



UNITED STATES  
ATOMIC ENERGY COMMISSION

OAK RIDGE OPERATIONS  
P.O. BOX E  
OAK RIDGE, TENNESSEE 37830 707789

594

(R)

AREA CODE 615  
TELEPHONE 483-8611

July 19, 1974

Charles W. Hill, Chief Counsel

REQUEST FOR CONTRACT ACTION

It is requested that you take the necessary steps to process the following described contract action (CA):

REPOSITORY Oak Ridge Operations  
COLLECTION Records Bldg. Area  
BOX NO. A-59-3 Bldg. 2714-H  
FOLDER cont. 3646 CA  
Univ. of Tenn. 7-19-74

1. Nature of Action Requested:

Selection of New Contractor and/or Negotiation of Contract  
Number:  
Contractor:

Modification of Contract  
Number: AT-(40-1)-3646  
Contractor: University of Tennessee

2. Nature of Services To Be Covered by Contract: Research

Title: "A Comparative Study of Radiation, Chemical and Aging Effects on Viral Transformation"

3. Type of Contract:

Support Agreement  Cost Type  Other

4. Amount of AEC Funds To Be Obligated by this CA: \$32,102

5. AEC Percentage of Est. Total Cost To Be Shown by this CA: 100%

6. Description of Other Changes To Be Covered by this CA: Modify contract to provide for the performance of additional research during the period July 1, 1974 through June 30, 1975. AEC Support Ceiling will be increased from \$113,042 to \$145,144. Title to the equipment, if any, shall vest in the Contractor under authority of Public Law 85-934.

7. Authority:

Form AEC-481 (CA) from  
J. L. Liverman, DBER, HQ,  
dated July 16, 1974.

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

ACR: LM

cc: Alice Brown  
File ✓  
Pink  
Reading

F 5002  
F 5042

F 5002

CONTRACTS - 3646 (2enn)  
C.A.

RCP&R BR  
MEDLEY:ejb  
8/19/74

1033976

U. S. ATOMIC ENERGY COMMISSION  
CONTRACT AUTHORIZATION

1. DATE

JUL 16 1974

2. AUTHORIZATION NO.

BER-75-39

3.A. TO

Robert J. Hart, Manager  
Oak Ridge Operations Office

3.B. FROM

James L. Liverman, Director  
Division of Biomedical and  
Environmental Research

4.A. CONTRACTOR (Name, Address, Department, etc.)

UNIVERSITY OF TENNESSEE  
Knoxville, Tennessee 37916  
Department of Microbiology

4.B. PRINCIPAL INVESTIGATOR(S)

JOSEPH H. COGGIN, Jr.

5.

NEW CONTRACT  RENEWAL  OTHER

6. TERM OF CONTRACT

7-1-74 thru 6-30-75

7. CONTRACT NUMBER

AT(40-1)3646

8. RECOMMENDED TYPE OF CONTRACT:

SPECIAL RESEARCH SUPPORT AGREEMENT (SRSA)  
 COST REIMBURSEMENT  
 FIXED PRICE  OTHER

9. PROPERTY TITLE TO VEST IN:

AEC  
 CONTRACTOR-DETERMINED  
TO BE IN THE PROGRAMMATIC  
INTEREST OF THE AEC

10. SECURITY CLASSIFICATION:

Work to be performed is under category I  
as defined by AEC Manual Appendix 3401.

11. PROJECT TITLE

A COMPARATIVE STUDY OF RADIATION, CHEMICAL AND AGING EFFECTS ON VIRAL TRANSFORMATION

12. HEADQUARTERS TECHNICAL CONTACT

George E. Stapleton

13. FINANCING

A. OPERATING EXPENSES

New AEC Funds .....	\$ 32,102
Estimated AEC Balance From Prior Term, if any .....	\$
Total Estimated AEC Support Cost for New Term .....	\$ 32,102
Estimated Contractor Contribution, On Proportionate Sharing Basis, if any .....	\$
Estimated Project Cost, For Pertinent Budget Period .....	\$ 32,102

Budget and Reporting Classification: EX 03 03  
Allotment Transfer: 06-51-91 (24)

B. PLANT AND CAPITAL EQUIPMENT .....

Budget and Reporting Classification:  
Allotment Transfer:

14. SPECIAL PROVISIONS AND INSTRUCTIONS:

The technical aspects of the proposed work have been reviewed and are approved. A need currently exists for the results of the research or other work that is to be undertaken. None of the AEC funds shall be used to confer a fellowship.  
Please keep us informed as to any problems encountered in your negotiations, as well as the date of execution of this contract and the amount of funds obligated. If the budget as negotiated differs substantially from that in the proposal, please forward a copy of the revised budget to Headquarters.  
If not already submitted, a 200-word summary of the proposed work should be forwarded by the contractor as soon as possible after negotiation of the contract.

15. SCOPE OF WORK

An investigation of the molecular events involved in radiation enhancement of viral oncogenesis in mammals and mammalian cells.

F 4016

CONTRACTS-3646 (Link) JUL 18 1974  
C.A.

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JUL 1 6 1974

Dr. Joseph H. Coggin, Jr.  
Department of Microbiology  
University of Tennessee  
Knoxville, Tennessee 37916

Dear Dr. Coggin:

I wish to advise you that the Research Committee has approved renewal of your Research Contract No. AT(40-1)3646, "A Comparative Study of Radiation, Chemical and Aging Effects on Viral Transformation," for an additional year at the level of research support requested.

You will be contacted by someone from the Oak Ridge Operations Office in the near future regarding negotiation of the renewal contract.

Sincerely,

George E. Stapleton  
Radiation Biologist  
Biomedical Programs  
Division of Biomedical and  
Environmental Research

cc: Hilton A. Smith  
Vice Chancellor for Graduate  
Studies and Research

bcc: Oak Ridge Operations Office

F 4046

JUL 1 8 1974

CONTRACTS-3646 (2200)  
C.A.

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APPENDIX "A"

CONTRACTOR: UNIVERSITY OF TENNESSEE  
CONTRACT NO.: AT-(40-1)-3646

For the contract period: July 1, 1974 through June 30, 1975

ARTICLE A-I. RESEARCH TO BE PERFORMED BY CONTRACTOR

The Contractor will conduct an investigation of the molecular events involved in radiation enhancement of viral oncogenesis in mammals and mammalian cells.

The Principal Investigator, Dr. J. H. Coggin, Jr., expects to devote approximately 15% of his time or effort to the project.

ARTICLE A-II. WAYS AND MEANS OF PERFORMANCE

(a) Items included in total estimated cost:

- |  |          |
|--|----------|
| (1) Salaries and wages:  | \$ 6,637 |
| (2) Equipment to be purchased or fabricated by the Contractor: | \$ 3,760 |

a Equipment estimated to cost less than \$1,000:

Animal Holding Equipment

b Equipment estimated to cost in excess of \$1,000:

None

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CONTRACTOR: UNIVERSITY OF TENNESSEE  
CONTRACT NO.: AT-(40-1)-3646

(3) Travel: \$ 1,000  
Domestic-----\$ 1,000  
Foreign-----\$ 0  
(4) Other direct costs: \$16,437  
(5) Indirect costs (based on a predetermined  
rate of 64.3 % of salaries and wages): \$ 4,268

(b) Items, if any, significant to the performance  
of this contract, but excluded from computation  
of support cost and from consideration in  
proportioning costs:

(1) Items to be contributed by the Contractor:

None

(2) Items to be contributed by the Government:

None

(c) Time or effort of Principal Investigator(s)  
contributed by Contractor, but excluded from  
computation of support cost and from consider-  
ation in proportioning costs:

All costs including salary, fringe benefits and  
related overhead.

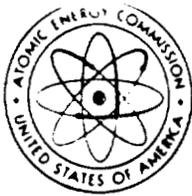
ARTICLE A-III. The total estimated cost of items under A-II (a) above  
for the contract period stated in this Appendix "A" is  
\$ 32,102; the Commission will pay 100 % of the  
actual costs of these items incurred during the contract  
period stated in this Appendix "A", subject to the  
provisions of Article III and Article B-XXIX. The es-  
timated AEC Support Cost for the contract period stated  
in this Appendix "A" is \$ 32,102.

CONTRACTOR: UNIVERSITY OF TENNESSEE  
CONTRACT NO.: AT-(40-1)-3646

The estimated AEC Support Cost is funded as follows:

(a) Estimated unexpended balance from the prior period(s):	\$ 0
(b) New funds for the current period:	\$32,102

The new funds being added in A-III (b) constitute the basis for advance payments provided under Article B-XI.



UNITED STATES  
ATOMIC ENERGY COMMISSION

OAK RIDGE OPERATIONS  
P.O. BOX E  
OAK RIDGE, TENNESSEE 37830

AREA CODE 615  
TELEPHONE 483-5000

April 4, 1974

J. L. Liverman, Director, Division of Biomedical & Environmental Research, HQ

RENEWAL OF CONTRACT NO. AT-(40-1)- 3646 - THE UNIVERSITY OF TENNESSEE

We are submitting for your review and appropriate action the following information concerning the contract which will expire on 6/30/74.

