UNITED STATES GOVERNMENT

Memorandum

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595

APR 1 1968

DATE:

TO : Ralph Elson, Director
Contract Division

FROM : Herman M. Roth, Director
Laboratory and University Division

SUBJECT: REQUEST FOR CONTRACT ACTION

OLE:LM

REPOSITORY Oak Ridge Operations Office Records Holding Area
COLLECTION Documents 1944-1994
BOX No. A-59-3 Bldg 2714-H
FOLDER Cont 3646 Tenn. CA 4-1-68

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

1. Nature of Action Requested:

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

   [X] Modification of Contract
   Number: AT-(40-1)-3646
   Contractor: University of Tennessee
   Knoxville, Tennessee

2. Nature of Services to be Covered by Contract: Research

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

3. Type of Contract: [X] Support Agreement [ ] Cost Type [ ] Other

4. Amount of AEC Funds to be Obligated by this Contract Action: $21,349.00

5. AEC Percentage of Estimated Total Cost to be Shown by this C/A: 76%

6. Description of Other Changes to be Covered by Contract Action:

   Modify contract to provide for the performance of additional research to be completed during the period April 1, 1968 through March 31, 1969. The AEC support ceiling for the extended period is $21,349. Title to equipment shall vest in the contractor under authority of the Atomic Energy Act of 1954 since the contractor's contribution is expected to equal or exceed the value of the equipment.

7. Authority:

   Form AEC-481 (Contract Authorization) from John R. Totter to S. R. Sapirie dated March 20, 1968.

Herman M. Roth

Herman M. Roth

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4-1-68

CONTRACTS 3646 (Tenn)

1034035

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

APPENDIX "A"

For the Contract Period April 1, 1968 through March 31, 1969.

A-I RESEARCH TO BE PERFORMED BY CONTRACTOR

The Contractor will continue studies of the effects of ionizing radiation, chemical effects, and aging on the viral transformation of cells *in vitro* to include especially factors that potentiate cellular transformation by SV40 virus, particularly in hamster embryo cells. Clarification will be made of the roles of X-irradiation, DNA antagonistic chemicals, aging, etc. Tissue culture methods will be used as well as other appropriate systems. Changes in DNA synthesis, virus concentrations, metabolic states, and other aspects of conversion of normal cells into neoplastic cells in embryonic cell populations will be quantitatively studied.

A-II WAYS AND MEANS OF PERFORMANCE

(a) Items Included in Total Estimated Cost:

(1)	Salaries and Wages:	$10,550.00
	Dr. J. H. Coggin, Principal Investigator (40% of time) Research Assistant (85% of time) Laboratory Assistant (50% of time) Animal Maintenance Helper (20% of time)	
(2)	Employee Benefits:	480.00
(3)	Materials and Supplies:	8,650.00
	Chemicals, isotopes, cages and racks, animals, animal food and bedding, media and serum, culture dishes, tubes, gas, and animal tissues.	
(4)	Equipment:	5,380.00
	Analytical balance, top loading balance, centrifuge, blender, and two tissue culture roller drum assembly.	
(5)	Publications and Communications:	320.00
(6)	Travel:	500.00
(7)	Indirect Costs (50.4% of Salaries and Wages Excluding Principal Investigator's salary):	2,419.00

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(b) Items Significant to the Performance of This Contract, But Excluded From Computation of Total Cost and From Consideration in Proportioning Costs:

None

A-III The total estimated project cost of A-II (a) above for the contract period stated above is $28,299.00.

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Form AEC-481
(6-67)

AECPR 9-4.51

U. S. ATOMIC ENERGY COMMISSION

CONTRACT AUTHORIZATION

1. DATE: MAR 2 0 1968

2. AUTHORIZATION NO.: BM-68-420

3.A. TO
S. R. Sapirie, Manager
Oak Ridge Operations Office

3.B. FROM
John R. Totter, Director
Division of Biology and Medicine

4.A. CONTRACTOR (Name, Address, Department, etc.)
UNIVERSITY OF TENNESSEE
Knoxville, Tennessee 37916
Department of Microbiology

4.B. PRINCIPAL INVESTIGATOR(S)
JOSEPH E. COGGIN, JR.

