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UNITED STATES
ATOMIC ENERGY COMMISSION
OAK RIDGE OPERATIONS
P.O. BOX E
OAK RIDGE, TENNESSEE 37830

AREA CODE 615
TELEPHONE 483-8611

January 10, 1973

707834

J. L. Liverman, Director, Division of
Biomedical and Environmental Research, AEC HQ
RENEWAL OF CONTRACT NO. AT-(40-1)-3646 - UNIVERSITY OF TENNESSEE

We are submitting for your review and appropriate action the following information concerning the contract which will expire on 3/31/73.

1. Renewal Proposal
2. Progress Report (4)
3. Financial Statemen(4)
4. 200-Word Summary (4)
(3)

We shall appreciate your advising us of your decision so that we may proceed with the necessary contract action at the earliest possible date.

A. H. Frost, Jr., Chief
Research Contracts, Procedures
and Reports Branch
Contract Division

ACR:
LM
Enclosures:
As stated above

bcc: D. S. Zachry, w/Prog Rpt (2),
& Form AEC-427
J. L. Medley, w/encls
Tickler, w/Ren Prop, & Fin Stmt
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THE UNIVERSITY OF TENNESSEE
120 HESLER BIOLOGY BUILDING
KNOXVILLE, TENNESSEE
37916

BIOLOGY BUSINESS OFFICE
PHONE 615-974-5081

MEMORANDUM

To: Dr. J. H. Coggin, Associate Professor, Department of Microbiology

From: Roy A. Dean, Jr., Administrative Assistant

Date: January 10, 1973

Subject: Financial Report Correcting Report as of 12/7/72 for AEC Contract AT (40-1) 3646

Please be advised as of this date our records indicate that:

1.	Total actual project cost to date for the current period	$17,471.00
2.	Estimated total cost for remainder of period (includes carry over ($5,772.97)	17,364.37
3.	Total actual and estimated cost chargeable to AEC for current period based on percentage of cost agreed upon as contained in A-III of Appendix "A" to Contract	34,835.37
4.	Accumulated costs chargeable to AEC (include costs reported in certified statement for preceding period(s) and the costs stated in Item (3) above)	76,714.00
5.	Accumulated AEC Support Ceiling as stated in Article III of Contract	
6.	Total estimated AEC funds remaining under Contract (subtract Item (4) from (5) which may be used to reduce amount of new funds required from AEC for proposed renewal period	None

If more detailed information is required please contact me at your convenience.

RAD:hr

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BY AEC AUTHORIZED ☐

SMITHSONIAN INSTITUTION

U.S. ATOMIC ENERGY COMMISSION

AEC CONTRACT NO. AT(40-1) 3646

SUPPORTING DIV. OR OFFICE:

NAME & ADDRESS OF CONTRACTOR OR INSTITUTION: (State the division, department, or professional school, medical, graduate or other, with which this project should be identified.)

The University of Tennessee, Department of Microbiology
Knoxville, Tennessee 37916

TITLE OF PROJECT:

A Comparative Study of Radiation, Chemical and Aging Effects on Viral Transformation.

NAMES, DEPARTMENT, AND OFFICIAL TITLES OF PRINCIPAL INVESTIGATORS AND OTHER PROFESSIONAL SCIENTIFIC PERSONNEL: (not including graduate students) engaged on the project, and fraction of man-year devoted to the project by each person.

Joseph H. Coggin, Jr. Ph.D.-Microbiology-Principle Investigator-Professor-0.33 MY
E. G. Rogan, Ph.D.-Microbiology, Research Biochemist - 1.0 MY
W. H. Hannon, Ph.D.-Microbiology- Research Biochemist- 0.5 MY
M. P. Schafer, M. S.-Microbiology- Research Biochemist - 0.5 MY

NO. OF GRADUATE STUDENTS ON PROJECT: 2 NO. OF GRADUATE STUDENT MAN-YEARS: 2

SUMMARY OF PROPOSED WORK: (200-300 words, omit Confidential Data). Summaries are exchanged with government and private agencies supporting research, are supplied to investigaters upon request, and may be published in AEC documents. Make summaries substantive, giving initially and for each annual revision the following: OBJECTIVE; SCIENTIFIC BACKGROUND FOR STUDY; PROPOSED PROCEDURE; TEST OBJECTS AND AGENTS.

This project is designed to determine how low-level x-irradiation, chemicals and aging promote enhanced viral transformation in vitro and in vivo in syngeneic hamsters. The early events in integration of the oncodnavirus genome (SV40 and adenovirus) into the radiation damaged genetic apparatus of the target host cell are of prime concern. The regulatory mechanisms employed by the virus to produce augmented plasma membrane in the transformed cell are also being examined. Fetal antigens have been discovered on hamster and mouse tumor cells transformed by many viruses. These immunologic markers are used to detect the earliest symptoms of viral transformation in the plasma membrane of the cell. Specific questions asked are: Among what class of cell DNA (unique or repetitive) is the viral genome located? Are increased numbers of virus genomes incorporated following increasing exposures to x-ray? Can fetal specific mRNA's be found in viral transformed tumor cells? In fetal development fetal antigens are observed to be exposed only through the 10th gestational day in the hamster. Antigen phasing (+ to -) occurs with a 4 fold increase in sialic acid deposition in the membrane and a 7 fold increase in cAMP levels. These findings afford meaningful biochemical approaches to explain the specific biochemical changes which occur in viral transformed cells.

RESULTS TO DATE:

BUDGET	PROGRAM CATEGORY NO.
PRIMARY	
SECONDARY	

Signature of Principal Investigator

DATE: 12-27-72

INVESTIGATOR – DO NOT USE THIS SPACE

1036228

CONTRACTS - 3646 C 3605 (2 cys) C.A.

1. Title of Project

A Comparative Study of Radiation, Chemical and Aging Effects on Viral Transformation.

2. Institution

University of Tennessee
Department of Microbiology
Knoxville, Tennessee 37916

Telephone: 615 - 974 - 2356

3. Project Abstract

Radiation, pyrimidine analogues and cell "aging" in vitro markedly sensitize hamster embryo cells to simian virus 40 (SV40) and adenovirus 31 stimulated transformation. All three methods employed for sensitizing normal cells to virus transformation are observed to render lesions in the target cell DNA, suggesting that a common mechanism may be involved in the enhancement of viral tumorigenesis both in vitro and in vivo. Radiation similarly potentiates SV40 oncogenesis in vivo. Disclosure of the mechanism(s) for (1) effecting and (2) enhancing neoplastic transformation by these viruses is the overall objective of this research effort. Approaching the problem of how virus promotes specific malignant conversion of normal cells with both biochemical and immunologic techniques, several significant observations have been described in this laboratory. Recent data suggest that the virus specifically positions all or a portion of its genome into unique segments (non-repetitive regions) of the cellular DNA. One significant result of the incorporation of viral genome is to alter the composition of cell membrane, specifically the content of glycoprotein. Regulatory functions of cyclic AMP (cAMP) in cell metabolism associated with viral integration are being studied. Insertion of the viral genome leads not only to specific antigenic changes in the membranes of the cell but, significantly, to the acquisition of behavioral properties constituting the malignant state. A remarkable similarity between phase specific antigenic changes in the developing fetal membrane of rodents and humans and parallel changes induced by the viruses in the cells they transform has been documented in our laboratory with a growing body of immunologic and biochemical evidence. We are presently describing specific regulatory and macromolecular changes which occur in the transformed tumor

cell and in the normal fetal cell employing nucleic acid hybridization techniques, biochemical studies of the cell surface, and experiments defining the regulatory control exerted by cyclic AMP at various concentrations in the cell. The mechanism by which irradiation potentiates the viral transformation process to inititate cellular retrogression is a prime concern in our present study plan. We are uniquely equipped and staffed to describe the immunologic markers and delineate the basic biochemical events of transformation of a cell to the malignant state.

4. Scientific Background

(See previous applications for consideration of the older literature, please)

A. Status of information regarding SV40 integration into cellular DNA

Stoker (1) cited four lines of evidence to suggest the presence of the virus genome in stable, transformed cells.

(a) Covalently linked to the chromosomal DNA is a fraction of DNA which hybridizes with purified viral nucleic acid and contains identical base sequences to those of the infecting virus (2-4).

