified the amount of lysis obtained, possibly through involvement of the C3b-dependent feedback mechanism. Activation of complement was accompanied by deposition of complements on the virus surface and lysis occurred on completion of the complement reaction sequence. Thus, in this system, the C1q subunit of C1 subserved a specific recognition function normally associated with antibody. This ability of human serum to inactivate oncornaviruses may represent a natural defense mechanism operative in vivo which deters expression of intact oncornaviruses in human malignancies.

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 determinantion of cause, early detection of risk, and eventual 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, particles have been observed in human placentas in numbers suggesting that they are 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, virus 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 contractor will continue ongoing studies, as follows: (1) Characterization of the products of endogenous type C viral genomes and their interaction with host immunologic defense mechanisms. (2) Analysis of the molecular nature of the viral genome products, and definition of the

relationship of their production to host genetic or developmental events, including cancer. (3) Definition of the spontaneous immune responses of mice to their endogenous type C oncornaviruses and any associated immunopathologic events. (4) Attempts to manipulate natural production and control of endogenous type C viruses or non-virion-associated viral molecules to determine immunopathologic complications, effect on control of natural cancers and/or normal growth and development. (5) Definition of the potentially important direct neutralizing effect of primate complement on type C viruses and associated neoplastic events.

Date Contract Initiated: June 29,1972

SIDNEY FARBER CANCER CENTER (NO1-CP7-1028) (Formerly CP5-3539)

Title: Attempt to Isolate Type C Virus from Cultured Human Leukemia Cells

Contractor's Project Director: Dr. David M. Livingston

Project Officer (NCI): Dr. George Todaro

Objectives: To attempt to isolate an intact type C virus from freshly-cultured human leukemia cells, either by finding such cells which spontaneously produce virus, or by inducing virus production by physical, chemical or immunological means.

Major Findings: Rabbit anti-human Fab immunoadsorbents were used to remove populations of normal B lymphocytes from relatively large aliquots of Ficoll-Hypaque purified leukemic white cells from both marrow and peripheral blood. Standardization experiments showed that established T cell lines passed through the column with 99% efficiency while Ig^+ human B cell lines adhered to the column with 60-90% efficiency in the first pass and greater than 95% efficiency in in the second pass.

Superior growth of leukemic cell cultures was obtained using reduced concentrations of oxygen. The success rat at which bona fide leukemia cell lines are established may be substantially increased by culture conditions combining T-cell purification with reduced oxygen tension.

Attempts to "supertransform" leukemic cells with simian virus 40 (SV40) were not successful. Thus far, SV40 infection of cells yielded no advantage in the establishment of cell lines.

In an effort to identify, unequivocally, the phenotype of an established cell line and to determine if it was the same as that of the leukemic cells in the specimen from which it was derived, anti-sera were developed which were highly specific for various types of normal and malignant human hematopoietic cells. The antigens identified with these sera have never before been recognized in human cells. Their recognition offers significant new opportunities to identify, purify, establish lines and study specifically differentiated forms of both