5. ☐ NEW CONTRACT ☒ RENEWAL ☐ OTHER

6. TERM OF CONTRACT: 4/1/68 thru 3/31/69

7. CONTRACT NUMBER: AT(40-1)3646

8. RECOMMENDED TYPE OF CONTRACT:
☐ FIXED PRICE ☐ OTHER
☐ COST REIMBURSEMENT
☒ SPECIAL RESEARCH SUPPORT AGREEMENT (SRSA)

9. PROPERTY TITLE TO VEST IN:
☐ AEC
☒ CONTRACTOR

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 In Vitro"

12. HEADQUARTERS TECHNICAL CONTACT
Dr. David Pittman

13. FINANCING

A. OPERATING EXPENSES

New AEC Funds (Initial AEC Support ceiling, if SRSA)	$ 21,349
Estimated AEC Balance From Prior Term, if any	$ -
Total AEC Funding (If SRSA, 76 % of Total Cost, up to Maximum of)	$ 21,349
Estimated Contractor Contribution, if any	$ 6,950
Total Estimated Project Cost	$ 28,399

Budget and Reporting Classification: 06 02
Allotment Transfer: 06-81-91(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 Studies on the radiation, chemical, and aging effects on viral transformation in mammalian cells cultured in vitro.

CONTRACTS — 3646 (Tenn)
J 2893
MAR 22 1968

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BMB:BMT

MAR 2 0 1968

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

Dear Dr. Coggin:

I am pleased to advise you that the Research Committee has approved renewal of your Research Contract No. AT(40-1)3646 entitled "A Comparative Study of Radiation, Chemical and Aging Effects on Viral Transformation In Vitro" for an additional year at approximately 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 yours,

David Pittman
Geneticist, Biology Branch
Division of Biology and Medicine

cc: Hilton A. Smith, Ph.D.
Vice President for Graduate
Studies and Research

J 2893

1034039

THE UNIVERSITY OF TENNESSEE
KNOXVILLE 37916
DEPARTMENT OF MICROBIOLOGY

March 26, 1968

Dr. C. S. Shoup, Chief
Research Contracts Branch
Laboratory and University Division
United States Atomic Energy Commission
Post Office Box E
Oak Ridge, Tennessee 37830

Subject: RENEWAL OF CONTRACT NO. AT-(40-1)-3646

Final Budget Statement for Contract period ending March 31, 1968

Dear Dr. Shoup:

As of March 31, 1968, all funds available for Contract AT-(40-1)-3646 will have been expended.

Sincerely,

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

JHC/mln

J 3962
MAR 27 1968
CONTRACTS— 3646 (Tenn)

1034040

OPTIONAL FORM NO. 10
5010-104

UNITED STATES GOVERNMENT

Memorandum

I & U files

TO : **John R. Totter, Director**
Division of Biology and Medicine, HQ

DATE: **January 5, 1968**

FROM : Herman M. Roth, Director
Laboratory and University Division, OR

SUBJECT: RENEWAL OF CONTRACT NO. AT-(40-1)-**3646 WITH THE UNIVERSITY OF TENNESSEE (DR. JOSEPH H. COGGIN, JR.)**

OLE:LM

We are submitting for your review and appropriate action the following information concerning the contract which will expire on **March 31, 1968:**

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.

Herman M. Roth
Herman M. Roth

Enclosures:
As Listed Above

BC: C. S. Shoup, w/cy encls.
D. S. Zachry, w/cy progress report
Alice Brown
R. L. Shannon, DTIE
w/cy progress report & Forms AEC-427

Adm. Ser., Br.
Medley
Medley:nhb
1/5/68

CONTRACTS – 3646 (Term)

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THE UNIVERSITY OF TENNESSEE
KNOXVILLE 37916
DEPARTMENT OF MICROBIOLOGY

December 28,1967

C.S. Shoup, Chief
Research Contracts Branch
U.S. Atomic Energy Commission
Laboratory and University Division
Post Box E
Oak Ridge, Tennessee 37830

Subject: Renewal of Contract No. AT(40-1)-3646

Dear Dr. Shoup:

Enclosed please find a renewal proposal for Contract No. AT(40-1)3646, and an expenditure statement for the present year as well as seven copies of progress report ORO-3646-1, three copies of the ten percent voucher and four copies of a 200 word summary of the project.

We look forward to another year of research association with the Atomic Energy Commission.

OK-MR

Respectfully yours,

Joseph H Coggin Jr.

Joseph H. Coggin,Jr., Ph.D
Assistant Professor of
Microbiology

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CONTRACTS-3646 (Tenn)
1034042

Expenditure Report
AEC Contract No. AT(40-1)3646
March 15, 1967 to December 15, 1967

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

Original Signed By
HILTON A. SMITH

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

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FINANCIAL STATEMENT

AEC Contract AT-(40-1)-3646

Prepared December 15, 1967

	EXPENDED ~~May 15,~~ April 1 1967 - Dec. 15, 1967	COMMITTED OR ANTICIPATED Dec. 16, 1967 - ~~April 14, 1967~~ March 31, 1968	UNIVERSITY CONTRIBUTION Expended to Dec. 15, 1967
Personnel	$2,640.00	$1,760.00	$6,240.00
Supplies	8,505.00	---	800.00
Equipment	6,089.21	615.79	6,800.00**
Travel	422.79	127.21	---
Telephone, Publication costs	50.25	324.75	---
Overhead	738.00	246.00*	---
	$18, 445.25	$3,073.75	$13,840.00

Total AEC Funds Allocated (~~4/15/67 - 4/14/68~~ 4-1-67 - 3-31-68) $21,519.00
Expenditures estimated (through ~~4/14~~ 3/31/68)AEC 21,519.00
Anticipated Balance (as of ~~4/14/68~~ 3-31-68) 0
(See renewal proposal for new budget for ~~4/15/68 - 4/14/69~~ 4-1-68 - 3-31-69)

*Overhead computed on basis of 24.6% of salaries and wages.
Estimated on the basis of 75% of expected total.