(b) Rapidly labelled RNA (mRNA) which hybridizes with purified viral DNA is found in the transformed cell (5-7).

(c) Virus specific antigens may usually be found in the transformed cell. (T, TSTA).

(d) Fusion studies and mitomycin C treatment can result in virus "release" from stably transformed SV40 tumor cells (8, 9).

Added to this list one could now include the observations that different transformed cell lines may possess different amounts of virus DNA (genome equivalents) associated with their chromosomal DNA (2-4, 10). Only transformed cell lines containing T antigen were observed to actively

synthesize viral RNA suggesting that S or surface antigen is not a direct virus specific function (11).

Integration of virus DNA in the cellular DNA (nuclear DNA) has been shown to occur in SV40 transformed mouse and hamster cells (4, 47). Aloni, et al (15, 16) warned against the use of SV40 DNA preparations to conduct hybridization studies with certain monkey cell lines transformed by SV40. Cellular DNA packaged in virions with SV40 DNA can produce confusing data. It is not known whether such integration or fixation occurs in other transformed lines or in primary transformed cells although this might generally be the case (10). The problem is to identify the site or sites of insertion and to establish the uniformity of the mechanism involved. Certain chromosomes seem to be involved in at least two systems examined to date (13, 14).

In lytic infection, three to four of the supposed ten viral genes are transcribed in the early phase of infection as mRNA (prior to viral DNA replication). About one-third of the viral DNA is apparently transcribed in early infection. Irradiation of viral particles suggests that only two to three viral genes seem required for transformation. Late functions in virus maturation occupy about 3 to 4 viral genes and these genes seem to be unimportant in transformation. These and other findings suggest that, at most, only one or two viral genes seem to be intimately involved in inducing transformation (12). One additional viral gene seems related to the induction of cellular DNA synthesis occurring at the same time as viral DNA replication is initiated (12).

In the past few years several reports have been most enlightening regarding the number of viral equivalents per genome. Employing com-

plementary (c) RNA produced in vitro from SV40 or polyoma DNA template, Westphal and Dulbecco (3) examined a number of polyoma and SV40 transformed tumor lines to determine the number of equivalents of SV40 per cell type. Results using the cRNA:DNA hybridization technique indicated that each line of tumor cell contained a different number of "viral equivalents" ranging from 5 to 60 equivalents within the nucleus of the cells. These workers asserted that no biological significance could be attributed to a cell line containing 60 equivalents of virus rather than 5 with respect to being "more" transformed. Tai and O'Brien (10) confirmed that a large number of viral equivalents existed in SV40 tumor cells using a cRNA:DNA hybridization technique at 24° for 18 hours in formamide. However, in a recent paper Haas, Vogt, and Dulbecco (53) demonstrated why many earlier estimates of viral equivalents were too high and reiterated the finding of about 5 viral equivalents per SV40 transformed cell.

Gelb, Kohne, and Martin (personal communication, 47) have devised a DNA:DNA hybridization technique which is sufficiently sensitive to detect one molecule or less of viral DNA per genome of the transformed cell using hydroxyapatite rather than DNA trapped on nitrocellulose filters. They also find the high number of viral equivalents per genome reported above for different SV40 tumor lines employing the cRNA:DNA technique to be erroneous. Results using the technique of Gelb, Kohne and Martin indicate that SV40 transformed clones actually contain an average of one or less SV40 genome per cell. We have available transformed tumor clones with vary numbers of viral equivalents per cell for our studies. We currently employ the hydroxyapatite method for all determinations for our DNA:DNA association experiments designed to

determine the number of viral equivalents per SV40 transformed hamster cell. Dr. Gelb has been most helpful in assisting us with this work.

Martin (18) examined the possibility that SV40, polyoma or adenovirus 12 might alter the normal pattern of RNA synthesis in cells transformed by these agents. As in most differentiated mammalian cells, only a small fraction (5%) of the cellular DNA is transcribed. Employing competition hybridization techniques no significant alteration in randomly labelled RNA from transformed hamster tumor lines was noted, suggesting that viral transformation did not produce a change in the pattern of DNA transcription. Some ambiguity arose, however, since control DNA was not representative of the same tissue type as the tumor cells. Under the conditions employed only RNA from repetitive DNA was examined and no data are available on changes stimulated among unique, non-repetitive DNA by transformation.

B. Role of Cyclic AMP in Viral Transformation

In seeking the fundamental events of viral transformation of a cell, changes in the cell's regulatory processes appear highly significant. Loss of some controls in basic biosynthetic and catabolic pathways may well be the ultimate cause of characteristic behavior patterns in transformed cells, such as loss of contact inhibition of growth. Cyclic AMP has been implicated in the general control of cell surface properties. Sheppard (52) has shown that dibutyryl cAMP treatment of SV40 transformed cells reinstated contact inhibited growth and that this can be reversed by removal of the cAMP analog. In addition to these changes in growth characteristics, dibutyryl cAMP also decreased the agglutinability of the virally transformed cells by wheat germ agglutin, indicating cAMP-induced changes in the plasma membrane. Otten, Johnson, and Pastan (54)

demonstrated an inverse relationship between growth rate of fibroblasts in tissue culture and the level of cAMP. Using 3T3 and transformed 3T3 cells they found that the measurable level of cAMP rose during contact inhibition of growth, suggesting that contact inhibition is mediated through cAMP. Further studies by Johnson, Friedman, and Pastan (55) demonstrated that treatment of tumor cells with cAMP analogs enables these transformed cells to regain normal morphology and growth characteristics. This response was reversibly controlled in tissue culture by the addition or depletion of cAMP. The lower than normal levels of cAMP found by Sheppard (57) in transformed cells may underlie the antisocial behavior of these cells.

More recently dibutyryl cAMP has been shown to affect the rate synethesis of sulfated acid mucopolysaccarides by transformed fibroblasts (56). Since mucopolysaccharides are laid down on the cell surface, this strongly suggests one possible role for cAMP in affecting the behavior of cells. Alterations in the amount of synthesis of mucopolysaccharides in transformed cells could explain loss of contact inhibition of growth, increased motility, increased agglutinability by plant agglutinins and decreased adhesiveness to the growing surface. The literature documents a growing body of evidence defining the role of cAMP in expression of transformed characteristics of cells. This leads us toward an understanding of the transformational event.

C. Fetal Expression in Cancer Cells

A growing list of human cancers are known to possess fetal components not expressed in homologous adult tissues (colonic, hepatic and lung cancers 23, 24, 25). We have demonstrated (26, ORO-3646-11) that hamster, mouse, and human fetal cells contain surface membrane antigens

cross-reactive with SV40, adenovirus 31 and certain chemically-induced tumor specific transplantation antigens (TSTA) in syngeneic hamsters. Fetal cells also induce a specific antibody reactive with the TSTA present in the tumor cell membrane, termed cytostatic or C antibody. The role of C antibody in tumor progression or rejection has been characterized in our laboratory under a research program supported in part by the A.E.C. (27, 28, 29). Soluble extracts of 72 different mouse tumors have been examined and found to be cross-reactive with antiserum produced in rabbits against mouse embryo cells confirming, indirectly, that a parallel situation to that found in hamsters exists in the mouse (30). Our laboratory has shown that not only are fetal membranes masked in the latter stages or gestation by a three-fold increase in sialic acid content and these membrane (14-day fetal cells) do not induce transplantation immunity, but also that SV40 transformed kidney tumor cells are observed to have one-third less sialic acid than normal adult kidney cells. The concentration of cAMP is found to be low during the period of fetal development when antigen cross-reactive with tumor antibody is present; cAMP levels rise later in gestation to normal adult levels. This parallels the low levels of cAMP seen in transformed cells. These and other findings suggest the following summarial facts regarding fetal antigen and cancer antigens:

(a) Mounting evidence indicates that all neoplastic conversions results in a spectrum of measurable changes in the cancer cells and some of the changes are associated with the expression of fetal antigens.

(b) Biochemical data confirm that an unmasking of sub-membrane components may account for the behavioral characters of cancer cells (Wallach, 31; Burger and Noonan, 32). These unmasked components may be synonymous with fetal antigens.

(c) SV40 produces the same TSTA in mouse, hamster and human cells transformed by the virus, and embryo cells from

all three species of fetus have antigens cross-reactive with this transplantation antigen.

