Cells of a Moloney virus-induced lymphoma (YAC) were agglutinated by Con. A, wheatgerm and soybean agglutinins. However, only Con. A showed a marked toxic effect on YAC cells in vitro and in vivo. In vitro incubation with 125 µg Con. A for 24 hours lysed 95% of the cells. Intraperitoneal injection of 1 mg Con. A at 1 hour, 2 days, and 5 days after intraperitoneal inoculation of 10^2 YAC cells into adult mice resulted in an inhibition of tumor formation in 70%, 50%, and 20% of the animals, respectively.

Amino acid and carbohydrate transport in normal and malignant transformed hamster cells was studied after equal binding Con. A to the cell surface. The transport of a number of amino acids was inhibited after Con. A binding in the transformed cells but not in the normal cells (e.g., L-leucine, L-arginine, L-glutamic acid, L-glutamine, cycloleucine, and α-aminoisobutyric acid). Transport of D-glucose and D-galactose was more inhibited by Con. A in transformed than in normal cells. There was no effect on the transport of L-fucose or 3-O-methyl-D-glucose in either normal or transformed cells.

Significance to Biomedical Research and the Program of the Institute: Compounds that interact differentially with the surface 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 potential therapeutic value of Con. A will be further explored in three main directions: (1) Chemical modifications of Con. A to attempt to improve the degree of previously shown differential toxic effect on normal and tumor cells. (2) Studies of what enables Con. A to produce cell toxicity (how it differs from other lectins which bind but do not kill). (3) Further studies of the ability of Con. A to induce the activation of lymphocytes.

Date Contract Initiated: April 22, 1969

Current Contract Level: $51,600

WISTAR INSTITUTE OF ANATOMY AND BIOLOGY (NIH-NCI-E-71-2092)

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. Charles W. Boone

Objectives: To extract and characterize tumor-specific transplantation antigens induced by selected DNA and RNA tumor viruses.

Major Findings: (1) In cooperation with Dr. Berge Hampar (NCI), the peroxidase staining technique for localization of murine gs antigen is being evaluated. The technique is both more sensitive and more specific than the fluorescent antibody technique. (2) Examination of early fetal hamster tissue for antigens shared with SV40-induced tumor cells has confirmed Coggin's findings that such a common antigen(s) exists, but does not support the concept that it is the important transplantation type antigen since it protects only male animals. His findings have been extended to show the embryos of primaparous females were effective immunogens against SV40 tumorigenesis while those from multiparous females were not. (3) Rescue attempts have been initiated employing human sarcomas and leukemias in a variety of combinations designed to activate latent viral genomes. These include co-cultivation and fusion with either BPL inactivated Sendai virus or lysolecithin.

Significance to Biomedical Research and the Program of the Institute: The treatment of cancer by immunologic methods has been an attractive hypothesis for decades, but it is only recently that new and fundamental discoveries in immunobiology have made cancer immunotherapy a real possibility. Many tumors possess individually distinct transplantation antigens against which the host mounts an immune response. The transplantation antigens of most virus-caused tumors in different animal species are the same for a given virus. The work being conducted by the contractor is part of a larger effort of the SVCP to isolate and test virus-induced tumor-specific transplantation antigens in animal model systems.

Proposed Course: (1) Improve use of the peroxidase reaction technique as a routine assay method for gs antigens and for intracellular localization of reverse transcriptase. (2) Study of the relationship of fetal antigen to transplantation antigen. (3) Attempt to rescue human oncogenic viruses using co-cultivation, fusion, and chemical activation with BrdU and IdU. (4) Continuation of attempts to prevent spontaneous tumors of mice by immunological techniques using direct immunization with vaccines, non-specific stimulation, and abrogation of tolerance to isogeneic tissues. (5) Continuation of attempts to immunize against neoplasia induced by known laboratory strains of oncorna viruses. (6) Continuation of attempts to purify transplantation antigen extracted by

high molarity salt solutions or neuraminidase treatment, and characterization of the isolated component(s).

Date Contract Initiated: February 1, 1971

Current Contract Level: $115,400

THE WORCESTER FOUNDATION FOR EXPERIMENTAL BIOLOGY
NIH-NCI-E-69-2007)

Title: Isolation and Purification of Transplantation Antigens

Contractor's Project Director: Dr. Donald F. H. Wallach

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

Objectives: To investigate the biochemical and antigenic changes which occur at the cell surface during and after transformation by oncogenic viruses.

Major Findings: This program continued to search for new proteins in monkey kidney cells during early stages of permissive, lytic infection by the oncogenic DNA virus SV40. It relied heavily upon various methods of cell fractionation and upon polyacrylamide gel electrophoresis for visualizing the protein components of isolated cell fractions. Repeated experiments produced consistent protein patterns for uninfected cell fractions. The latest and best-controlled experiments show no new gel bands visible in fractions of infected cells if gels are stained in the Coomasie blue or Stains-All dyes. However, if the cells are labeled with glucosamine-1-^{14}C, microsome and microsome wash fractions of infected cells display at least one confirmed ^{14}C band not found in comparable fractions of control cells. The microsome "washes" were not innocuous rinses, but removed materials whose protein spectrum was different from the soluble fraction and whose ^{14}C specific activity was considerably higher. Inasmuch as by far the highest specific activity of any cellular component was found in the most buoyant microsome fraction, the activity of the material in the washes suggests that it is rich in membrane glycoprotein or in an export material which can be found on cell surface, or both. This "new" glucosamine-labeled component of microsomes from infected cells will be investigated further, using non-SVCP support.

Significance to Biomedical Research and the Program of the Institute: N/A

Proposed Course: This contract was terminated in September, 1971.