**University has supplied-Forma Ultracold Unit ($1400), Cost-share on Ultracentrifuge with NASA ($3000), Automatic recorded ($1,100), Incubators ($1000)

OKO
R.M.

1034044

1. Title of Project.

A Comparative Study of Radiation, Chemical and Aging Effects on Viral Transformation *In Vitro*

2. Institution.

University of Tennessee
Department of Microbiology
Knoxville, Tennessee 37916
Telephone: 615- 974-5466

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3. Project Abstract.

A study of SV40 transformation was initiated in this laboratory eleven months ago in an effort to define the sequence of molecular events which result in the conversion of normal hamster embryo cells to neoplastic cells *in vitro*. A unique system has been developed for this purpose. SV40 virus transforms normal hamster embryo cells in culture within an efficiency range of 0.1 to 0.4%. If the cells were subcultured until they became senescent (15 to 17 passages in vitro or 87 days) and then infected with SV40 virus, the efficiency of transformation increased to 14.3%. Pretreatment of young embryo cells with 150R of x-ray just prior to infection with SV40 resulted in rapid transformation of surviving cells with an efficiency of 27.8%. Similar results were obtained by pretreatment of cells with a radiomimetic thymidine analogue. The similarities between the reported biological effects (chromosome damage) of x-irradiation, thymidine analogue exposure and aging suggested that a common mechanism in enhancing the viral transformation "process" may be operating for all three treatments.

The experimental approach selected for this program seeks to clarify the role of x-irradiation, DNA antagonists (5-iododeoxyuridine) and aging in potentiating SV40 transformation of hamster cells *in vitro*. Our first efforts have been directed to a definitive examination of the parameters of the irradiated cell test system which exhibits enhanced transforming efficiency. A titration of x-ray exposure on embryonal cell survival has been completed and experiments are presently underway to evaluate the effects of increasing radiation exposure on the frequency of transformation. A tissue culture method has been developed for performing all studies with cells from primary explant, avoiding the use of subcultured cells and eliminating many selection events. Periods of active DNA synthesis post-irradiation have been defined and studies are now in progress to correlate these periods with sensitivity to SV40 transformation. An evaluation of several DNA antagonists (other than IUDR) for potential to enhance SV40 transformation has been initiated and cytotoxic titrations of these agents are nearly completed. Additionally, the role of virus concentration and type, metabolic state of cells and other parameters of the test system are being investigated as time permits.

The planned study will make it possible to gain a quantitative understanding of many of the conditions which exist and events which occur in the conversion of normal cells to neoplastic cells following exposure to oncogenic viruses. The unique feature of the system is that these studies can be performed on primary embryonal cell populations with greatly increased "resolution" of oncogenicity since transformation occurs as a frequent event in sublethally irradiated cells rather than as the rare event, often observed in many non-tumor cell-virus model systems.

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4. Scientific Background.

A. Current Review.* An examination of the recent literature indicates that several laboratories are actively investigating molecular mechanisms of mammalian virus transformation in vitro. Results from these studies are most enlightening and correlate well with our research findings and program of investigation.

Todaro and Green continue to gain increasing insight into the mechanism of SV40 transformation of the spontaneously transformed 3T3 mouse fibroblast line. Extending the earlier observation (1) that non-growing cells were not susceptible to SV40 transformation, they recently reported that interferon or statalon protected the mouse line against SV40 transformation in replicating cultures (2). Exponentially growing cultures became increasingly more sensitive to SV40 transformation in the presence of interferon and the results indicated that an irreversible step in viral transformation occurred in cells actively synthesizing DNA. Todaro and Green conclude that replicating cellular DNA is the target for viral action in neoplastic conversion. Borek and Sachs (3-4) obtained results recently which complement the findings obtained with interferon. Employing primary hamster embryo cells in subculture with a 3.5 to 7.5% cloning efficiency 300 r of x-ray produced a 2.5% transforming frequency. The percentage of transformed colonies dropped rapidly when the cells were passaged prior to irradiation and no transformed clones were observed after three subpassages. Thus, only a select portion of the embryonal population (growing cells) was susceptible to transformation. The conclusion drawn from their investigation was that radiation induced transformation is an event "fixed" during active DNA synthesis, and that DNA is the affected cellular constitutent.