(d) It seems reasonable that SV40 must be integrated into the cellular DNA of the transformed cell and the result of this interaction is specifically to promote the altered synthesis of surface membrane (diminished sialic acid deposition). We anticipate that the virus may specifically produce a regulatory change by producing a regulatory product or, alternatively insert its genome in a specific site among unique cellular genes "destroying" an important cell function relating to membrane synthesis. The search for an answer to these questions is a prime concern of our immediate research effort.

D. Surface Changes Associated with Viral Transformed Tumors

Several recent reports (48, 49) have suggested that some tumor cells transformed by oncodnaviruses contain Forssman antigen at the cell surface in agreement with the earlier findings of others (50, 51). We have demonstrated that SV40, adenovirus, Rauscher virus and spontaneously transformed tumors of the hamster or mouse contain surface antigens (fetal antigens) present on hamster, mouse and human fetal cells during the normal course of differentiation. These antigens in fetal membranes uniformly disappear in the latter period of gestation and are not reexpressed normally in neonate or adult cells. Viral transformation activates these antigens. A growing body evidence suggests that studies of the biochemistry of cell surfaces will aid in understanding how transformation alters the expression of surface antigens.

Alterations in the deposition of sialic acid in glycoprotein and glycolipid components of mammalian cell membranes have been related to the modifications of membranes that occur with viral transformation of cells. Ohta et al (58) found lower levels of sialic acid in malignant cells than in normal cells. Glycolipids and glycoproteins with incomplete carbohydrate chains have been demonstrated in spontaneous

and virus transformed hamster fiborblast cells (59). These carbohydrate chains appeared to lack sialic acid as well as having reduced levels of other carbohydrates (6). The concentration of sialic acid has been shown by Smith and Walburg (60) to decrease with morphological progression of tumors. Malfunction or repression of biosynthetic processes, especially glycosyltransferase, has been implicated in explaining reduction of sialic acid and other surface changes or transformed cells (61, 62, 63). Experimental procedures to remove sialic acid from cell surfaces have been reported to uncover transplantation antigens (64) and to alter the response of tumor cells to phytoagglutinins used to monitor changes in surface membranes (65).

Because of the work in our laboratory relating fetal and tumor antigens, we are also interested in patterns of glycolipid synthesis during gestation. McQuiddy and Lilien (66) have reported that between the seventh to fourteenth day of embryonic development the amount of sialic acid measured in chick neural retina rose 50%. Changes in surface sialic acid of embryonal retina cells have been seen to correspond to alterations in available phytoagglutinin binding sites (67). Although these binding sites for phytoagglutinins have not been related to known embryonal antigens, they do constitute a measure of exposed sites on the cell surface.

In these studies it must be remembered that changes in sialic acid levels or other parameters may be occurring in localized areas too small to be detected by present procedures. Thus it is possible that antigenically significant changes in sialic acid content may be occurring on a microscopic scale while no change is seen in total level of sialic acid. Specifically we can picture 10-day fetal or transformed

cells having exposed antigenic sites which are rendered unavailable by sialic acid on a 12-day fetal or non-transformed cells, respectively. The idea of localized alterations was demonstrated by Warren et al (68), who found a glycoprotein in component of transformed cells which could be converted to a component resembling that found in normal cells by enzymic removal of sialic acid. This type of event might not be reflected in measurements of total cellular sialic acid.

As more is known about the biochemical processes involved in antigenicity, measurement of components like sialic acid will become more informative. Removal of sialic acid has been reported to increase the strain specificity of the transplantation antigen of tumors (69). Thus immunologic and biochemical studies can go hand in hand in elucidating the basis for antigenic communication between cells or normal adult, transformed, or fetal state. Here, as in other studies, use of cells from developing organisms will aid in understanding the molecular events which transform a normal adult cell into a tumor cell.

5. Scientific Scope.

This research project was organized in 1968 *to explore the mechanisms involved in the potentiation of viral carcinogenesis* in animals *caused by sublethal irradiation, selected radiomimetic chemicals and natural aging processes.* Inherent in this objective is the discovery of how virus produces neoplastic alterations in cells. Early work focused on establishing that, indeed, radiation, chemicals and in vitro cell aging promoted viral oncogenesis in vitro (Coggin, J. H., J. Virology, 3, 458-462, 1969); in addition considerable effort was vested in determining the radiation responses of normal embryo cells used as target cells in the transformation studies (Coggin, J. H. and Kouri, R. E. Proc. Soc. Exptl. Biol. Med., 129, 609-620, 1968). *Subsequent studies were designed to determine whether the marked potentiation of virus induced neoplastic transformation by sublethal x-irradiation which we observed in vitro could actually be demonstrated in vivo.* Clear demonstration of radiation-potentiated SV40 and adenovirus 31 oncogenesis was reported in neonatally infected hamsters in 1970 (Coggin, J. H., et al. Proc. Soc. Exptl. Biol. Med., 134, 1109-1111, 1970). Thus, several of the basic objectives of the program were reasonably well established within two years of the initiation of the project:

(1) Radiation, radiomimetic agents and "aging" promoted the efficiency of SV40 oncogenesis in vitro. Dose determinations were obtained.

(2) Radiation at "low-levels" was quite effective in potentiating oncogenesis by other oncodnaviruses as well. Essential information about the possible molecular mechanisms for facilitating integration of the viral genome into host chromosome was obtained by evaluating radiation effects on cellular DNA replication.

(3) Marked stimulation in oncodnavirus oncogenesis in hamsters could be achieved in vivo with non-lethal doses of x-irradiation.

(4) Repair of radiation damage prior to infection restricted the efficiency of viral transformation.

The focus of the project subsequently centered on developing a molecular and biological understanding of why the radiation-damaged nuclear apparatus of the cell was more susceptible to SV40 transformation. Two approaches were selected. One involved an attempt to measure the efficiency of viral genome integration into the host cell chromatin coordinate with increasing pre-infection exposures to x-irradiation. Nucleic acid hybridization techniques were developed and are being used to exploit this question. The other approach was to *develop biological markers* for detecting cell transformation which could serve as indices of viral transformation for denoting the *early events* in *virus-cell interaction.* Clearly this early period in infection holds the essential clues which will ultimately reveal how radiation damage facilitates viral transformation. Immunologic markers (tumor neoantigens) at the surface of the plasma membrane of the transforming cell afforded the most useful tools for characterizing the earliest "symptoms" that target cells were undergoing viral transformation. Fortunately we had a strong program in tumor immunology at the University of Tennessee in collaboration with the MAN Program at Oak Ridge National Laboratories.

Initially we attempted to use antibody raised against tumor-specific transplantation antigen (TSTA) which was known to appear in the serum of animals developing autochthonous tumors (Coggin, et al. Proc. Soc. Exptl. Biol. Med., 132, 246-252, 1969; Ambrose, et al, Proc. Soc. Exptl. Biol. Med., 132, 1013-1020, 1969; Ambrose, et al, Nature, 233, 321-327, 1971). The appearance of this immunoglobulin indicated that transformation had occurred and the newly formed

tumor cells possessed TSTA. This antibody was believed to be tumor specific and did not occur in *normal* male or female animals nor in animals bearing tumors induced by other viruses or occurring spontaneously. We were prepared to use this antibody to monitor the surface of normal cells for the earliest changes in the transformed cell membrane which would reflect cellular changes induced by the transforming virus, hopefully within 8 to 10 hours post-infection. Once we could establish that transformation had occurred, we wanted to determine whether viral genome had been incorporated into cellular DNA and we wished to monitor the cell population for altered membrane biosynthesis. Membrane alterations are believed to be responsible for the major cancerous properties of malignant cells. We knew that integration of viral genome occurs within the first several rounds of host DNA replication and we were seeking to measure these changes using a new instrument (cytoflurograf) which permits *quantitative* determination of the binding of immunoglobulin to cell surfaces demonstrating TSTA. Since non-infected *fetal* cells were our "normal" target cells for viral transformation they were used as control cells. Quite to our surprise non-infected *10-day fetal* cells were observed to bind antibody believed to be tumor specific. Term fetus did not bind anti-TSTA antibody nor did adult tissue.