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

ACRIM

Enclosures:  
As stated above

RCP&R BR  
MEDLEY:ejb  
4-4-74

bcc: D. S. Zachry, w/Prog Rpt (2) & Form 427  
Tickler, w/Ren Prop & Fin Stmt  
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CONTRACTS-3646 (Jenn)  
C.A.

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PUBLICATION  
BY AEC  
AUTHORIZED

NOTICE OF RESEARCH PROJECT  
SCIENCE INFORMATION EXCHANGE

SMITHSONIAN INSTITUTION

U.S. ATOMIC ENERGY COMMISSION

SIE NO.

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., Principal Investigator - 1.5 M.Y.

NO. OF GRADUATE STUDENTS ON PROJECT: 2 NO. OF GRADUATE STUDENT MAN-YEARS: 1

SUMMARY OF PROPOSED WORK: (200-300 words, omit Confidential Data). Summaries are exchanged with government and private agencies supporting research, are supplied to investigators 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 contract is aimed at disclosing how low-level (50-150R) X-ray exposure or exposure to radiomimetic chemicals potentiate SV40 oncogenesis *in vitro* and *in vivo*. The key to this work is the description of the molecular and biological mechanism(s) by which oncogenic viruses trigger retrogressive changes in adult target cells leading to immature, fetal-like plasma membranes in the transformed cells. These studies focus on the role of sublethal irradiation in stimulating virus induced cancer. Immunologic assays of embryonic antigen expression are being used to detect the earliest membrane manifestation of neoplastic change. Biochemical assays of changes in sialoglycoprotein distribution are being correlated with immunologic changes in radiation sensitized cells undergoing SV40 induced transformation. Studies are conducted in hamsters.

RESULTS TO DATE: Available data indicate that radiation induced trauma to the cellular DNA potentiates the integration of the viral genome into target cells. Following integration, viral regulatory activities suppress normal patterns of host membrane synthesis and cause the reexpression of cryptic embryonic antigens.

	PROGRAM CATEGORY NO.
BUDGET	
PRIMARY	
SECONDARY	

Signature of Principal Investigator

DATE: April 1, 1974

INVESTIGATOR - DO NOT USE THIS SPACE

1033983

Financial Statement

April 2, 1974

AT-(40-1)3646

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

(1) Total actual project cost to date for the current period	<u>\$19, 444</u>
(2) Estimated total cost for remainder of period	<u>\$16, 884</u>
(3) Total actual and estimated cost chargeable to AEC for current period based on percentage of cost agreed upon as contained in A-111 of Appendix "A" to Contract	<u>\$36, 328</u>
(4) Accumulated costs chargeable to AEC (include costs reported in certified statement for preceding period(s) and the costs stated in Item (3) above	<u>\$113, 042</u>
(5) Accumulated AEC Support Ceiling as stated in Article III of Contract	<u>\$113, 042</u>
(6) Total estimated AEC funds remaining under Contract (subcontract Item (4) from (5) which may be used to reduce amount of new funds required from AEC for proposed renewal period	<u>\$ None</u>

 4/2/74

Mr. Roy A. Dean, Administrative Assistant

1033984

Renewal Proposal

Eighth Year

Contract

AT(40-1)3646

The University of Tennessee  
Department of Microbiology  
Knoxville, Tennessee 37916

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

Principal Investigator  
Professor of Microbiology

April 1, 1974

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CONTRACTS - 3646 (Jenn)<sup>F</sup> 1886  
O.A.

1. *Title of Project*

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

2. *Institution*

The University of Tennessee  
Department of Microbiology  
Knoxville, Tennessee 37916

Telephone: 615-974-2356

### 3. Project Abstract.

The current focus of this proposal is to continue ongoing efforts to discover how tumor viruses regulate and control the expression of embryo-associated cellular products as an integral activity in transforming normal cells. These studies are feasible because exposure of normal target cells to sublethal X-irradiation greatly sensitizes the cells to viral oncogenesis. We have observed that coordinate with the induction of neoplastic characteristics in viral transformed cells, embryonic moieties (EA) appear in the plasma membrane of the tumor cells and act as autoantigens. Radiation (25 to 350R) alone does not cause transformation of normal hamster or human cells *in vitro*, however, these levels of exposure do promote 50 to 1000-fold increases in viral oncogenesis induced by oncoviruses. Under support from this contract we have successfully demonstrated that early embryo tissue of the hamster, mouse, and human possess antigens (EA) which are identical to those occurring on viral transformed cells. During the terminal stages of embryonic or fetal development *in vivo* these antigenic entities are masked by biochemical processes associated with membrane maturation and subsequently reside cryptically, insofar as we can tell, in adult cell membrane. Viral oncogenesis serves to cause defective biosynthesis and results in the reexpression of EA. The central problem in our current work is to discover (1) whether we can successfully use embryonic markers expressed at the cell surface to reliably detect the earliest stages of viral induced neoplastic traits in the tumor cells, (2) to determine the biochemical events related to membrane maturation involved in the control and expression of EA in fetal development and in cancer induction and , (3) to determine how low-level radiation serves to potentiate this virus-host cell interaction. If we are successful in developing a reliable assay for fetal marker expression (EA) associated with the very early events in neoplasia, a valuable tool for detecting radiation potentiated oncogenesis will be available for understanding and analyzing the cancer induction.

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#### 4. Scientific Background.

##### A. Historical Survey of This Contract.

This project was initiated eight years ago in an effort to discover whether exposure to sublethal levels of radiation, pyrimidine analogues, or cell "aging" processes *in vitro* served to potentiate oncovirus oncogenesis. This program requires a brief historical review and accounting. Since the initiation of this work we have reported that radiation and radiomimetic chemicals markedly potentiate viral oncogenesis in primary cell culture systems *in vitro* and in animals treated locally with X-ray before infection with tumor viruses. Recall that in this work the target cells, not the transforming virus, are presensitized by irradiation. In the time since these initial reports, research on this project has settled into a study of the molecular mechanisms by which "low-level" radiation of normal target cells leads to increased viral oncogenesis *in vitro*.

More recently our efforts were focused on determining whether X-irradiation served to potentiate SV40 oncogenesis by inducing "break and repair" reactions which facilitated the integration of the transforming virus genome into cellular DNA. DNA:DNA hybridization techniques were adapted and developed to determine the relationship between radiation dose (used to presensitize the cells prior to infection) and the number of viral genome equivalents incorporated into a given tumor clone. The results of these experiments have been published with the general conclusion that an absolute linear relationship did not exist between increasing radiation doses and increased number of viral equivalents present in a tumor clone; however a general increase in the number of viral equivalents was found covalently-linked in association with transformed cell DNA in radiation presensitized cells at exposure levels exceeding 100R when compared to unirradiated, SV40 transformed clones. Briefly stated, X-ray pretreatment of target cells seemed to cause a greater *number* of infected target cells to "accept" and incorporate an SV40 genome leading to stable transformation.

Three years ago, as a "spin-off" from this project, we discovered that embryonic antigens were almost universally expressed in virally and chemically induced tumor cells. This new observation from rodent model system studies was quickly recognized to relate to human cancer since studies in many laboratories now show quite unequivocally that human cancers carry histologically related, cross-reacting antigens. Many of these antigenic determinants are recognized to be embryonic in character and the possibility stands that all are embryonic antigens (EA). Norman Anderson at ORNL and the principal investigator of this contract (J.H.C.) have reviewed the central problems associated with retrogenic expression in neoplasia this past year and reprints or preprints of several reviews\* are included in the 1974 Progress Report for your perusal.

Last year, in our proposal for an additional three years support from the A.E.C. to study the relationship of radiation damage to viral oncogenesis and retrogenesis in neoplasia, we outlined a series of new objectives aimed at discovering the interrelatedness of EA expression with radiation-stimulated oncogenesis. Our central theme was then and continues to be to develop immunologic and molecular tools for discerning the *earliest events* in viral oncogenesis, potentiated by radiation, which can be used as "markers" of neoplastic conversion leading to the malignant state. We have worked hard these past 12 months to

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\*Cancer, Differentiation, and Embryonic Antigens:Some Central Problems. *Adv. in Cancer Res.*, 19:105, 1974.

Proposed Mechanisms by Which Autochthonous Neoplasms Escape Immune Rejection. *Cancer Res.*, 1974. (In press.)

An Evaluation of the Isotopic Antiglobulin Assay and the Cytostatic Assay for Detecting SV40 Tumor Immunity. *Immunological Parameters of Host-Tumor Relationships.* June, 1974. (In press).

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develop reliable assays for embryonic antigens and we feel that we are on the right track and that our ideas were correct and experimentally testable. Funds made available for this work through the A.E.C. support only graduate student research.