Borek and Sachs proposed that radiation induced error can be repaired if DNA replication is postponed until the error correction mechanism has sufficient time to effect repair. Repair mechanisms to lethal and sub-lethal x-irradiation appear to be equivalent (5). Similarly, the requirement for DNA synthesis to "fix" SV40 transformation can best be explained by the need to incorporate the viral genome or some functional derivative during this period. Otherwise, delay in incorporation may lengthen exposure of the viral genome to enzymatic degradation intracellularly and result in permanent inactivation of the virus genome.

In numerous experiments performed in our laboratory, x-ray exposures of 150 R have never been observed to produce transformed foci in vitro, whereas, infection of these cells with SV40 immediately post-irradiation routinely produces 25 or more transformed clones per 100 infected clones examined. Higher exposures to 2500 R have similarly never produced any detectable radiation-induced, transformed clones. No feeder layer was used in our procedures and the conditions are not identical to those of Borek and Sacks possibly accounting for the failure of x-ray-induced transformed clones to develop.

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* January 1967-December, 1967. For general review of scientific background to this system see part B this section.

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Sinclair reported that when Chinese hamster cells in the G_1 period were inhibited from further growth, they became more sensitive to x-irradiation and, following apparent repair, resistance to radiation returned (6). Cullen (7) observed that the transition of mouse ascites cells from in vivo to in vitro culture conditions caused an immediate increase in radiation sensitivity. Along with other investigators we observed that normal cells in culture became increasingly sensitive to SV40 transformation with aging and subculture in vitro.

SV40 transformed 3T3 mouse cells produced T antigen induced by the virus in the presence of persistent interferon treatment (8). Pretreatment of normal 3T3 cells with interferon, however, prevented T antigen synthesis. These findings suggest that, following incorporation of the SV40 genome into some stable form, interaction and stable association between host and viral genomes leads to the transcription of RNA which is interferon insensitive (a host cell characteristic) and is possibly the product of a hybrid template. A similar interferon insensitivity of T antigen synthesis was observed to adenovirus-SV40 hybrid viruses (9).

Calcium ion concentration has recently been found to directly effect adenovirus 12 transformation of rat embryo cells in vitro--more foci appeared in 0.1 mM Ca^{++} than in medium containing 1.8 mM Ca^{++} (10). In a recent study of the effects of x-ray pretreatment on the transformation frequency of hamster embryo cells by adenovirus 31, we noted that in culture medium with high calcium ion content a generalized cytopathic effect was observed. This CPE did not occur in lower calcium ion medium. The relationship of calcium to adenovirus proliferation in transformation induction is unclear, but seems highly related to the "choice" situation that may exist in bacterial cells infected with some lycogenic phage.

In a polyoma virus-hamster cell line system (11) Marin and Basilico demonstrated that puromycin inhibited cells were two to three times more sensitive to transformation than were non-inhibited cells. The effect of the protein inhibitor was entirely dependent on the time of addition of the drug, suggesting that inhibition of the synthesis of some cellular protein enhanced the potential of the virus to achieve intergration or to escape inactivation.

A series of recent reports by Fujinaga and Green (12-14) demonstrate the intimate interaction between tumor virus and host cell in the transformed state. Employing a series of elegant hybridization procedures, these workers have indicated that adenovirus transformed hamster cells which do not shed infectious virus produced mRNA which is complementary to the original infecting adenovirus DNA. This new species of polyribosome-associated mRNA is not present in normal hamster cells and, thus, provides a clear indication of the persistence of viral genetic material in transformed cells. Other data show that the new class of mRNA occurs in the nucleus of the transformed cell as well. The techniques described by these workers should greatly facilitate our own studies of SV40 transformation in the system we are employing.

These recent findings, correlated with numberous others not cited, demonstrate that many inroads are currently being made into the obscure biochemical events of viral transformation. The interaction between tumor

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virus and "susceptible" cell leading to transformation is an event occurring rapidly after viral penetration in many of the models examined in vitro and DNA synthesis is required to "fix" the transforming event. Findings and ideas advanced by these recent results from other laboratories show that the enhancing principal of x-irradiation, analogue-induced damage and aging in SV40 transformation under investigation in our laboratory relate directly to the mechanism of transformation operating in other DNA tumor virus systems and are most encouraging.

4.A References.

1. Todaro, G. J. and H. Green. 1966. Cell growth and the initiation of transformation by SV40. Proc. Natl. Acad. Sci., U. S., 55: 302-308.

2. Todaro, G. J. and H. Green. 1967. Semian virus 40 transformation and the period of cellular DNA synthesis. J. Virology, 1:115-119.

3. Borek, C. and L. Sachs. 1967. Cell susceptibility to transformation by x-irradiation and fixation of the transformed state. Proc. Natl. Acad. Sci., U. S., 57: 1522-1527.