This basic observation has prompted a chain-reaction of research focusing on a fundamental approach to understanding the true nature of the cancer cell and affording meaningful prospects for early cancer detection and therapy monitoring (N. G. Anderson and J. H. Coggin, "Models of Differentiation, Retrogression and Cancer" in Embryonic and Fetal Antigens in Cancer. I, 7-37, 1971 U.S. Dept. Commerce, Springfield, Va.).

In 1969 we observed that it was feasible to protect susceptible animals against virus cancer by immunizing the animal with syngeneic or heterologous fetus. The significance of this finding for this report is that we had discovered that SV40 and many other tumor cells possessed a cross-reacting cell

surface antigen which was fetal or embryonic in origin. Viruses such as the oncodnavirus were capable of infecting target cells (fetal or adult) and of specifically "fixing" or promoting retrogression to a neoplastic-like state; in the adult host these virus-controlled changes resulted in successful malignant cancer production. The virus could specifically regulate a constant cancer-type in every cell successfully transformed.

Some sixteen published pieces of research, supported significantly by this contract, have been generated from our laboratory between 1970 and 1972 as a result of the initial discovery in 1970 that virus-tumors contain embryonic, fetal or phase-specific autoantigens (see section 6 , p 31 for a synopsis of this work).

What is significant about this finding in relation to the objectives of this contract? First, we now have the important new knowledge about how virus may produce cancer cells and the monitoring tools to describe the molecular interaction between virus and target cell which lead to transformation as well; specifically we know that infection of mature hamster and mouse cells with oncodnaviruses results in alterations in the normal formation of sialoglycoproteins in the cell covering. Underlying fetal autoantigens are exposed, the tumor cells lose "contact-inhibition" traits, grow in disoriented patterns, and are invasive in vivo. We have a reliable, quantitative technique to detect these changes in the transformed cells using the indirect immunofluorescence assay employing the *Cytoflurograf* (Biophysics Systems, Inc). It is now possible to detect fetal antigens, the earliest "symptoms" that predict malignant conversion of the cell following viral transformation. We know specific biochemical changes which occur in the cell's normal synthesis of the cell covering. We hope that we can now determine by what regulatory means the virus specifically controls these changes. We have developed suitably sensitive techniques for detecting and isolating fetal mRNA reexpression in

adult cells and can now conduct competition hybridization studies to demonstrate whether tumor specific or viral-specific RNA's are indeed fetal-like. *Our objective then is to characterize the molecular events (biochemical and regulatory) resulting in conversion to neoplasia and to correlate this phenomenon to retrogression or dedifferentiative processes.*

Biochemical and molecular studies of the *early events* of transformation would be severely limited if our studies were dependent upon the normal efficiency of transformation (1 transformant per 1000 infected cells). Fortunately, we have established that radiation potentiates transformation markedly. (150 R of x-ray administered prior to infection permits the appearance an average of 33 transformants per 100 infected cells). The availability of this many transformants in the total population makes many of the planned studies plausible using mass culture. We are able to proceed.

Immediate Objectives

Our immediate objectives are:

(1) To obtain *conclusive* evidence which will reveal whether radiation pretreatment facilitates an increase in the absolute *number* of viral equivalents integrated per transformed cell or rather serves to increase the *probability* that a genome will be integrated.

(2) To characterize the biochemical events leading to the production of immature plasma membranes and fetal antigen reexpression in radiation-stimulated oncogenesis. Sialic acid synthesis and incorporation of sialoglycoprotein into membranes of tumor cells compared to synthesis and incorporation into normal control cell membranes will be investigated.

(3) Employing purification procedures developed in our lab-

oratory fetal, phase-specific mRNA's will be isolated and tested for their identity to "tumor-specific" mRNA. The competitive hybridization procedure is being used.

(4) The role of cyclic AMP concentration, synthesis and degradation in retrogression induced by SV40 virus will be investigated.

(5) The "masking" of fetal antigen in the normal developing hamster fetus will be evaluated. Such studies provide not only specific parameters to measure when viruses stimulate retrogressive or dedifferentiative changes in normal cells leading to neoplasia but also are most suitable for evaluating subtle changes in fetal development which radiation exposure might introduce.

Specific Experimentation and Methods of Procedure

I. Early Events in SV40 Transformation In Vitro.

Purpose:

The purpose of this study will be to determine whether the SV40 genome becomes associated with term, fetal cell DNA in a stable manner within 24-72 hours post-infection and whether changes in the surface membrane occur early, paralleling viral integration or later, after several cell divisions. Also we will determine whether increasing radiation doses between 25 R and 150 R lead to the early "incorporation" of more virus genome equivalents per transformed cells or simply facilitates the incorporation of a single genome.

Methods:

Fourteen day fetal cells in primary culture are exposed to radiation levels between 0 R and 150R and infected with SV40 virus (1 PFU/cell). Uninfected cells and non-infected, non-irradiated cells serve as controls. High titer SV40 antiserum is added following virus adsorption. Sample plates are washed at selected times post-infection (24, 48, and 72 hours post-infection) and target cells are harvested by aspiration and exposed to either fetal antibody or IgG from hyperimmune serum from hamsters immunized against SV40 tumors. Normal serum and/or virgin serum serves as control sera. Following adsorption and washing highly specific, fluorescent-tagged rabbit anti-hamster IgG is added, incubated with target cells and washed. The cells are then scored in the Cytoflurograf for (a) viability (b) percentage cells labeled with a level of immunofluorence above the selected threshold (c) estimated moles of immunoglobulin bound per cell for three population classes. These data reflect the changes in cell surface or plasma membrane indicating the unmasking of fetal antigen. Challenge studies with graded doses of these cells reflect relative oncogenic potential of the cell population.

The fluorescent labeling procedure indicates the *population fraction* (estimated number of transformants) which has undergone membrane change sufficient to react with test immunoglobulin. Parallel plates are overlaid with agar and stained at 12 days post-infection to confirm morphologic transformation and relative frequency by this standard procedure.

Other similar plates are collected and the cells harvested and cellular DNA separated from viral DNA by procedures previously described (ORO -3646 14). Cellular DNA is then "cleaned up" and serves as test template in a "Gelb"-type study for viral DNA equivalents. The technique is suitably sensitive to

detect 0.3 ±0.2 of a virus genome per cell when labeled viral DNA is used as a "homologue" probe. Controls include artifically reconstituted mixtures of viral DNA with normal cellular DNA. By determining the effects of irradiation pretreatment on the number of transformants appearing post-infection and the number of viral DNA equivalents associates strongly with the host cell DNA we will obtain an interesting picture of the early events in SV40 oncogenesis.

We anticipate, from initial studies, that little useful data on viral integration can be obtained from non-irradiated infected control cultures because of the extremely low efficiency of SV40 transformation; however, comparison of low and high level X-ray pretreatment will suffice to obtain the desired data.

Follow up studies will be conducted to determine the nature of the association between viral and cellular DNA. In addition, early effects of transformation on sialic acid content of the transformed cell membranes compared to non-infected cells and on cAMP synthesis will be examined.

We recognize that these studies are complicated and are potentially ladened with many experimental pitfalls but we have proceeded carefully and have worked out both the biological test parameters and the DNA: DNA hybridization procedures to the desired reliability point.

II. Identification of Specific mRNA's During Fetal Hamster Development and Tumor Formation.

Purpose:

This laboratory has documented through immunological data the appearance in fetal cells of antigen which are cross-reactive with SV40-induced surface neo-antigens. This work has led us to study the fetal cell synthetic processes for two purposes. One, synthesis of fetal antigens which can protect adult antigens against SV40 oncogenicity is of interest itself; and, two, study of

the synthesis of fetal antigens indirectly furthers our studies of tumor antigens and their synthesis. We are working toward the goal of elucidating synthesis of significant tumor antigens using the simpler model system of the developing hamster fetus.

In this research project our specific goal is to identify and utilize the messenger RNA's (mRNA's) responsible for the antigenic expression of 10-day fetal hamster cells which protect adult hamsters against SV40 oncogenicity. We have defined the period of fetal antigen phasing (+ to-) to occur between 10.5 days post-conception and 11.5 days. This observation is most helpful since the available data suggests that new cellular synthesis of the sialoglycoprotein covering accounts for antigen masking; one can anticipate de novo processes to account for the phenomenon mRNA's present at day 11 but absent at day 10 must exist.