Specifically, we have developed both a radioimmunoassay procedure for the detection of small quantities of embryonic antigen and a quantitative immunofluorescence assay for detecting the very early expression of EA at the surface of "normal" cells undergoing transformation *in vitro*. The studies are made experimentally feasible because low-level sublethal X-irradiation presensitized cells to oncogenesis by oncovirus and yields large populations of transformed cells *in vitro* in a given test population. Simultaneously, under support from this contract, we have been examining the biochemical events associated with the expression and regulation of embryonic antigens (EA) in the plasma membrane of the developing fetus (hamster and mouse). Biochemical studies have, so far, supported the concept that EA expression in the fetal cell membrane is silenced at the 11th day of gestation because of maturation processes associated "adult" membrane formation. EA's are now known to be present on unfertilized eggs *in situ* in the ovary and EA expression continues through gamete fusion and the first ten days of gestational development.\* Thus we will remain with our research plan, submitted in detail last year, seeking to delineate specific biochemical and regulatory changes associated with the reexpression of cryptic EA residing in adult cell membrane as an essential function in cellular transformation.

#### B. Relationship of This Study to Other Work in Viral Oncogenesis.

Papovaviruses are the best characterized tumor viruses particularly when we consider the wealth of information now available regarding the molecular

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\*Cancer, Differentiation, and Embryonic Antigens: Some Central Problems. *Adv. in Cancer Res.*, 19:105, 1974.

interactions between these viruses and their transformed cell hosts. These viruses have been particularly amenable to study because they possess a small DNA genome which has a limited gene complement and many of the unique viral coded proteins have been identified. Viral genetic information responsible for tumor induction seems restricted to one or two genes. Intranuclear tumor or T antigen is now recognized to be coded for from the viral genome since T antigen expression occurs in cells lytically infected with SV40 and only mutations in the viral genome effect the synthesis of this antigen (see Robb, J. A., *J. Virol.*, 12:1187, 1973). Recent reports have shown that at least *one cellular gene* is required for maintaining some manifestation of the transformed state. These characteristics of transformation include atypical cellular DNA synthesis, excess cell saturation density, lack of growth factor requirements, and agglutinin binding receptors on tumor cells. Thus, at least one viral gene and one cellular gene are essential for regulating the *induction* of transformation. In the work we are doing we seek to add a quantitative parameter to indicate transformation, specifically by developing a reliable assay for EA expression using objective assays. Portugal and his colleagues (*J. Virol.*, 12:1616, 1973) recently showed that a number of new isoaccepting tRNA species (leucyl-, isoleucyl-, phenylalanyl-, and threonyl- tRNA's) thought originally to be associated with viral gene activity are now recognized to be *identical* to tRNA alterations accompanying *differentiation* of developing normal cells.

Renger and Basicilco reported this past May (*J. Virol.*, 11:702, 1973) that *cellular* rather than viral genes are responsible for the *maintenance* of the neoplastic state in SV40 transformed cells. These data were obtained from elegant studies using SV40 transformed cell lines which were temperature sensitive, exhibiting neoplastic traits at 32° but losing these characteristics at 39°C. Infection of these SV40 transformed cells with *wild-type* SV40 (non-ts mutants) does not alter this control pattern suggesting clearly that cellular rather than

viral genes are ultimately responsible for maintaining the transformed state.

Our observation that tumor rejection antigens present on SV40 tumor cells were embryonic in character rather than viral specific has been confirmed in many tumor systems in the past 24 months (Table 1). These data too fit the idea that a major action of oncogenic viruses is to actuate regulatory shifts in cells which cause the plasma membranes to revert to an immature form.

In summary the mounting evidence suggests that oncogenic viruses cause neoplasia by becoming heritably associated with the cellular genome and by producing some key regulatory substances which causes the cell to undergo dedifferentiation processes. Many of the neoplastic characteristics which are now associated with tumor cells are related to cell-coded activities whose reexpression is under viral regulation.

## Reported Observations of Induction of Tumor Immunity Following

## Direct Immunization with Fetal Tissue.

System	Tumor Immunity Induced Against	Fetal Vaccine Effective in Males	Females	Route of Vaccination	Direct challenge	Bendich, et al., (1973)
Mouse	QUA	N.D.*	+	S.C.	Direct challenge	Bendich, et al., (1973)
Mouse	MCA-10	N.D.	+	?	Direct challenge, serum cytotoxicity with <sup>125</sup> I Iododeoxyuridine	LeMevel and Well (1973)
Mouse	MCA-10	+	+	I.P.	Cell challenge	Castro, et al., (1974)
Mouse	PCT	+	N.D.*	I.P.	Spleen colony assay (FCFU)	F.A. Salinas, et (1972)
Rat	MCA-R	+	+	?	Direct challenge	Grant, et al., (1973)
Guinea pig	MCA-A, MCA-25	+	+	I.D. (+CFA) I.P.	DTHR's Direct challenge	Grant, et al., (1973)
Hamster	SV40	+	-	I.P.	Interrupt SV40 Oncogenesis	Girardi, et al., (1973)

REFERENCES: Bendich, A., Borenfreund, E., and Stonehill, E. H., *J. Immunol.*, 111:285, 1973.  
 Castro, J.E., Hunt, R., Lance, E.M., Medawar, P.B., and Zanelli, J., *Can. Res.*, In press, 1974.  
 Grant, J., Ladisch, S., and Wells, S.A., *Cancer*, 33:376, 1974.  
 Grant, J. and Wells, S.A., *J. of Surgical Res.*, In press, 1973.  
 LeMevel, B.P. and Wells, S.A., *Nature, New Biol.*, 244:183, 1973.  
 Salinas, F.A., Smith, Jane A., and Hanna, M.G., *Nature*, 240:41, 1973.  
 Girardi, A.J., Reppucci, P., Dierlam, P., Rutala, W., and Coggin, J.H., *Proc. Nat. Acad. Sci.*, 70:183, 1973.

N.D. = Not done. I.D. (CFA) = Intradermal injection (complete Freund's adjuvant).

S.C. = Subcutaneous injection. FCFU = Fetal colony forming unit.

I.P. = Intraperitoneal injection. DTHR's = Delayed type hypersensitivity reactions.

## 5. *Scientific Scope.*

### A. Overall Objectives.

(1) Characterize the biochemical events leading to the production of immature plasma membranes exposing embryonic antigen in radiation-stimulated SV40 oncogenesis.

(2) Evaluate the early events of neoplastic transformation using embryonic antigen markers as indices of virus stimulated retrogenesis.

The current research effort is following closely the workscope presented last year. We have achieved several of the proposed objectives in the past 12 months and have narrowed our focus to *two project areas* which now seem most promising. We have evaluated several assays for quantitating the presence of embryonic antigens at the surface of tumor cells. The results, currently being published\*, showed that the radioimmunoassay for cell surface antigen (IAT method) measured cross-reacting (EA) antigens rather than tumor specific transplantation antigen (Burdick *et al.*, *Int. J. Cancer*, 12:474, 1973) and this assay is most suitable for our purpose in detecting the early expression of EA at the surface of cells undergoing SV40 transformation.

We reviewed the historical and theoretical basis for these biochemical and immunological studies last year in the large proposal. Hopefully it will be sufficient to say here that the overall objective is to correlate biochemical changes in plasma membrane induced by SV40 in normal adult cells with the development of cell surface antigens of the embryonic type and the onset of the transformed state.

### B. Specific Projects.

(1). Biochemical Studies. (see page 19-23, 1973 proposal for protocols and details)

\*An Evaluation of the Isotopic Antiglobulin Assay and the Cytostatic Assay for Detecting SV40 Tumor Immunity. *In: Immunological Parameters of Host-Tumor Relationships.* June, 1974. (Ed.) David Weiss, Academic Press, New York. (In press).

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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 (150R 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 *vass culture*. We are able to proceed with the following specific studies:

- a. 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.
- b. Employing purification procedures developed in our laboratory fetal, phase-specific mRNA's will be isolated and tested for their identity to "tumor-specific" mRNA. The competitive hybridization procedure is being used.
- c. The role of cyclic AMP concentration, synthesis and degradation in retrogenesis induced by SV40 virus will be investigated.
- d. 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 more suitable for evaluating subtle changes in fetal development which radiation exposure might introduce.

(2) Immunological Studies.

These studies are ongoing and are being conducted with the basic format described last year with certain specific revisions. We have developed a suitable

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quantitative system for detecting extremely low quantities of EA at the surface of tumor cells during transformation using the isotopic antiglobulin test (IAT), a modified radioimmunoassay.