4. Borek, C. and L. Sachs. 1966. In vitro cell transformation by x-irradiation. Nature, 210: 276-278.

5. Elkind, M. M., Sutton-Gilbert, H., Moses, W. B. and C. Kamper. 1967. Sub-lethal and lethal radiation damage. Nature, 214: 1088-1092.

6. Sinclair, W. K. 1967. X-ray survival and DNA synthesis in Chinese hamster cells I. The effect of inhibitors added before x-irradiation. Proc. Natl. Acad. Sci., U. S., 58: 115-122.

7. Cullen, B. 1967. Transient changes in the radio-sensitivity of mammalian cells on transfer from in vivo to in vitro culture conditions. Nature 213: 1268-1270.

8. Oxman, M. N., Baron, S., Black, P. H., Takemoto, K. K. Habel, K. and W. P. Rowe. 1967. The effect of interferon on SV40 T antigen production in SV40 transformed cells. Virology, 32: 122-127.

9. Oxman, M. N., Rowe, W. P. and P. H. Black. 1967. Studies of adenovirus-SV40 hybrid viruses, VI. Differential effects of interferon on SV40 and adenovirus T antigen formation in cells infected with SV40 virus, adenovirus, and adenovirus-SV40 hybrid viruses. Proc. Natl. Acad. Sci. U. S. 57: 941-948.

10. Freeman, A. E., Black, P. H., Wolford, R. and R. J. Huebner. 1967. Adenovirus type 12-rat embryo transformation system. J. Virology, 1: 362-367.

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11. Marin, G. and C. Basilico. Effect of the inhibition of protein synthesis on the establishment of transformation by polyoma virus. J. Virology, 1: 120-127

12. Fujinaga, K. and M. Green. 1967. Mechanism of viral carcinogenesis by DNA mammalian viruses. IV. Related virus-specific RNA in tumor cells induced by highly oncogenic adenovirus types 12, 18 and 31. J. Virology, 1: 576-582.

13. Fujinaga, K. and M. Green. 1967. Mechanism of viral carcinogenesis by DNA mammalian viruses II. Viral specific RNA in tumor cells induced by weakly oncogenic human adenoviruses. Proc. Natl. Acad. Sci., U. S. 57: 806-812.

14. Fujinaga, K. and M. Green. 1966.1 Mechanism of viral specific carcinogenesis by DNA mammalian viruses. I. Viral specific RNA in polysomes of adenovirus tumor and transformed cells. Proc. Natl. Acad. Sci., U. S., 55: 1567-1573.

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B. General Review of Scientific Background. (From Original Proposal)

The transformation of mammalian cells by oncogenic viruses has been shown to be either direct and rapid or indirect and slow depending on the virus and type of cell selected for study. In recent years conclusive investigations have demonstrated that certain viruses which produce tumors in vivo can also induce in vitro alterations and neoplastic transformation of cells. Rodent tissue culture cells infected with polyoma virus undergo a sequence of changes resulting in the uniform transformation of normal cells to neoplastic cells (1-2). SV40 induced the transformation of human (3) and hamster (4-5) renal cells in vitro. Cell culture-virus systems have become widely used as models for studying neoplastic conversion or transformation. The methods used and achievements made from these studies are the subject matter of recent reviews; [MacPherson (6), Temin (7), Defendi (8), Defendi and Lehman (9) and Habel and Axelrod (10)].

The three DNA-containing oncogenic viruses SV40, polyoma, and adenovirus have been used extensively for in vitro transformation studies. This proposal outlines results obtained for SV40 virus transformation and this virus will be discussed in some detail although contemplated work in the second and third year of this study would employ polyoma and certain strains of adenovirus as test agents.

Moorhead and Weinstein (11) recently restated Fould's concept (12) of progressive tumor development which serve as an excellent definition of neoplastic change that may occur in cells infected with tumor-inducing viruses. These neoplastic changes have been used as indices of in vitro transformation for the SV40-hamster cell model system. Fould's "unit characters" of tumor progression include cell morphology, culture morphology as expressed by the property of contact inhibition, antigenic changes, karyologic changes, growth characteristics and malignancy The remainder of this section of the proposal is devoted to a citation of those facts from the available literature which point to the usefulness of SV40 virus and embryonic hamster cells as an ideal model system for studying cellular transformation in vitro.

SV40 virus isolated by Sweet and Hilleman (13) produces tumors with regularity when inoculated into newborn hamsters (14). In order to examine and define the changes induced by SV40 virus in the hamster cell in vitro so as to be able to recognize any type of specific effect directly attributable to the virus, it is desirable that the host cell population be stable. That is, ideally the cells should not spontaneously demonstrate any "unit character" of the transformed cell in the absence of virus inducement.