We have already worked out suitable procedures for purification of DNA and RNA and for competition hybridization (see our Fifth and Sixth Annual Progress Reports, ORO-3646-14 & ORO-3646-18). Recent results have demonstrated, surprisingly, that mRNA species are present in 10-day fetal tissues but absent in 12- and 14- day fetal tissue (Figure 2, ORO-3646-18). The data indicates that about 35% of the RNA present at 10 days of gestation is no longer being synthesized by 12 days. We have begun studies to isolate these 10-day specific fetal mRNA's.

Methods:

Our initial approach to the problem is to utilize polyacrylamide gel electrophoresis (see ORO-3646-14 for procedure) to separate 10-day fetal RNA into fractions. The fractions then will be tested for ability to complement 12-day fetal RNA in completely competing out hybridization of ^{3}H-10day RNA with 10 day fetal DNA. By these procedures we will identify those mRNA's

specific to 10-day fetal RNA.

Once we have identified and purified such specific mRNA's, we shall proceed to utilize them for in vitro synthesis of protein (G.C. Rosenfeld, et al, 1972 Biochem, Biophys. Res. Commun. 47, 387). We have available in this laboratory our standard immunologic tests for fetal and tumor antigens, which should aid us in identifying the products of such in vitro synthesis. We have the potential of describing synthesis of fetal antigens cross-reactive with SV40 induced tumor antigens. We already have collected a large number of SV40 induced hamster tumors and have purified RNA and DNA from them. We expect that the procedures and concepts necessary for our work with RNA synthesis in developing fetal hamsters will be directly carried over into work with the tumor materials.

III. Analysis of Sialic Acid Content in Fetal Membranes.

Purpose:

To investigate the production of sialoglycoproteins in fetal and tumor cells to determine if sialoglycoproteins cause "masking" of fetal antigens (See ORO-3646 for specific accomplishments to date).

Approach:

Determine sialic acid content in:

1. Membrane mitochondrial, microsomal, and soluble fractions of homogenates of whole hamsters at various ages, including fetal, neonatal, and postnatal.
2. Small SV40 tumor induced fibrosarcomas ($\sim 10^4$ cells, 50 days) and normal muscle tissues from the same animal.
3. Age-matched (10-day) hamster fetus from multiparous and primiparous mothers.
4. Whole untreated hamster fetus and hamster fetus treated as follows--bled, and hepatectomized (whole).

5. Intact cells from 10-, 12-, and 14-day hamsters treated with neuraminidase.

Specific Questions Asked Experimentally:

1. Is there a difference in sialic acid content in membrane, mitochondrial, microsomal, and soluble fractions from fetal (various ages), neonatal, and postnatal hamsters?
2. Is the sialic acid content of fibrosarcoma different from that of normal muscle tissue?
3. How does the number of pregnancies of the mother affect the sialic acid level of the fetus?
4. Is the change in sialic acid level in a fetus related to the development of a specific organ?
5. Will treatment of 10-, 12-, and 14-day hamsters with neuraminidase remove sialic acid and unmask fetal antigens?

IV. Cyclic AMP (cAMP) in Differentiating Fetus and in Tumor Cells

Purpose:

To determine whether membrane maturation and processes associated with fetal antigen masking can be related to the cAMP content of the cell. (See ORO-3646 for results obtained to date).

Approach:

We have established that there is a very significant increase in cAMP in the developing fetal membrane coordinate with antigen phasing and maturation. We seek to establish its role in controlling membrane structure using the following steps:

1. Monitor morphological characteristics and cell density (viable cell count) of four test systems -- control (untreated), control plus dibutryryl cAMP, control plus theophylline, and control plus a com-

bination of dibutyryl and theophylline.

2. Repeat experiment 1 with low passage tumor cells from SV40-transformed or -induced fibrosarcomas (only if Experiment 1 does not produce density-dependent growth).
3. If growth is produced in 1 and/or 2 which is not density dependent, grow cells in presence and absence of dibutyryl cAMP, irradiate (in presence of cAMP), immunize hamsters with these cell preparations, challenge with untreated SV40 tumor cells (later), perform an in vivo transplantation immunity test.

Specific Questions Asked Experimentally:

1. Does cAMP concentration change coordinate with the early stages of SV40 oncogensis?
2. Can cibutyryl cAMP and theophylline restore contact-inhibited growth to SV40-transformed hamster cells in culture?
3. If so, is the new growth density dependent?
4. Can such growth be restored in SV40-induced fibrosarcoma cells in culture?
5. Is dibutyryl cAMP able to suppress TSTA expression in SV40-transformed hamster cells?
6. Are sialic acid (bound and free) levels different in contact-inhibited and non-contact-inhibited SV40-transformed cells grown in the presence and absence of dibutyryl cAMP?

Significance of Proposed Work

The research effort is designed to investigate the precise conditions and parameters of enhanced virus transformation of normal cells stimulated by radiation, radiomimetic chemicals and associated natural processes (cell aging). As before, the program involves a quantitative examination of radiation or chemically-induced alterations in cells which sensitize them to the transforming potential of SV40 and adenoviruses. An important result to be simultaneously gleaned from the present approach will be information about the mechanism for virus transformation of cells under conditions of normal infection. Previously we have emphasized the importance of using normal target cells for these studies; cells which possess none of the "unit characters" of tumor progression including loss of contact inhibition, changes in cell and culture morphology, antigenic alterations, karologic changes, growth characters and malignancy prior to infection with virus. Researchers in other laboratories employ cell systems which have already undergone certain spontaneous changes to study virus transformation (e.g., 3T3 cells). These changes make the established cells useful for the study of certain aspects of transformation but always with qualification since they are not normal cells. A system has been developed in this laboratory which permits the study of transformation mechanisms using hamster cells from primary tissue with no unit character of the transformed cell prior to infection with the oncogenic agent. We have recently demonstrated that radiation stimulated SV40 tumorigenicity at the cellular level in hamsters paralleling our in vitro findings.

We are now concerned with describing and understanding the basic biochemical events of transformation evidenced by morphological and immunologic parameters. We have overwhelming evidence that the result of the interaction between oncogenic virus and target cell is to augment chemical composition

of the normal cell membrane causing the cell membranes to have antigenic qualities identical to those of fetal cells. Associated with this change are the multitude of characters common to cancer cells. A portion of our effort in the coming contract period will be devoted to establishing this point by comparing the transcriptional products of tumor cells and fetal cells.

Describing the mechanism for the insertion of SV40 DNA would provide invaluable clues for exploring the mechanisms of activation and insertion of the DNA form of "oncogenes" and "virogenes" for RNA cancer viruses. Appropriate materials (DNA) for such a study with RNA viruses are impossible to acquire at present. In short whether or not reverse transcription of oncogenic RNA genomes to a DNA storage form contitutes a valid picture of malignant conversion or expression, we are still faced with the real problem of how and where the oncogenic information is integrated and stored in the genetic complement of the cell and by what means it promotes neoplasia. Disclosure of the regulatory interplay between viral expression and host cell expression must be characterized. Hence, it seems fruitful and worthwhile to continue to investigate this problem with the well-characterized SV40 system, a virus which can promote neoplasia by insertion of its DNA into the host chromosome.

Further, we have observed that low-level radiation and subtoxic chemical exposure potentiate the oncogenic qualities of SV40 and adenovirus both in vitro and in vivo presumably by damaging the cellular DNA (in either a physical sense producing a gap or by impairing a regulatory gene) and facilitating insertion of the viral genome into host cell genetic material. This mechanism must be understood because of the widespread application of radiation in medicine and its growing use in industry and

because of the strong association between certain forms of neoplasia and DNA-containing herpes viruses. We have carefully prepared ourselves to conduct the proper experiments to answer these questions and have prepared the necessary biological reagents to proceed with a series of experiments utilizing DNA hybridization techniques with transformed and radiation-potentiated transformed cells. We have shown that an identity exists between membrane components expressed in the fetuses of the hamster, mouse and human during the normal course of fetal development and tumor antigens present as specific transplantation antigens on SV40 and adenovirus 31 hamster tumor cell membranes, chemically-induced hamster tumor cells, and in mouse spleen cells infected with Rauscher leukemia virus. Other investigators have subsequently shown similar antigens in mouse lymphomas, chemically-induced rat sarcomas and in other tumors. These observations provide strong evidence that both chemical and biological carcinogenic agents act to promote the expression of cellular products in adult cells which are normally restricted to fetal life. At least four major types of human malignancy are now known to possess fetal antigens.