*IAT Procedure:*

The isotopic antiglobulin assay (Harder and McKhann, 1968) as modified for use with monolayer tissue culture cells has been previously described (Burdick *et al.*, 1973). Target, 14 day fetal cell suspensions (EA<sup>-</sup> cells) with or without irradiation and infection were plated one day before use in the individual wells of Microtest-II plates (Falcon Plastics, Los Angeles, Calif., USA) in RPMI 1640 plus 20% FCS, and for the test, the adherent monolayers were incubated for 30 min, appropriately diluted, washed five times, then incubated for 30 min with anti-globulin diluted in RPMI ± 10% FCS and then washed six times. The wells were sprayed with Aeroplast (Parke-Davis and Co., Detroit, Mich., USA), cut apart on a bandsaw and counted in Packard Auto-Gamma counter. Antiglobulin controls (AGC) were performed by adding medium + 10% FCS for the first incubation and antiglobulin for the second. Results were expressed as counts per min (CPM) and as:  
the absorption ratio =

$$AR = \frac{\text{CPM in wells with Immune or Pregnant Serum}}{\text{CPM in wells with Control Serum}}$$

There are some biological variables inherent to the tissue culture techniques employed which cannot be totally controlled. Therefore, the results are not exactly quantitatively reproducible from day to day, and inferences of exact amounts of globulin bound to the cell would be unreliable (Burdick *et al.*, 1973) in comparing percentage values from one day's results with those of another run. Comparison of the AR between different radiation doses and virus concentrations will provide a ranking, but quantitative comparison of the antigenic content of various cell lines requires parallel absorptions of the same serum run on the same day.

### *Absorption*

Absorption of sera prior to use in the isotopic antiglobulin assay was performed in a standard fashion (Ting and Herberman, 1971). Cells to be used for absorption were removed from tissue culture flasks by trypsinization (0.25% trypsin - 0.1 EDTA), suspended and maintained overnight in spinner culture flasks. Aliquots containing the indicated number of cells were centrifuged at 800 x g for 10 min, the supernatant was aspirated, and 0.2 ml of the serum to be absorbed was added. After incubation for 60 min at 37°C and 5% CO<sub>2</sub> with frequent agitation, the samples were centrifuged first at 800 x g then the supernatant was aspirated and centrifuged at 2,000 x g for 10 min and this supernatant was employed in the isotopic antiglobulin test. Single-cell suspensions of various fetuses of 10-15 days gestation are prepared by extensive mincing, then used for absorption as described above.

### *Serum fractionation and iodination*

Fractionation of an anti-hamster gamma globulin (AG), was performed by ammonium sulfate precipitation, and then separation on G-200 Sephadex, using 0.2M Tris-NaCl-HCl buffer at pH 8.0. Aliquots containing 1 mg of the 7S fraction of the antiglobulin were labeled with <sup>125</sup>I (New England Nuclear, Boston, Mass., USA) by the modified chloramine-T method (McConahev and Dickson, 1966). The molar iodine: gamma-globulin ratio was always below 0.25:1. The 7S fraction was suspended in RPMI 1640 with 10% FCS and tested in the isotopic antiglobulin assay as described above.

Sera for detecting EA are derived from pregnant, multiparous, inbred hamsters primed on 5 occasions with 10 day gestation hamster embryo cells.

### Experimental Design:

Fourteen day fetal cells (EA<sup>-</sup>) 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

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antiserum is added following virus adsorption. Samples plates are washed at selected times post-infection (24, 28, 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  $^{125}\text{I}$ -tagged anti-hamster IgG is added, incubated with target cells and washed. The wells are collected as described and scored. Immuno-fluorescence studies run in parallel are being conducted as described last year. 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 IAT results and the fluorescent labeling procedure indicate 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.

Titration of radiation dose, cell target levels transformation frequency and other parameters are nearly completed. The ultimate application of this approach is to determine how early EA expression is noted and how the quantitative distribution of EA correlates with transformation frequency in the sample population. Biochemical changes in the transforming cell population (sialic acid synthesis, eg.) will subsequently be correlated with the expression of EA.

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6. Scientific Personnel.

<u>NAME</u>	<u>TITLE</u>	<u>BIRTHDATE (Mo., Day, Yr.)</u>
Coggin, J. H., Jr.	Professor	[REDACTED]

<u>PLACE OF BIRTH</u>	<u>PRESENT NATIONALITY</u>	<u>SEX</u>
[REDACTED]	USA	Male

<u>EDUCATION: INSTITUTION &amp; LOCATION</u>	<u>DEGREE</u>	<u>YEAR CONFERRED</u>	<u>SCIENTIFIC FIELD</u>
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]

HONORS

U. S. Public Health Service Predoctoral Fellowship 1962-1965, Grant No. 2G503;  
U. S. Public Health Service Premasters Traineeship 1960-1961; American Academy of  
Microbiology 1973

MAJOR RESEARCH INTEREST

Tumor Immunology

ROLE IN PROPOSED PROJECT

Principle Investigator

RESEARCH SUPPORT

15% of Dr. Coggin's time is provided by the University as cost sharing.

RESEARCH AND/OR PROFESSIONAL EXPERIENCE

1973	Professor of Microbiology
1967-Present	Section Chief of Transplantation Antigen Study and Consultant Virologist to Biophysical Separation Laboratory, Molecular Anatomy Program, ORNL.
1966-Present	Virologist, University of Tennessee, Knoxville. 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).
1965-1966	Senior Research Virologist. Virus and Cell Biology Division, Merck Institute for Therapeutic Research (Merck, Sharp, 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.

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RESEARCH AND/OR PROFESSIONAL EXPERIENCE (Continued)

- 1962-1965 U. S. Public Health Service, Predoctoral Trainee. Department of Microbiology, University of Chicago, Chicago, Illinois. Investigated the mode of action and mechanism of cellular resistance to antitumor drugs.
- 1965 Acting Principal Investigator, NIH Grant CA-07525. Responsibilities included supervision and planning of research for laboratory personnel in a biochemical investigation of 6-mercaptopurine degradation by leukemia cells and Escherichia coli.
- 1961-1962 Research Associate, Kettering-Meyer Cancer Laboratory, Birmingham, Alabama. Responsibilities as group leader included coordination of research within a group of 35 junior personnel and administrative responsibility.
- 1960-1961 U. S. Public Health Service Trainee (Premasters), University of Tennessee, Knoxville, Investigated growth and proliferation of Streptococcus faecalis on plant tissues as graduate student.
- 1959-1960 Senior Microbiologist, State of Tennessee Department of Public Health, Nashville.

PUBLICATIONS

- Coggin, Joseph H., Jr., Kathleen R. Ambrose, Peggy J. Dierlam, and Norman G. Anderson. Proposed Mechanisms by Which Autochthonous Neoplasms Escape Immune Reaction. *Cancer Research*, June, 1974. In press.
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- Rogan, E. G., M. P. Schafer, N. G. Anderson, and J. H. Coggin, Jr. Cyclic AMP Levels in the Developing Hamster Fetus: A Correlation with the Phasing of Fetal Antigens in Membrane Maturation. *Differentiation*, 1:199, 1973.
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- Girardi, A. J., P. Reppucci, P. J. Dierlam, W. Rutala, and J. H. Coggin, Jr. 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. Natl. Acad. Sci.*, 70, No. 1:183, 1973.

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Anderson, N. G., J. H. Coggin, E. B. Cole, and J. W. Holleman (eds.) *Embryonic and Fetal Antigens in Cancer, II*. U. S. Department of Commerce, Springfield, Va. 1972.

Anderson, N. G. and J. H. Coggin, Jr. Phase-Specific Autoantigens (Fetal) in Model Tumor Systems. *Embryonic and Fetal Antigens in Cancer, II*. (eds.) N. G. Anderson, J. H. Coggin, E. B. Cole, and J. W. Holleman. U. S. Department of Commerce, Springfield, Va., p. 91, 1972.

Coggin, J. H., Jr., K. R. Ambrose, B. B. Bellomy, and N. G. Anderson. Tumor Immunity in Hamsters Immunized with Fetal Tissues. *J. Immunology*, 107:526, 1971.

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

Hanna, M. G., R. W. Tennant, and J. H. Coggin, Jr. Suppressive Effect of Immunization with Mouse Fetal Antigen on Growth of Cells Infected with Rauscher Leukemia Virus and on Plasma-Cell Tumors. *Proc. Natl. Acad. Sci.*, 68:1748, 1971.

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

Anderson, N. G. and J. H. Coggin, Jr. Models of Differentiation, Retrogression and Cancer. *Proceedings of the First Conference and Workshop on Embryonic and Fetal Antigens in Cancer, I*. (eds.) N. G. Anderson and J. H. Coggin, Jr. U. S. Department of Commerce, Springfield, Va. p. 7, 1971.

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Hanna, M. G., R. W. Tennant, J. A. Treber, and J. H. Coggin, Jr. Immunization with Mouse Fetal Antigens: Suppressive Effect on Growth of Leukemia-Virus-Infected Cells and on Plasma Cell Tumors. *Proceedings of the First Conference and Workshop on Embryonic and Fetal Antigens in Cancer, I*. N. G. Anderson and J. H. Coggin, Jr. (eds.) U. S. Department of Commerce, Springfield, Va. p. 267, 1971.

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

Krueger, R. G., J. H. Coggin, Jr., and N. Gilliam. *Introductory Microbiology*. MacMillan and Company, New York, 1971.