The discovery that the oncogenic viruses produce transformation in vitro was first made using cell populations obtained from recently disaggregated tissues of mammalian origin. These cell populations have the characteristics of cell strains described by Hayflick (15); that is, they have relatively short culture life-times (50 passages or less), low-plating efficiency, and are composed of heterogenous types of cells. Cloning is achieved with the greatest of difficulty and quantitative studies of the events of transformation are not easy to perform. This fact alone has limited the usefulness of embryonic or adult hamster cells for definitive studies of the transforming process.

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When primary fibroblasts are cultured and repeatedly subcultured in vitro, their eventual fate depends on the species of mammal from which they are derived. Explanted mouse embryo fibroblasts undergo an initial phase of growth decline and then uniformly give rise to established transformed cell lines (16-17). Mouse-cells are, therefore, considered to be highly unstable, undergoing predictable spontaneous transformation. The 3T3 established mouse embryo cell line has been used recently to study the SV40 transformation process (18-20). One attractive feature in using established cell lines is that they usually are subjected to cloning with reasonable efficiency (30-40 per cent).

Todaro, Green and Black showed several other advantages in using 3T3 mouse cells recently (19-20) and reported the highest rate of transforming efficiency ever attained with DNA-containing tumor virus. The spontaneously transformed 3T3 cell exhibits contact inhibition "normally" and viral-transformants can easily be detected within the cell population as foci of piled-up epitheloid cells on a confluent monolayer cell background or as cells capable of growing in agar overlay. They achieved this high rate of transformation by employing high titer, purified SV40 preparations (22) containing 3×10^{11} $TCID_{50}$ of virus and found the number of transformations to be proportional to the virus dose through virus concentrations of 5×10^{8} $TCID_{50}$'s (virus: cell multiplicity of 600) (20). The highest efficiency of transformation obtained was 50 per cent and the efficiency advantage of the SV40 - 3T3 cell system over the polyoma-rat embryo cell system was ten-fold. The 3T3 line is heteroploid, has a high in vitro plating efficiency (12-60 per cent) as mentioned previously but retains its "normal" capacity to show contact inhibition. Twelve to eighteen days were required to detect SV40-transformed cells (20). Although conclusive evidence is lacking, it would appear that transformation does not occur in 3T3 cells unless they are actively synthesizing DNA (23). Green postulates that transformation may be "fixed" in the cell as early as one generation after virus exposure (23).

Hamster cells in culture offer suitable tools for a study of SV40 transformation in vitro (8). In contrast to mouse cells, hamster embryo cells are quite stable and survive through 40-50 generations as do human cells in culture making for an attractive experimental analogy. Primary Syrian hamster cells have low cloning efficiency (one per cent or less), demonstrate contact inhibition, remain diploid until the culture becomes senescent (9, 24) and very rarely produce spontaneous transformants. Several reports employing established hamster cells in culture described the morphological transitions observed for these cells after infection with unconcentrated SV40 preparations and suggestive evidence was found for the persistence of viral influence (antigen) in the absence of infectious virus (25-28).

The plating efficiency of the C-13 clone of the BHK/21 hamster cell line was 19-24 per cent (27). SV40-infected primary hamster lung and kidney cells showed progressive changes in metabolism and morphology and one to three months post-infection demonstrated greatly enhanced plating efficiency (40-60 per cent) (28).

The use of established mouse and hamster lines possessing at least one or more of the "unit characters" of the transformed cell described above to study SV40 transformation has been done out of necessity. The low plating efficiency of primary cells has been one of the main drawbacks to a quantitative

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study of transformation mechanisms in "normal" cells and for this reason investigators have turned to established cell lines where the transforming event can be observed with greater regularity. Transformed lines have been selected for use based upon their retention of at least one or more characters of the normal cell. The loss of this unit character of the normal cell following exposure to virus is then taken as an index of virus induced transformation. Moorhead and Weinstein (11) recently issued a statement of caution concerning conclusions that can be drawn from transformation studies using established cell lines rather than primary cell explants: "Each of these continuous lines provides an excellent assay system for studies of "transformation", but, it must be kept in mind that each has [already] acquired certain of the "unit characters" which define transformation in other systems and in some instances even in hamster and mouse systems". Ideally then, one would desire to study transformation in primary embryonic cells in culture with the hope that these cells would possess none of the "unit characters" of the transformed cell prior to exposure to SV40.

The study of SV40-transformation of the human fibroblast has received much attention in this regard in recent years (29-35, 4, 5). Embryonic or adult human fibroblast cell cultures have the advantage of a zero rate of in vitro spontaneous mutation under the usual conditions of culture and a low background of chromosome aberrations (34) during most of their lifetime in vitro. Embryonic hamster cells have a similar in vitro culture profile; Chinese hamster cells excepted (11).