Recently we have established that classes of RNA, unique to fetal tissues or absent from these tissues during gestation are also uniquely present, or conversely absent, in SV40-transformed cells when compared to the RNA present in normal cells. Stringent competition hybridization procedures being conducted with RNA and DNA from in vitro derived transformed clones from normal hamster tissues and fetal hamster tissues giving rise to the adult target cells. The techniques developed for this work will afford not only a clarification of the significance and character of fetal expression in cancer in our hamster and mouse model system, but,

more importantly, will outline the parameters for a meaningful molecular study of fetal antigens occurring in several forms of human malignancy.

We have added to our scope research studies designed to elucidate the basic biochemistry of transformation. Our experiments measuring cAMP during development and transformation and utilizing cAMP to affect expression of transformed characteristics lead us to a greater understanding of regulatory problems in transformation. This work complements our efforts to characterize the role of glycolipids and glycoprotein in the expression of fetal and tumor antigens on the cell surface.

6. Scientific Personnel

Principal Investigator:

Joseph H. Coggin, Jr. - 33% time on project
Social Security No. [redacted]
Business Address Department of Microbiology
University of Tennessee
Knoxville, Tennessee 37916
Telephone 615 - 974 - 2356
Born [redacted]

Education:

[redacted]

Current Memberships:

American Society for Microbiology
American Association for the Advancement of Science
Tissue Culture Association
Sigma Xi
Society for Experimental Biology and Medicine
American Association for Cancer Research
Radiation Research Society

Experience:

Section Chief of Transplantation Antigen Study, MAN Program, Oak Ridge National Laboratories and Consultant Virologist to Biophysical Separation Laboratory, 1967 to present.
Consultant virologist to East Tennessee Children"s Hospital and Fort Sanders Hospital, 1966 to present.
Professor of Microbiology, 1973.

Virologist and Associate Professor, University of Tennessee, 1966 to present.
Lecturer in virology and molecular biology at graduate and undergraduate level.
Principle Investigator - AEC contract No. AT(40-1) 3646.
Principle Investigator - NIH Grant CA-10429-02. National Cancer Institute. Principle Investigator - NCI Tumor Transplantation Antigen Study FS-7, ORNL.

Senior Research Virologist. 1965-1966. Virus and Cell Biology Division, Merck Institute for Therapeutic Research (Merck, Sharpe, and Dohme Laboratories)- Planned and supervised research in cancer virology and immunology in conjunction with Dr. Maurice Hilleman. The program involved experimentation into problems of tumor antigen assay and purification,

the viral etiology of cancer, recovery of viruses from transformed cells and immune mechanisms operative in hamsters infected with oncogenic viruses.

U. S. Public Health Service Predoctoral Trainee. 1962-1965. University of Chicago, Department of Microbiology. Investigated the mode of action and mechanism of cellular resistance to anti-tumor drugs.

Acting Principle Investigator, 1965. NIH Grant CA-07525. Responsibilities included supervision and planning of research for labor atory personnel in a biochemical investigation of 6-mercaptopurine degradation by leukemic cells and Escherichia coli.

Research Associate, 1961-1962. Kettering-Meyer Cancer Laboratory. Birmingham, Alabama. Responsibilities as group leader included coordination of research within group of 35 junior personnel and administrative responsibility.

U. S. Public Health Service Trainee (Premasters) 1960-1961. University of Tennessee. Investigated growth and proliferation of Streptococcus faecalis on plant tissues as graduate student.

Senior Microbiologist, 1959-1960. State of Tennessee Department of Public Health, Nashville, Tennessee.

Pertinent Publications:

Radiation-Enhanced Oncogenesis by SV40. Proc. Soc. Exp. Biol. and Med., 134, 1109-1111. 1970. Coggin, J. H., Harwood, S. E. and Anderson, N. G.

Enhanced Virus Transformation of Hamster Embryo Cells in Vitro. J. Virology 3, 458-462, 1969. Coggin, J. H.

Radiation Responses of Embryonal and SV40 Transformed Hamster Cells in Culture. Proc. Soc. Exptl. Biol. Med. 129, 609-620. 1968. Kouri, R. E. and Coggin, J. H.

Prevention of SV40 Virus Tumorigenesis by Irradiated, Disrupted and 5-Idodeoxyuridine Treated Tumor Cell Antigen. Proc. Soc. Exptl. Biol. Med. 124, 774-784. 1967. Coggin, J. H., Larson, V. M. and Hilleman, M.R.

Immunologic Responses in Hamsters to Homologous Tumor Antigens Measured in Vitro and in Vivo. Proc. Soc. Exptl. Biol. Med., 124, 1295-1302, 1967. Coggin, J. H., Larson, V. M. and Hilleman, M. R.

SV40 Tumor Antigen Purification. Recent Results in Cancer Research. Malignant Transformation by Viruses VI, 142, 1966. Larson, V. M., Coggin, J. H., and Hilleman, M. R.

Metabolism of 5-Mercaptopurine by Resistant Escherichia coli. J. Bacteriology, 92, 446-454. 1966. Coggin, J. H. Loosemore, M., and Martin, W. R.

6-Diazo-5-OXO-L-norleucine Inhibition of Escherichia coli. J. Bacteriology, 89, 1348-1353. 1965. Coggin, J. H., and Martin, W. R.

Part A. Fetal Antigens.

Fetal Antigen Capable of Inducing Transplantation Immunity Against SV40 Hamster Tumor Cells. Journal of Immunology., Vol. 105. pp. 524-526. 1970. J. H. Coggin, K. R. Ambrose, N. G. Anderson.

1. Established that hamster and mouse fetus of early but not late gestation induced cytostatic antibody against SV40 tumor cells in a syngeneic model.
2. Established that X-irradiated embryo cells produced tumor resistance against SV40 tumors.

Tumor Immunity in Hamsters Immunized with Fetal Tissues. Journal of Immunology., Vol. 107, pp. 526-533. 1971. J. H. Coggin, K. R. Ambrose, B. B. Bellomy, N. G. Anderson.

1. Hamster and mouse fetus of early but not late gestation possessed antigen capable of interrupting SV40 and adenovirus 31 oncogenesis.
2. Irradiation of the fetal tissues was essential to maintain antigen expression when cells were injected into adult hosts.
3. Antibody (S) against surface antigens on SV40 tumors was found in normal pregnant hamsters during the course of the second and third pregnancy.
4. Antibody could not be detected several days post-partum.
5. First demonstration that female vaccinee developed humoral immunity to fetal antigens but failed to develop cellular immunity. SV40 oncogenesis was not interrupted in females (100% tumors) whereas only 40% tumors occurred in fetal primed males.

Immunization Against Tumors with Fetal Antigens. In Fetal and Embryonic Antigens in Cancer, I. pp. 185-202. 1971. J. H. Coggin, Jr., K. R. Ambrose, N. G. Anderson. N. G. Anderson and J. H. Coggin, Jr., ed. U. S. Dept. Commerce Springfield, Va.
A Review of first year's progress:

1. First demonstration that irradiated syngeneic hamster fetus could be used to induced humoral and cell mediated immunity to SV40 tumors.
2. First demonstration that fetal antigens present on viral transformed tumor cells elicited cell mediated immunity.
3. First demonstration that fetal antigens were phase specific.
4. First demonstration that mouse as well as hamster fetus possessed fetal antigen cross-reactive in inducing immunity to SV40 tumor challenge.
5. Discussion suggesting that SV40 induced TSTA might be a fetal antigen reexpressed or might be a distinct

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4. First demonstration that mouse as well as hamster fetus possessed fetal antigen cross-reactive in inducing immunity to SV40 tumor challenge.
5. Discussion suggesting that SV40 induced TSTA might be a fetal antigen reexpressed or might be a distinct

entity. Idea was presented that each type of tumor might possess one or several embryonic antigens.

Models of Differentiation, Retrogression, and Cancer. In Embryonic and Fetal Antigens in Cancer, I. pp. 7-38. 1971. N. G. Anderson and J. H. Coggin, Jr. N. G. Anderson and J. H. Coggin, Jr., ed. U. S. Dept. Commerce, Springfield, Va.