Ambrose, K. R. and J. H. Coggin, Jr. Detection of Cytostatic Antibody Against SV40 Tumor in Immunized and Tumor Bearing Hamsters. *Bacteriol. Proc.*, 70:187, 1970.

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- Ambrose, K. R., E. L. Candler, and J. H. Coggin, Jr. Characterization of Tumor-Specific Transplantation Immunity in Diffusion Chambers In Vivo. *Proc. Soc. Exptl. Biol. Med.*, 132(2):1013, 1969.
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- Coggin, J. H., Jr., V. M. Larson, and M. R. Hilleman. Immunologic Responses in Hamsters to Homologous Tumor Antigens Measured In Vitro and In Vivo. *Proc. Exptl. Biol. Med.*, 124:1295, 1967.
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- Coggin, J. H., Jr. and W. R. Martin. 6-Diazo-5-OXO-L-norleucine Inhibition of Escherichia coli. *J. Bacteriol.*, 89:1348, 1965.

BIOGRAPHICAL SKETCH

<u>NAME</u>	<u>TITLE</u>	<u>BIRTHDATE (Mo., Day, Yr.)</u>
Ambrose, Kathleen R.	Research Associate	[REDACTED]

<u>PLACE OF BIRTH</u>	<u>PRESENT NATIONALITY</u>	<u>SEX</u>
[REDACTED]	USA	Female

<u>INSTITUTION AND LOCATION</u>	<u>DEGREE</u>	<u>YEAR CONFERRED</u>	<u>SCIENTIFIC FIELD</u>
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]

HONORS

Winter Garden Scholarship, 1967, AEC Fellowship, 1967-1968, Sigma XI Honorary Society

MAJOR RESEARCH INTEREST

Tumor Immunology, Viral Oncology

ROLE IN PROPOSED PROJECT

Supervision of tumor immunology experimental studies

RESEARCH SUPPORT

Supported by CP 73-210

RESEARCH AND/OR PROFESSIONAL EXPERIENCE

1970-Present	Research Associate, Department of Microbiology, University of Tennessee, Knoxville.
1969-1970	Research Assistant, Department of Microbiology, University of Tennessee, Knoxville.
1967-1969	Laboratory Technician, Department of Microbiology, University of Tennessee, Knoxville.

PUBLICATIONS:

Coggin, Joseph H., Jr., Kathleen R. Ambrose, Peggy J. Dierlam, and Norman G. Anderson. Proposed Mechanisms by Which Autochthonous Neoplasms Escape Immune Reaction. *Cancer Res.*, June, 1974. In press.

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RESEARCH AND/OR PROFESSIONAL EXPERIENCE (Continued)

Coggin, Joseph H., Jr., Kathleen R. Ambrose, and Edrick L. Candler. An Evaluation of the Isotopic Antiglobulin Assay for Detecting SV40 Tumor Immunity. In: *Immunologic Parameters of Host-Tumor Relationships*, 3. (Ed.) David Weiss. Academic Press, New York. 1974  
In press.

Coggin, J. H., Jr., K. R. Ambrose, B. B. Bellomy, and N. G. Anderson. Tumor Immunity in Hamsters Immunized with Fetal Tissue. *J. Immunology*, 107:526, 1971.

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

Coggin, J. H., Jr., K. R. Ambrose, and N. G. Anderson. Immunization Against Tumors with Fetal Antigens. *Proceedings First Conf. and Workshop on Embryonic and Fetal Antigens in Cancer. I.* (Eds.) N. G. Anderson and J. H. Coggin, Jr. U. S. Department of Commerce, Springfield, Va., p. 185, 1971.

Ambrose, K. R., N. G. Anderson, and J. H. Coggin, Jr. Concomitant and Sinecomitant Immunity to SV40 Tumor in Embryoma-Bearing Hamsters. *Proc. First Conf. and Workshop on Embryonic and Fetal Antigens in Cancer, I.* (Eds.) N. G. Anderson and J. H. Coggin, Jr. U. S. Department of Commerce, Springfield, Va., p. 281, 1971.

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

Coggin, J. H., Jr., K. R. Ambrose, and N. G. Anderson. Fetal Antigen Capable of Inducing Transplantation Immunity Against SV40 Hamster Tumor Cells. *J. Immunol*, 105:524, 1970.

Ambrose, K. R., and J. H. Coggin, Jr. Detection of Cytostatic Antibody Against SV40 Tumor in Immunized and Tumor Bearing Hamsters. *Bacteriol. Proc*, 70:187, 1970.

Ambrose, K. R., and J. H. Coggin, Jr. Role of Antibody in Inhibition of SV40 Tumor Cell Proliferation in Diffusion Chambers. *Bacteriol. Proc.*, 68:157, 1969.

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Ambrose, K. R., E. L. Candler, and J. H. Coggin, Jr. Characterization of Tumor-Specific Transplantation Immunity in Diffusion Chambers *In Vivo*. *Proc. Soc. Exptl. Biol. Med.*, 132(2):1013 (1969).

Coggin, J. H., Jr., and K. R. Ambrose. A Rapid *In Vivo* Assay for SV40 Tumor Immunity in Hamsters. *Proc. Soc. Exptl. Biol. Med.*, 130:246, 1969.

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In addition, this contract supports two predoctoral trainees, who devote their full research time to the objectives of this proposal. Current A.E.C. assistantships are held by Cleo Babeley and Korin Hearne.

7. *Other Personnel.*

2 Laboratory Aides - Dennis Ragsdale - 50% time

Barbara Bellomy - 50% 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. N.C.I. 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 Virus-Induced Tumor Rejection" has been in progress since July, 1970 and is funded at the present level of \$143,000 per year. This program is sponsored by the National Cancer Institute as a segment of contract CP 73-210 which is terminating at ORNL effective July, 1974. The objectives of this research are to develop immunotherapeutic techniques for controlling human cancer. N.C.I. funds cannot be used, to perform these basic studies since they are not directly mission oriented projects.

The University provides \$1500 in supplies and services to the A.E.C. project.

9. *Premises, Facilities, Equipment, and Materials.*

The Virology-Immunology Laboratory available for this project in the Department of Microbiology, University of Tennessee, Knoxville, consists of three contiguous, large laboratories occupying some 3500 square feet of useable space. In addition, 1,200 square feet of support space (washroom, prep. area, storage, etc.) is available. Animal facilities have been upgraded and all hamsters

1034005

are kept in two *containment* units separated completely from other rodents (1,500 square feet). A separate animal holding room (500 square feet) is available for breeding the large numbers of pregnant hamsters needed for fetal tissue. The largest supplier of pregnant rodents in the country cannot provide sufficient inbred hamsters or mice on a time-mated basis, hence we breed our own animals at a considerable cost savings. We currently have space to accomodate 6-7,000 hamsters in *new* facilities and some 3-500 breeders. Mice will be housed at Oak Ridge National Laboratory in excellent barrier facilities made available through the Molecular Anatomy Program, ORNL, on a "pay as needed basis".

We have excellent tissue culture facilities containing some 60 linear feet of Laminar flow work surface in eight units, walk-in incubators and essential cold storage and freezer space. All major items of equipment for conducting the work including Cytofluorograf, Cytograf, scintillation systems, preparative ultra-centrifuges and the like are available in our own laboratory. Additional facilities or equipment we require from the Molecular Anatomy Program are available to our personnel on a continuing basis. Tissue culture roller assemblies for large scale tissue production, purchased under the previous Interagency agreement (AEC-NCI) are available.

10.

## COST ESTIMATE

BUDGET  
*July 1*  
~~April 1~~, 1974 to ~~March 31~~, 1975  
*June 30*

AEC Contract AT-(40-1)3646

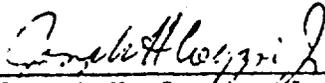
	<u>Estimated Requirements</u>	
	<u>Eighth Year</u>	<u>Ninth Year</u>
1. Salaries and Wages	<u>\$6,854.00</u>	<u>\$6,854.00</u>
Principal Investigator - 15% time	N/C	N/C
2 Graduate Students - 50% time	6,637.00	6,637.00
Fringe Benefits	217.00	217.00
2. Equipment	<u>\$3,760.00</u>	<u>\$3,760.00</u>
Animal Holding Equipment	3,760.00	3,760.00
3. Travel	<u>\$1,000.00</u>	<u>\$1,000.00</u>
4. Other Direct Costs	<u>\$16,220.00</u>	<u>\$18,220.00</u>
Supplies	11,043.00	13,043.00
Publications	240.00	240.00
Other	4,937.00	4,937.00
5. Indirect Costs	<u>\$4,268.00</u>	<u>\$4,268.00</u>
64.3% of \$6,637.00		
Total Project Costs	<u><u>\$32,102.00</u></u>	<u><u>\$34,102.00</u></u>

Principal Investigator agrees to spend 15% of academic year on project and the University agrees to cost share in accordance with their normal policy.