Human cells infected with SV40 in culture demonstrate extensive culture degeneration and virus is shed constantly (30, 35-36) by up to 100 per cent of the cells. This leads to the possibility that cells which survive this degeneration may have been selected for by the cytopathic growth of the virus and are subsequently transformed by the virus. Hamster cells, correspondingly, show no evidence of destruction following infection with SV40 (4-5, 8) making these cells very desirable for studying the transforming process if the equally important problem of plating and transforming efficiency could be circumvented. Defendi states (8) that large amounts of virus are needed to achieve one transforming event and that only a fraction of the cell population is susceptible to transformation when exposed to a maximum amount of virus. The efficiency of transformation is very low using either primary or established cells. Generally accepted values from numerous reports in the literature indicate that one to ten million functional virus particles are required to transform one normal cell to the neoplastic state. This means that one must "plate" and clone 2,000 infected cells to obtain 10 to 12 transformed clones. The problem is even more formidable when it is recognized that the usual cloning efficiency by the agar technique falls in the range of 0.5 to 1 per cent. The rarity with which the viral induced transforming event occurs has greatly limited any pragmatic analysis of the mechanism(s) involved or the sequential stages of the transforming process, if indeed such a sequence exists.

The recent findings of Todaro and Green (21) and Black (20) show that one can produce transforming efficiencies as high as 30-50 per cent using high titers of SV40 virus but the cell system (3T3 mouse line) involves the use of cells having already undergone a certain "degree" of transformation (heteroploidy and infinate growth characteristics). Several attempts have been made to modify the susceptibility of cells to transformation and some success has been obtained.

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Aged human cells in culture nearing the degenerative phase of growth show an enhanced efficiency of transformation by SV40 (32-34). Chromosomal studies have shown that these cells retain the diploid state until the 37th-41st subculture after which time spontaneous aneuploidy was noted (32). Similar results were obtained with hamster embryo cells (9). Chromosome breaks have frequently been associated with aging and SV40 or polyoma infection [see Moorhead and Weinstein (11)].

The hamster line BHK/21 has been used in attempts to alter the susceptiability of cells to transformation. The cells were pretreated with increasing doses of x-irradiation and subsequently infected with polyoma virus (38). Although the doses of irradiation used diminished the surviving population rapidly, Stoker was able to observe an apparent two to three-fold enhancement in the percentage of transformed cells detected out of the surviving population which had been reduced by approximately one log. No evidence was apparent to suggest that irradiation favored the survival of transformed cells. One possibility Stoker suggests to explain the enhancement of transformation post-irradiation was that the damaged chromosomes which resulted from radiation exposure might be more susceptible to the intergration of the viral genome. Radiation inactivation studies recently showed that when irradiated polyoma virus is used to transform BHK/21 cells, the radiation target of the viruses transforming ability is about one half the size of the target for the viruses lytic activity (39). This is consistent with the hypothesis that part of the viral genome is incorporated into the genome of the transformed cell. Treatments which stimulate chromosome breaks should greatly facilitate transformation if the "intergration" hypothesis is valid.

The variability in transforming efficiency observed by Stoker (38) using irradiated cells may result from the combined variations in the efficiency of the radiation-damage repair mechanism for each cell (40-42). It is known that for each cytologically observed chromosome break induced by irradiation as many as 100 breaks may have healed (11).

Todaro and Green (42) reported the enhancement of SV40 transformation of the 3T3 mouse line by 5-iododeoxyuridine (IUDR) and 5-bromodeoxyuridine. They obtained an enhancement factor of five-fold increase in transforming frequency when 3T3 cells were exposed to 5 μg/ml of IUDR for 24 hours prior to infection with SV40. The drug was only incorporated into growing cells (43-44). Morris and Crammer (44) reported that IUDR is incorporated into the DNA double helix and this suffices to injure the chromosome and thus, the cell. Trecently we observed chromosome breaks which were induced by exposure of SV40-transformed hamster tumor cells to IUDR in vitro (45)

The effectiveness of all these treatments seems limited to a brief period of time preceding the interaction of the virus with the cell. If the treatments described above and related phenomena occurring in senescent cells result in or involve chromosome breaks, the possibility clearly remains that the viral genome or some fraction of it may be intergrated into the chromosome of the cell before the onset of the morphologic or physiologic events of transformation can begin. Recent findings (18) suggest a two-step reaction in the transforming process supporting the initiator-promotor phenomenon in carcinogenesis postulated by Berenblum (46).

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Defendi (8) points out that in SV40-infected cells it is difficult to avoid the conclusion that the primary event in the possibly long series of steps leading to transformation of normal cells must necessarily involve chromatid or chromosome lesions. Chromosome damage may be the primary event in transformation. Whether this event must occur naturally, by virus induction, by processes related to aging, or is induced by physical factors is a question that needs definitive study.