A model for carcinogen stimulated cellular transformation is proposed. Specific test parameters for establishing the involvement of retrogression in carcinogenesis are proposed. A proposed mechanism of escape from immunologic rejection employing fetal antigens was suggested. Molecular control mechanisms for dedifferentiated cell programming are proposed.

Interruption of SV40 Oncogenesis with Human Fetal Antigen. Nature. Vol. 233, pp. 194-195. 1971. K. R. Ambrose, N. G. Anderson, and J. H. Coggin, Jr.

1. Human fetal kidney cells (1st trimester) in primary culture were immunogenic against SV40 induced autchthonous tumors. Adult kidney cells and late fetal kidney tissue were not similiarly immunogenic.
2. These findings suggest that fetal antigens cross-reactive with tumor neoantigens are:
   a. phase-specific
   b. present on a variety of fetal types including human as well as rodent.
   c. support the proposition that ontogeny recapitulates phylogeny.

Concomitant and Sinecomitant Immunity to SV40 Tumors in Embryoma-Bearing Hamsters. In Embryonic and Fetal Antigens in Cancer, I. pp. 281-289. 1971. K. R. Ambrose, N. G. Anderson, and J. H. Coggin, Jr., ed. U. S. Dept. Commerce Springfield, Va.

1. Non-irradiated hamster fetal cells produce embryomas in syngeneic adult male and female hamsters.
2. The induction of an embryoma was dependent on the dose of live fetal cells injected.
3. Hamsters bearing embryomas showed modest but reproducible cell mediated immunity and were resistant to tumor challenge.

4. Surgically cured hamsters freed of embryomas showed strong resistance to SV40 tumor challenge.

Proceedings of the First Conference and Workshop on Embryonic and Fetal Antigens in Cancer, I. 1971. N. G. Anderson and J. H. Coggin, Jr., ed. U. S. Dept. Commerce, Springfield, Va.

An edited collection of 29 papers concerning the expression of embryonic antigens in cancer cells, the biological significance of fetal antigens in cancer and future course of exploitation of these cross-reactive antigens in cancer detection and monitoring. Included are edited workshop discussions which focused on the more intense problems relating to the role of fetal antigens in anti-tumor reactivity and the nature of these antigens.

Suppressive Effect of Immunization with Mouse Fetal Antigen on Growth of Cells Infected with Rauscher Virus and on Plasma-Cell Tumors. Proc. Nat'l Academy of Science. Vol. 68, pp. 1748-1952. 1971. M. G. Hanna, R. W. Tennant, and J. H. Coggin, Jr.

1. BALB/c mice primed with syngeneic irradiated fetal tissues were observed to possess antibody which suppressed the proliferation of Rauscher induced tumors, reduced splenamegaly and the mortality of mice challenged with lymphoma transplants.
2. Proliferation or function of normal stimulated spleen cells was not impaired by fetal immunizations.

Immunization Against Tumors with Fetal Antigens: Detection of Immunity by the Colony Inhibition Test and Adoptive Transfer. Embryonic and Fetal Antigens in Cancer, I. pp. 203-214. 1971. P. Dierlam, N. G. Anderson, and J. H. Coggin, Jr.

1. Studies using the colony inhibition procedure demonstrated that male hamsters immunized with syngeneic fetal tissues developed peripheral and lymph node effector cells which were cytotoxic against several types of tumor cells.
2. Effector cell cytotoxicity in fetal primed animals could also be shown by the adoptive or passive transfer test.
3. Irradiation of the fetal cells was essential to produce cytotoxic effector cells.
4. Females injected with fetal antigen (syngeneic) were not immunized so that they developed cytotoxic effector cells although antibody responses in females were observed.

Cyclic AMP Levels in the Developing Hamster Fetus: A Correlation with the Phasing of Fetal Antigen in Membrane Maturation. Differentiation. 1972. E. G. Rogan, M. P. Schafer, N. G. Anderson, and J. H. Coggin, Jr.

1. The parallel increase in cAMP coordinate with fetal antigen phasing in development was described.
2. The role of cAMP in relation to fetal antigen expression was discussed.
3. The control of development of the fetal plasma membrane was discussed and the retrogressive stimulus of carcinogens was proposed.

Embryonic Antigens in Virally Transformed Cells. Proceedings of Symposium on Membranes, Viruses, and Immune Mechanisms in Experimental and Clinical Disease. Acad. Press, N. Y., 1972. N. G. Anderson and J. H. Coggin, Jr.

1. Retrogressive stimulation by oncodnaviruses was proposed as an important consideration in viral oncogenesis.
2. Control mechanisms for virus induced regulation of membrane synthesis was described and the proposal that abortive infection with oncogenic viruses results in immature plasma membranes in transformed cells was considered.
3. A review of embryonic re-expression in cancer cells transformed by SV40 was given.

Phase Specific Surface Autoantigens on Membranes of Fetus and Tumors. Proceedings of Fourth International Conference on Lymphatic Tissue and Germinal Centers in Immune Reactions. 1972. J. H. Coggin, Jr., Plenum Press.

1. A detailed analysis of the phase specific properties of fetal antigens against some 5 model tumors was described.
2. In Vitro and In Vivo assay models are described.
3. The role of cell-free embryonic antigens in effecting tumor progression was proposed.
4. The effectiveness of fetal immunization in female vacciners was demonstrated following splenectomy.
5. Autosensitization to autochthonous "embryonic" antigen expression was described in oophorectomized females.

Prevention of SV40 Tumors by Hamster Fetal Tissue: The Influence of Parity Status of the Donor Female on Immunogenicity of Fetal Tissue and on Immune Cell Cytotoxicity. Proc. Nat'l Academy

of Science, U.S. 1972. A. J. Girardi, P. Reppucci, P. Dierlam, W. Rutula, and J. H. Coggin, Jr. In the press.

1. The immunogenicity of 10 day hamster fetus (syngeneic model) against autochthonous SV40 induced tumors was confirmed.
2. Fetus (10 day gestation) from primaparous females was immunogenic.
3. Fetus (10 day gestation) from multiparous females was not immunogenic.
4. Fetus from multiparous donor females could be rendered immunogenic by treatment with proteolytic enzymes which removed immunoglobulins. It was assumed that the adherent IgG to fetal surface antigens masked the antigenic sites of the fetal antigens and "neutralized" their antigenicity.
5. The phasing of fetal antigen between 10 and 14 days of gestation was confirmed in the SV40 neonate model. Fourteen day hamster fetus could be rendered immunogenic by exposure to proteolytic enzymes.

The Relationship of Sialic Acid to the Expression of Fetal Antigens in the Developing Hamster Fetus. International Journal of Cancer. 1972. W. H. Hannon, N. G. Anderson, and J. H. Coggin, Jr. In the press.

1. The precise time of fetal antigen phasing was defined to be between 10.5 days and 11.0 days of gestation employing in vitro and in vivo assays procedures.
2. A coordinate relationship between sialic acid deposition in the developing plasma membrane endoplasmic reticulum and the "masking" or phasing of embryonic antigen cross-reactive with SV40 and other tumor cell transplantation resistance antigens was observed.
3. Tumors and cultured, transformed cells had low levels of sialic acid similar to the low levels found in immunogenic fetal membranes.

Phase Specific Autoantigens (Fetal) in Model Tumor Systems. Embryonic and Fetal Antigens in Cancer, II. J. H. Coggin, Jr. and N. G. Anderson, ed. U. S. Dept. Commerce, Springfield, Va.

An updated summary of recent results relating to fetal antigen expression in some 15 tumor models examined to date including the hamster, rat, mouse, guinea pig, and human systems was presented. The proposed role of soluble fetal antigens in "blocking" cellular reactivity to tumor-neoantigens was proposed as was the specific blocking reactions observed in pregnant animals coordinate

with parity. The analogy between the cross-reactivity was described. The prospect that fetal antigens present on tumor cells and TSTAs are unique entities was considered.

The Specificity of Antisera from Burkitt Lymphoma and Infectious Mononucleosis Patients: Cross Reaction with Embryonic Antigens. Embryonic and Fetal Antigens in Cancer. pp. 203-218. 1971. W. W. Harris, B. Harrell, and N. G. Anderson.