1034007

11. Authentication

Principal Investigator

  
\_\_\_\_\_  
Joseph H. Coggin, Jr., Ph.D.  
Professor

For The University

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

Financial Statement

April 2, 1974

AT-(40-1)3646

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

(1) Total actual project cost to date for the current period	<u>\$19, 444</u>
(2) Estimated total cost for remainder of period	<u>\$16, 884</u>
(3) Total actual and estimated cost chargeable to AEC for current period based on percentage of cost agreed upon as contained in A-111 of Appendix "A" to Contract	<u>\$36, 328</u>
(4) Accumulated costs chargeable to AEC (include costs reported in certified statement for preceding period(s) and the costs stated in Item (3) above	<u>\$113, 042</u>
(5) Accumulated AEC Support Ceiling as stated in Article III of Contract	<u>\$113, 042</u>
(6) Total estimated AEC funds remaining under Contract (subcontract Item (4) from (5) which may be used to reduce amount of new funds required from AEC for proposed renewal period	<u>\$ None</u>

 4/2/74

Mr. Roy A. Dean, Administrative Assistant

1034009

Renewal Proposal

Eighth Year

Contract

AT(40-1)3646

The University of Tennessee  
Department of Microbiology  
Knoxville, Tennessee 37916

Joseph H. Coggin, Jr., Ph.D.  
Principal Investigator  
Professor of Microbiology

April 1, 1974

1034010

F 1886

1. *Title of Project*

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

2. *Institution*

The University of Tennessee  
Department of Microbiology  
Knoxville, Tennessee 37916

Telephone: 615-974-2356

### 3. Project Abstract.

The current focus of this proposal is to continue ongoing efforts to discover how tumor viruses regulate and control the expression of embryo-associated cellular products as an integral activity in transforming normal cells. These studies are feasible because exposure of normal target cells to sublethal X-irradiation greatly sensitizes the cells to viral oncogenesis. We have observed that coordinate with the induction of neoplastic characteristics in viral transformed cells, embryonic moieties (EA) appear in the plasma membrane of the tumor cells and act as autoantigens. Radiation (25 to 350R) alone does not cause transformation of normal hamster or human cells *in vitro*, however, these levels of exposure do promote 50 to 1000-fold increases in viral oncogenesis induced by oncoviruses. Under support from this contract we have successfully demonstrated that early embryo tissue of the hamster, mouse, and human possess antigens (EA) which are identical to those occurring on viral transformed cells. During the terminal stages of embryonic or fetal development *in vivo* these antigenic entities are masked by biochemical processes associated with membrane maturation and subsequently reside cryptically, insofar as we can tell, in adult cell membrane. Viral oncogenesis serves to cause defective biosynthesis and results in the reexpression of EA. The central problem in our current work is to discover (1) whether we can successfully use embryonic markers expressed at the cell surface to reliably detect the earliest stages of viral induced neoplastic traits in the tumor cells, (2) to determine the biochemical events related to membrane maturation involved in the control and expression of EA in fetal development and in cancer induction and, (3) to determine how low-level radiation serves to potentiate this virus-host cell interaction. If we are successful in developing a reliable assay for fetal marker expression (EA) associated with the very early events in neoplasia, a valuable tool for detecting radiation potentiated oncogenesis will be available for understanding and analyzing the cancer induction.

1034012

#### 4. Scientific Background.

##### A. Historical Survey of This Contract.

This project was initiated eight years ago in an effort to discover whether exposure to sublethal levels of radiation, pyrimidine analogues, or cell "aging" processes *in vitro* served to potentiate oncovirus oncogenesis. This program requires a brief historical review and accounting. Since the initiation of this work we have reported that radiation and radiomimetic chemicals markedly potentiate viral oncogenesis in primary cell culture systems *in vitro* and in animals treated locally with X-ray before infection with tumor viruses. Recall that in this work the target cells, not the transforming virus, are presensitized by irradiation. In the time since these initial reports, research on this project has settled into a study of the molecular mechanisms by which "low-level" radiation of normal target cells leads to increased viral oncogenesis *in vitro*.

More recently our efforts were focused on determining whether X-irradiation served to potentiate SV40 oncogenesis by inducing "break and repair" reactions which facilitated the integration of the transforming virus genome into cellular DNA. DNA:DNA hybridization techniques were adapted and developed to determine the relationship between radiation dose (used to presensitize the cells prior to infection) and the number of viral genome equivalents incorporated into a given tumor clone. The results of these experiments have been published with the general conclusion that an absolute linear relationship did not exist between increasing radiation doses and increased number of viral equivalents present in a tumor clone; however a general increase in the number of viral equivalents was found covalently-linked in association with transformed cell DNA in radiation presensitized cells at exposure levels exceeding 100R when compared to unirradiated, SV40 transformed clones. Briefly stated, X-ray pretreatment of target cells seemed to cause a greater *number* of infected target cells to "accept" and incorporate an SV40 genome leading to stable transformation.

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Three years ago, as a "spin-off" from this project, we discovered that embryonic antigens were almost universally expressed in virally and chemically induced tumor cells. This new observation from rodent model system studies was quickly recognized to relate to human cancer since studies in many laboratories now show quite unequivocally that human cancers carry histologically related, cross-reacting antigens. Many of these antigenic determinants are recognized to be embryonic in character and the possibility stands that all are embryonic antigens (EA). Norman Anderson at ORNL and the principal investigator of this contract (J.H.C.) have reviewed the central problems associated with retrogenic expression in neoplasia this past year and reprints or preprints of several reviews\* are included in the 1974 Progress Report for your perusal.

Last year, in our proposal for an additional three years support from the A.E.C. to study the relationship of radiation damage to viral oncogenesis and retrogenesis in neoplasia, we outlined a series of new objectives aimed at discovering the interrelatedness of EA expression with radiation-stimulated oncogenesis. Our central theme was then and continues to be to develop immunologic and molecular tools for discerning the *earliest events* in viral oncogenesis, potentiated by radiation, which can be used as "markers" of neoplastic conversion leading to the malignant state. We have worked hard these past 12 months to

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\*Cancer, Differentiation, and Embryonic Antigens: Some Central Problems. *Adv. in Cancer Res.*, 19:105, 1974.

Proposed Mechanisms by Which Autochthonous Neoplasms Escape Immune Rejection. *Cancer Res.*, 1974. (In press.)

An Evaluation of the Isotopic Antiglobulin Assay and the Cytostatic Assay for Detecting SV40 Tumor Immunity. *Immunological Parameters of Host-Tumor Relationships*. June, 1974. (In press).

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develop reliable assays for embryonic antigens and we feel that we are on the right track and that our ideas were correct and experimentally testable. Funds made available for this work through the A.E.C. support only graduate student research.

Specifically, we have developed both a radioimmunoassay procedure for the detection of small quantities of embryonic antigen and a quantitative immunofluorescence assay for detecting the very early expression of EA at the surface of "normal" cells undergoing transformation *in vitro*. The studies are made experimentally feasible because low-level sublethal X-irradiation presensitized cells to oncogenesis by oncodnavirus and yields large populations of transformed cells *in vitro* in a given test population. Simultaneously, under support from this contract, we have been examining the biochemical events associated with the expression and regulation of embryonic antigens (EA) in the plasma membrane of the developing fetus (hamster and mouse). Biochemical studies have, so far, supported the concept that EA expression in the fetal cell membrane is silenced at the 11th day of gestation because of maturation processes associated "adult" membrane formation. EA's are now known to be present on unfertilized eggs *in situ* in the ovary and EA expression continues through gamete fusion and the first ten days of gestational development.\* Thus we will remain with our research plan, submitted in detail last year, seeking to delineate specific biochemical and regulatory changes associated with the reexpression of cryptic EA residing in adult cell membrane as an essential function in cellular transformation.

#### B. Relationship of This Study to Other Work in Viral Oncogenesis.

Papovaviruses are the best characterized tumor viruses particularly when we consider the wealth of information now available regarding the molecular

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\*Cancer, Differentiation, and Embryonic Antigens:Some Central Problems. *Adv. in Cancer Res.*, 19:105, 1974.

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interactions between these viruses and their transformed cell hosts. These viruses have been particularly amenable to study because they possess a small DNA genome which has a limited gene complement and many of the unique viral coded proteins have been identified. Viral genetic information responsible for tumor induction seems restricted to one or two genes. Intranuclear tumor or T antigen is now recognized to be coded for from the viral genome since T antigen expression occurs in cells lytically infected with SV40 and only mutations in the viral genome effect the synthesis of this antigen (see Robb, J. A., *J. Virol.*, 12:1187, 1973). Recent reports have shown that at least *one cellular gene* is required for maintaining some manifestation of the transformed state. These characteristics of transformation include atypical cellular DNA synthesis, excess cell saturation density, lack of growth factor requirements, and agglutinin binding receptors on tumor cells. Thus, at least one viral gene and one cellular gene are essential for regulating the *induction* of transformation. In the work we are doing we seek to add a quantitative parameter to indicate transformation, specifically by developing a reliable assay for EA expression using objective assays. Portugal and his colleagues (*J. Virol.*, 12:1616, 1973) recently showed that a number of new isoaccepting tRNA species (leucyl-, isoleucyl-, phenylalanyl-, and threonyl- tRNA's) thought originally to be associated with viral gene activity are now recognized to be *identical* to tRNA alterations accompanying *differentiation* of developing normal cells.