Cells transformed by the Epstein-Barr virus were observed to react with sera prepared in a syngeneic rodent model against mid-gestation fetal tissues.
The proposal is made that both virion associated antigens as well as derepressed fetal antigens appear in Burkitt tumor cells.

Proceedings of the Second Conference and Workshop on Embryonic and Fetal Antigens in Cancer, II. 1972. N. G. Anderson and J. H. Coggin, Jr., ed., U. S. Dept. Commerce, Springfield, Va.

A collection of 47 edited papers presented at the second conference on embryonic and fetal antigens re-expressed in tumor cells. Many model systems were reported to possess embryonic or fetal antigen reexpressed on tumor cells. The nature of these antigens and the character of the immune response to the fetal antigens was discussed.

Part B. Tumor Immunology

Induction of Tumor-Specific Transplantation Immunity in Hamsters with Cell Fractions from Adenovirus and SV40 Tumor Cells. Proceedings of Society for Experimental Biology and Medicine. Vol. 132, pp. 328-336. 1969. J. H. Coggin, Jr., L. H. Elrod, K. T. Ambrose, and N. G. Anderson.

1. Tumor immunity was induced with the plasma membranes of SV40 and adenovirus tumor cells.
2. Crude homogenates (unseparated) of these tumors produced enhanced tumor growth even with the plasma membrane fraction present.
3. The results suggested that certain fractions or components of tumor cells could impair the cell mediated response of the vaccinee to tumor neotransplantation antigens possibly by stimulating blocking humoral responses.

A Rapid In Vivo Assay for SV40 Tumor Immunity in Hamsters. Proc. of Society for Experimental Biology and Medicine., Vol. 132 pp. 246-252. 1969. J. H. Coggin, Jr. and K. R. Ambrose.

1. Antibody was induced against SV40 tumors by immunization of adult hamsters with X-irradiated tumor cells or SV40 virus.
2. Diffusion chamber studies revealed that the antibody was directed against neoantigens at the tumor cell surface.
3. Antibody was rarely cytotoxic to the tumor cells in chambers but was reproducibly cytostatic (growth inhibitory).
4. Evidence for specificity was given for the type of tumor employed.
5. Normal tissues nor heterologous tissues failed to elicit the antibody.
6. Immunofluoresence studies showed IgG to be present at the surface of inhibited target cells. Immunosuppression of IgG synthesis prevented the development of C-antibody induction.

Characterization of Tumor-Specific Transplantation Immunity Reactions in Immunodiffusion Chambers In Vivo. Proc. of Society for Experimental Biology and Medicine. Vol. 132, pp. 1013-1020. 1969. K. R. Ambrose, E. L. Candler, and J. H. Coggin, Jr.

1. The specificity of induction of cytostatic antibody by a given TSTA among different oncodnavirus tumor cells was described.
2. The antibody nature of the cytostatic factor was documented.
3. Statistical signifances of the level of detection of the antibodies are described.
4. C-antibody induction in several tumor systems was described.

Cytostatic Antibody and SV40 Tumor Immunity in Hamsters. Nature., Vol. 233, pp. 321-327. 1971. K. R. Ambrose, N. G. Anderson, and J. H. Coggin, Jr.

1. Cytostatic antibody presence and disappearance was equated with the appearance of SV40 induced tumors in neonatally infected animals. Antibody was present during the latent period in viral tumorigenesis but disappeared from circulation as the tumor mass became detectable. Similar results were obtained in the live cell challenge system.
2. Surgical removal of the tumor resulted in the reinstatement of detectable circulating levels of the antigen in surgically cured animals whereas

surgical failure never led to reappearance of antibody.

2. Surgical removal of the tumor resulted in the reinstatement of detectable circulating levels of the antigen in surgically cured animals whereas surgical failure never led to reappearance of antibody.
3. The role of C-antibody in tumor progression is proposed.
4. The early induction of transformed cells in SV40 infected neonates is described for the in situ tumor focus.

Associate Investigators:

E. R. Rogan, Ph. D., Research Associate. 50% time on project. Supported 50% by National Cancer Institute Contract FS-7 effective April 1973. A.E.C. funding $6000. is badly needed to permit Dr. Rogan's continued work on the A.E.C. Program.
M. Schafer, M. S., Laboratory Technician. Supported by National Cancer Institute Contract FS-7. 100% time on project.

S. G. Winslow, M. S. Graduate Student. 50% time on project.

C. Babelay, B. S. Research Assistant. 50% time on project.

7. Other Personnel:

1 Laboratory Aide - Mrs. Cindy Smith - 50% time
1 Glassware Washer - Miss Sarah Frazer - 20% time
1 Animal Caretaker - Mr. Steve Pershing - 25% time

8. Other Financial Assistance

A.E.C. alone sponsors our study of virus transformation described here. We cannot over emphasize the importance of continued A.E.C. support at this critical phase of our research. NCI support listed below cannot be used to fund basic research as described here. The above personnel are permitted to assist in this research only if this A.E.C. contract is in force as a collaborative venture.

A new contract with Union Carbide (MAN Program) under subcontract No. 3379 to study "Immune Reactions in Virud-Induced Tumor Rejection" has been in progress since July 1970 and is funded at the present level of $106,000 per year. This program is sponsored by The National Cancer Institute as a segment of contract FS-7. The objectives of this research are to develop immunotherapeutic techniques for controlling human cancer. N.C.I. funds cannot be used, to perform the sialic acid or cAMP work or the hybridization studies since they are not directly mission oriented projects.

The University provides $1200 in supplies and services to the A.E.C. project.

K. T. Ambrose devotes 10% time to the project in cell preparation procedures and is sponsored by subcontract 3379.

9. Premises, Facilities, Equipment and Materials

These remain essentially the same as in previous years. Our research facilities have recently been expanded from 7,500 square feet to 15,500 square feet. Of this newly expanded area two new rooms have been added to virologic research increasing the total research area to 5000 square feet exclusive of animal facilities. Some $30,000. have been expended in the past 24 months to generally upgrade our research area providing spectrophotometers, wash area facilities and animal room improvements. Additionally, a new transfer hood work station and special equipment for hybridization

studies were obtained. An electron microscope (RCA-EMU-4) has recently been given to me for the conduct of reserach in my laboratory from the National Institute of Allergy and Infectious Disease. Additionally, the MAN Program recently provided me with $15,000. in special equipment to make the hybridization studies feasible.

10. COST ESTIMATE

BUDGET

April 1, 1973 to March 31, 1974

1. SALARIES AND WAGES:		
One Research Associate-E.G. Rogan 50% time		$6,000.00
One Laboratory Aide-100% time		3,700.00
Two Graduate Assistants-50% time at $3500.00 each*		7,000.00
Student Assistants*		1,000.00
Subtotal S & W		17,700.00
Fringe Benefits		1,370.00
Overhead-Indirect Costs	Apr. 1, 1973-June 30, 1974 61.4% S & W	2,716.95
	July 1, 1973-March 31, 1974 64.3% S & W	8,535.83
		30,322.78

* Part-Time - only 5% fringe benefits asked for Social Security

2. SUPPLIES AND MATERIALS:	
Chemicals	$1,000.00
Isotopes	1,000.00
Animals	500.00
Media and Sera	800.00
Expendables (Plastic Ware)	1,500.00
Subtotal: Supplies and Materials	$4,800.00
3. EQUIPMENT:	-0-
4. PUBLICATION COSTS:	$ 300.00
5. TRAVEL:	$ 500.00
6. OTHER: Animal Maintenance Costs	$2,800.00
Service Contract on Scintillation System	650.00
Request A.E.C. contribution for continued AT(40-1)3646	$39,372.78

University Contribution:

As in the past five contracts on AT(40-1)3646 the University proposes the released time of the Principle Investigator during the academic year so that 33% of his effort can be devoted to this research program. This contribution of time and the usual $1,200.00 in services constitute a substantial cost sharing by the University of Tennessee toward the continuation of AT(40-1)3646.

The estimated project costs for 1974 and 1975 would be the above amount plus a 5% cost increase.

11. Authentication

Principal Investigator

Joseph H. Coggin, Jr., Ph.D.
Associate Professor

For the University

Hilton A. Smith, Ph.D.
Vice Chancellor for Graduate
Studies and Research

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