Renger and Basicilco reported this past May (*J. Virol.*, 11:702, 1973) that *cellular* rather than viral genes are responsible for the *maintenance* of the neoplastic state in SV40 transformed cells. These data were obtained from elegant studies using SV40 transformed cell lines which were temperature sensitive, exhibiting neoplastic traits at 32° but losing these characteristics at 39°C. Infection of these SV40 transformed cells with *wild-type* SV40 (non-ts mutants) does not alter this control pattern suggesting clearly that cellular rather than

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viral genes are ultimately responsible for maintaining the transformed state.

Our observation that tumor rejection antigens present on SV40 tumor cells were embryonic in character rather than viral specific has been confirmed in many tumor systems in the past 24 months (Table 1). These data too fit the idea that a major action of oncogenic viruses is to actuate regulatory shifts in cells which cause the plasma membranes to revert to an immature form.

In summary the mounting evidence suggests that oncogenic viruses cause neoplasia by becoming heritably associated with the cellular genome and by producing some key regulatory substances which causes the cell to undergo dedifferentiation processes. Many of the neoplastic characteristics which are now associated with tumor cells are related to cell-coded activities whose reexpression is under viral regulation.

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Table 1

Reported Observations of Induction of Tumor Immunity Following  
Direct Immunization with Fetal Tissue.

System	Tumor Immunity Induced Against	Fetal Vaccine Effective in Males	Females	Route of Vaccination	Direct challenge	Bendich, et al., (1973)
Mouse	QUA	N.D.*	+	S.C.	Direct challenge	Bendich, et al., (1973)
Mouse	MCA-10	N.D.	+	?	Direct challenge, serum cytotoxicity with <sup>125</sup> I Iododeoxyuridine	LeMevel and Well (1973)
Mouse	MCA-10	+	+	I.P.	Cell challenge	Castro, et al., (1974)
Mouse	PCT	+	N.D.*	I.P.	Spleen colony assay (FCFU)	F.A. Salinas, et (1972)
Rat	MCA-R	+	+	?	Direct challenge	Grant, et al., (1973)
Guinea pig	MCA-A, MCA-25	+	+	I.D. (+CFA) I.P.	DTHR's Direct challenge	Grant, et al., (1973)
Hamster	SV40	+	-	I.P.	Interrupt SV40 Oncogenesis	Girardi, et al., (1973)

REFERENCES: Bendich, A., Borenfreund, E., and Stonehill, E. H., *J. Immunol.*, 111:285, 1973.  
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 Grant, J. and Wells, S.A., *J. of Surgical Res.*, In press, 1973.  
 LeMevel, B.P. and Wells, S.A., *Nature, New Biol.*, 244:183, 1973.  
 Salinas, F.A., Smith, Jane A., and Hanna, M.G., *Nature*, 240:41, 1973.  
 Girardi, A.J., Reppucci, P., Dierlam, P., Rutala, W., and Coggin, J.H., *Proc. Nat. Acad. Sci.*, 70:183, 1973.

N.D. = Not done. I.D. (CFA) = Intradermal injection (complete Freund's adjuvant).

S.C. = Subcutaneous injection. FCFU = Fetal colony forming unit.

I.P. = Intraperitoneal injection. DTHR's = Delayed type hypersensitivity reactions.

## 5. Scientific Scope.

### A. Overall Objectives.

(1) Characterize the biochemical events leading to the production of immature plasma membranes exposing embryonic antigen in radiation-stimulated SV40 oncogenesis.

(2) Evaluate the early events of neoplastic transformation using embryonic antigen markers as indices of virus stimulated retrogenesis.

The current research effort is following closely the workscope presented last year. We have achieved several of the proposed objectives in the past 12 months and have narrowed our focus to *two project areas* which now seem most promising. We have evaluated several assays for quantitating the presence of embryonic antigens at the surface of tumor cells. The results, currently being published\*, showed that the radioimmunoassay for cell surface antigen (IAT method) measured cross-reacting (EA) antigens rather than tumor specific transplantation antigen (Burdick *et al.*, *Int. J. Cancer*, 12:474, 1973) and this assay is most suitable for our purpose in detecting the early expression of EA at the surface of cells undergoing SV40 transformation.

We reviewed the historical and theoretical basis for these biochemical and immunological studies last year in the large proposal. Hopefully it will be sufficient to say here that the overall objective is to correlate biochemical changes in plasma membrane induced by SV40 in normal adult cells with the development of cell surface antigens of the embryonic type and the onset of the transformed state.

### B. Specific Projects.

(1). Biochemical Studies. (see page 19-23, 1973 proposal for protocols and deta:

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\*An Evaluation of the Isotopic Antiglobulin Assay and the Cytostatic Assay for Detecting SV40 Tumor Immunity. In: *Immunological Parameters of Host-Tumor Relationships*. June, 1974. (Ed.) David Weiss, Academic Press, New York. (In press).

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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 (150R 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 with the following specific studies:

- a. 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.
- b. Employing purification procedures developed in our laboratory fetal, phase-specific mRNA's will be isolated and tested for their identity to "tumor-specific" mRNA. The competitive hybridization procedure is being used.
- c. The role of cyclic AMP concentration, synthesis and degradation in retrogenesis induced by SV40 virus will be investigated.
- d. 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 more suitable for evaluating subtle changes in fetal development which radiation exposure might introduce.

(2) Immunological Studies.

These studies are ongoing and are being conducted with the basic format described last year with certain specific revisions. We have developed a suitable

quantitative system for detecting extremely low quantities of EA at the surface of tumor cells during transformation using the isotopic antiglobulin test (IAT), a modified radioimmunoassay.

*IAT Procedure:*

The isotopic antiglobulin assay (Harder and McKhann, 1968) as modified for use with monolayer tissue culture cells has been previously described (Burdick *et al.*, 1973). Target, 14 day fetal cell suspensions (EA<sup>-</sup> cells) with or without irradiation and infection were plated one day before use in the individual wells of Microtest-II plates (Falcon Plastics, Los Angeles, Calif., USA) in RPMI 1640 plus 20% FCS, and for the test, the adherent monolayers were incubated for 30 min, appropriately diluted, washed five times, then incubated for 30 min with anti-globulin diluted in RPMI ± 10% FCS and then washed six times. The wells were sprayed with Aeroplast (Parke-Davis and Co., Detroit, Mich., USA), cut apart on a bandsaw and counted in Packard Auto-Gamma counter. Antiglobulin controls (AGC) were performed by adding medium + 10% FCS for the first incubation and antiglobulin for the second. Results were expressed as counts per min (CPM) and as:

the absorption ratio =

$$AR = \frac{\text{CPM in wells with Immune or Pregnant Serum}}{\text{CPM in wells with Control Serum}}$$

There are some biological variables inherent to the tissue culture techniques employed which cannot be totally controlled. Therefore, the results are not exactly quantitatively reproducible from day to day, and inferences of exact amounts of globulin bound to the cell would be unreliable (Burdick *et al.*, 1973) in comparing percentage values from one day's results with those of another run. Comparison of the AR between different radiation doses and virus concentrations will provide a ranking, but quantitative comparison of the antigenic content of various cell lines requires parallel absorptions of the same serum run on the same day.

### *Absorption*

Absorption of sera prior to use in the isotopic antiglobulin assay was performed in a standard fashion (Ting and Herberman, 1971). Cells to be used for absorption were removed from tissue culture flasks by trypsinization (0.25% trypsin - 0.1 EDTA), suspended and maintained overnight in spinner culture flasks. Aliquots containing the indicated number of cells were centrifuged at 800 x g for 10 min, the supernatant was aspirated, and 0.2 ml of the serum to be absorbed was added. After incubation for 60 min at 37°C and 5% CO<sub>2</sub> with frequent agitation, the samples were centrifuged first at 800 x g then the supernatant was aspirated and centrifuged at 2,000 x g for 10 min and this supernatant was employed in the isotopic antiglobulin test. Single-cell suspensions of various fetuses of 10-15 days gestation are prepared by extensive mincing, then used for absorption as described above.

### *Serum fractionation and iodination*

Fractionation of an anti-hamster gamma globulin (AG), was performed by ammonium sulfate precipitation, and then separation on G-200 Sephadex, using 0.2M Tris-NaCl-HCl buffer at pH 8.0. Aliquots containing 1 mg of the 7S fraction of the antiglobulin were labeled with <sup>125</sup>I (New England Nuclear, Boston, Mass., USA) by the modified chloramine-T method (McConahey and Dickson, 1966). The molar iodine: gamma-globulin ratio was always below 0.25:1. The 7S fraction was suspended in RPMI 1640 with 10% FCS and tested in the isotopic antiglobulin assay as described above.

Sera for detecting EA are derived from pregnant, multiparous, inbred hamsters primed on 5 occasions with 10 day gestation hamster embryo cells.

### Experimental Design:

Fourteen day fetal cells (EA<sup>-</sup>) 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

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antiserum is added following virus adsorption. Samples plates are washed at selected times post-infection (24, 28, 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  $^{125}\text{I}$ -tagged anti-hamster IgG is added, incubated with target cells and washed. The wells are collected as described and scored. Immunofluorescence studies run in parallel are being conducted as described last year. 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 IAT results and the fluorescent labeling procedure indicate 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.

Titration of radiation dose, cell target levels transformation frequency and other parameters are nearly completed. The ultimate application of this approach is to determine how early EA expression is noted and how the quantitative distribution of EA correlates with transformation frequency in the sample population. Biochemical changes in the transforming cell population (sialic acid synthesis, eg.) will subsequently be correlated with the expression of EA.

6. *Scientific Personnel.*

<u>NAME</u>	<u>TITLE</u>	<u>BIRTHDATE (Mo., Day, Yr.)</u>
Coggin, J. H., Jr.	Professor	[REDACTED]

<u>PLACE OF BIRTH</u>	<u>PRESENT NATIONALITY</u>	<u>SEX</u>
[REDACTED]	USA	Male

<u>EDUCATION: INSTITUTION &amp; LOCATION</u>	<u>DEGREE</u>	<u>YEAR CONFERRED</u>	<u>SCIENTIFIC FIELD</u>
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]

HONORS

U. S. Public Health Service Predoctoral Fellowship 1962-1965, Grant No. 2G503;  
 U. S. Public Health Service Premasters Traineeship 1960-1961; American Academy of  
 Microbiology 1973

MAJOR RESEARCH INTEREST

Tumor Immunology

ROLE IN PROPOSED PROJECT

Principle Investigator

RESEARCH SUPPORT

15% of Dr. Coggin's time is provided by the University as cost sharing.

RESEARCH AND/OR PROFESSIONAL EXPERIENCE

1973	Professor of Microbiology
1967-Present	Section Chief of Transplantation Antigen Study and Consultant Virologist to Biophysical Separation Laboratory, Molecular Anatomy Program, ORNL.
1966-Present	Virologist, University of Tennessee, Knoxville. 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).
1965-1966	Senior Research Virologist. Virus and Cell Biology Division, Merck Institute for Therapeutic Research (Merck, Sharp, 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.

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RESEARCH AND/OR PROFESSIONAL EXPERIENCE (Continued)

- 1962-1965 U. S. Public Health Service, Predoctoral Trainee. Department of Microbiology, University of Chicago, Chicago, Illinois. Investigated the mode of action and mechanism of cellular resistance to antitumor drugs.
- 1965 Acting Principal Investigator, NIH Grant CA-07525. Responsibilities included supervision and planning of research for laboratory personnel in a biochemical investigation of 6-mercaptopurine degradation by leukemia cells and Escherichia coli.
- 1961-1962 Research Associate, Kettering-Meyer Cancer Laboratory, Birmingham, Alabama. Responsibilities as group leader included coordination of research within a group of 35 junior personnel and administrative responsibility.
- 1960-1961 U. S. Public Health Service Trainee (Premasters), University of Tennessee, Knoxville, Investigated growth and proliferation of Streptococcus faecalis on plant tissues as graduate student.
- 1959-1960 Senior Microbiologist, State of Tennessee Department of Public Health, Nashville.

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BIOGRAPHICAL SKETCH

NAME Ambrose, Kathleen R.      TITLE Research Associate      BIRTHDATE (Mo., Day, Yr.) [REDACTED]

PLACE OF BIRTH [REDACTED]      PRESENT NATIONALITY USA      SEX Female

INSTITUTION AND LOCATION [REDACTED]      DEGREE [REDACTED]      YEAR CONFERRED [REDACTED]      SCIENTIFIC FIELD [REDACTED]

HONORS

Winter Garden Scholarship, 1967, AEC Fellowship, 1967-1968, Sigma XI Honorary Society

MAJOR RESEARCH INTEREST

Tumor Immunology, Viral Oncology

ROLE IN PROPOSED PROJECT

Supervision of tumor immunology experiment studies

RESEARCH SUPPORT

Supported by CP 73-210

RESEARCH AND/OR PROFESSIONAL EXPERIENCE

1970-Present      Research Associate, Department of Microbiology, University of Tennessee, Knoxville.

1969-1970      Research Assistant, Department of Microbiology, University of Tennessee, Knoxville.

1967-1969      Laboratory Technician, Department of Microbiology, University of Tennessee Knoxville.

PUBLICATIONS:

Coggin, Joseph H., Jr., Kathleen R. Ambrose, Peggy J. Dierlam, and Norman G. Anderson. Proposed Mechanisms by Which Autochthonous Neoplasms Escape Immune Reaction. *Cancer Res.*, June, 1974. In press.

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In addition, this contract supports two predoctoral trainees, who devote their full research time to the objectives of this proposal. Current A.E.C. assistantships are held by Cleo Babeley and Korin Hearne.

7. *Other Personnel.*

2 Laboratory Aides - Dennis Ragsdale - 50% time

Barbara Bellomy - 50% 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. N.C.I. 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 Virus-Induced Tumor Rejection" has been in progress since July, 1970 and is funded at the present level of \$143,000 per year. This program is sponsored by the National Cancer Institute as a segment of contract CP 73-210 which is terminating at ORNL effective July, 1974. The objectives of this research are to develop immunotherapeutic techniques for controlling human cancer. N.C.I. funds cannot be used, to perform these basic studies since they are not directly mission oriented projects.

The University provides \$1500 in supplies and services to the A.E.C. project.

9. *Premises, Facilities, Equipment, and Materials.*

The Virology-Immunology Laboratory available for this project in the Department of Microbiology, University of Tennessee, Knoxville, consists of three contiguous, large laboratories occupying some 3500 square feet of useable space. In addition, 1,200 square feet of support space (washroom, prep. area, storage, etc.) is available. Animal facilities have been upgraded and all hamsters

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7. *Other Personnel.*

2 Laboratory Aides - Dennis Ragsdale - 50% time

Barbara Bellomy - 50% time

8. *Other Financial Assistance:*

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are kept in two *containment* units separated completely from other rodents (1,500 square feet). A separate animal holding room (500 square feet) is available for breeding the large numbers of pregnant hamsters needed for fetal tissue. The largest supplier of pregnant rodents in the country cannot provide sufficient inbred hamsters or mice on a time-mated basis, hence we breed our own animals at a considerable cost savings. We currently have space to accommodate 6-7,000 hamsters in *new* facilities and some 3-500 breeders. Mice will be housed at Oak Ridge National Laboratory in excellent barrier facilities made available through the Molecular Anatomy Program, ORNL, on a "pay as needed basis".

We have excellent tissue culture facilities containing some 60 linear feet of Laminar flow work surface in eight units, walk-in incubators and essential cold storage and freezer space. All major items of equipment for conducting the work including Cytofluorograf, Cytograf, scintillation systems, preparative ultra-centrifuges and the like are available in our own laboratory. Additional facilities or equipment we require from the Molecular Anatomy Program are available to our personnel on a continuing basis. Tissue culture roller assemblies for large scale tissue production, purchased under the previous Interagency agreement (AEC-NCI) are available.

10.

COST ESTIMATE

BUDGET

*July 1*  
~~April 1~~, 1974 to ~~March 31~~, 1975

AEC Contract AT-(40-1)3646

	<u>Estimated Requirements</u>	
	<u>Eighth Year</u>	<u>Ninth Year</u>
1. Salaries and Wages	<u>\$6,854.00</u>	<u>\$6,854.00</u>
Principal Investigator - 15% time	N/C	N/C
2 Graduate Students - 50% time	6,637.00	6,637.00
Fringe Benefits	217.00	217.00
2. Equipment	<u>\$3,760.00</u>	<u>\$3,760.00</u>
Animal Holding Equipment	3,760.00	3,760.00
3. Travel	<u>\$1,000.00</u>	<u>\$1,000.00</u>
4. Other Direct Costs	<u>\$16,220.00</u>	<u>\$18,220.00</u>
Supplies	11,043.00	13,043.00
Publications	240.00	240.00
Other	4,937.00	4,937.00
5. Indirect Costs	<u>\$4,268.00</u>	<u>\$4,268.00</u>
64.3% of \$6,637.00		
Total Project Costs	<u><u>\$32,102.00</u></u>	<u><u>\$34,102.00</u></u>

Principal Investigator agrees to spend 15% of academic year on project and the University agrees to cost share in accordance with their normal policy.

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11. Authentication

Principal Investigator

  
\_\_\_\_\_  
Joseph H. Coggin, Jr., Ph.D.  
Professor

For The University

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