Two lines of evidence support his contention although certainly they do not by any means prove it. First, SV40 virus induced chromosome damage and normal human cells in the degenerative phase are very prone to transformation when compared to "young cells". Second, treatments or compounds (47) which produce DNA instability or chromosome change enhance the frequency of transformation.

The significance of this literature review is that circumstantial but factual evidence suggest that chromosome damage, if not necessarily a primary step in natural SV40 transformation, still leads to enhancement of transforming efficiency *in vitro*. Advantage of this fact can be taken to obtain a test system where transformation is a frequent rather than a rare occurrence using primary hamster cells. Such a system can then be used to make a quantitative investigation of the transformation process with the advantage that normal cells rather than cells which have undergone certain "degrees" of transformation can be used as part of the test system. Such a system has been developed in this laboratory and is the subject of the following section.

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4C. Funding from other agencies.

AEC alone sponsors our program of study in the SV40-induced transformation program. We did receive $5000 from the National Space and Aeronautics Administration Contingency Fund this past year to purchase an ultracentrifuge for this program. Permission was requested from C. S. Shoup to use the $7,835 given us by the AEC for the purpose of purchasing an ultracentrifuge for other equipment needs and was granted. We are not applying for other funds for this research.

We also are investigating the immunity mechanism in hamsters to SV40 transplantation antigen as another phase of our overall research program and this research is sponsored by the National Cancer Institute, Grant No. CA 10429-01

5. Scientific Scope.

This research effort is designed to investigate the precise parameters of an enhanced transformation phenomenon recently observed in this laboratory. The unusual aspect of the study is that it involves a quantitative investigation of virus transformation in normal cells. Quantitative studies by other investigators which deal with viral transformation have, of necessity, involved cell systems which have already undergone some degree of neoplastic change in that they clone readily and usually exhibit abnormal karyotype before exposure to virus. Recent experiments in this laboratory demonstrate that the plating efficiency of primary hamster embryo cells can be greatly increased by employing modified culture techniques and that SV40-transformed embryo cells exhibit good cloning efficiency in agar overlay. In our laboratory we have observed that pretreatment of embryonic hamster cells with x-irradiation, 5-iododeoxyuridine (IUDR) or aging enhances the efficiency of SV40 transformation of primary hamster cells. Our immediate objective in this regard will be to define the conditions of culture, virus dose effects, radiation dose responses, periods of DNA synthesis, and metabolic conditions of the cell which relate to the phenomenon of transformation enhancement.

A. Rationale.

Stoker (38)*, Todaro and Green (43) and others (32-34) have demonstrated that established cell lines or diploid human cells in culture are rendered more susceptible to viral transformation by x-irradiation, analogue exposure or by simply ageing the cells to a degenerate stage prior to infection with oncogenic viruses. We have recently extended this observation, for all three types of treatment, to normal hamster embryo lung and kidney cells in culture.

The importance of studying the mechanism of viral transformation in vitro using a cell system with no unit character of the malignant or transformed cell prior to virus exposure cannot be over-emphasized. Great strides in our understanding of viral transformation are indeed being made with systems such as the 3T3 cell line system, but it is imperative to correlate these observations with neoplastic conversion occurring in normal cells. Such a correlation is one of the major objectives of the present line of research in this laboratory using normal embryonal cells

* Reference numbers relate to section 4, part B listing.

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rendered susceptible to SV40 transformation by agents which damage DNA.

Two factors make the system, presently in use, suitable for the study outlined in this proposal. First, by employing new methods of cell harvest and by carefully controling culture conditions (Please see 1967 progress report Appendix A for details), high plating and cloning efficiency of embryonal cells has been achieved. In a recent report Borek and Sachs investigated x-irradiation-induced transformation of embryonal cells using preparations with a cloning efficiency of 3.4 to 7.2%. Our method yields plating efficiencies up to 95% and cloning efficiencies as high as 63%.

Second, employing cells with good plating efficiency, we obtain 15 to 50-fold increases in SV40 transformation of embryonal cells (taken directly as explant cells) when cells are exposed to 150 R of x-ray just prior to infection.

Employing this system where 25-30 percent of infected cells produce transformed clones we have initiated an investigation of the parameters and conditions of enhanced SV40 transformation.

The results cited below and the research planned will clarify and define a number of conditions which affect the transformation mechanism in vitro. Planned studies should also provide a better understanding of the biochemical sequence of events in transformation. Finally, an increased understanding of the biological effects of radiation and radiomimetic chemicals should be forthcoming from this study.

B. Recent Results.

Specific findings in the past nine months show:

(1) SV40 transformed embryo cells given 150 R of x-ray prior to infection do not appear to be unique transformants; that is, the majority of cells were found to produce clones containing T antigen. Most clones were malignant upon transplant into syngeneic hamster strains and were generally virus-free after several subpassages in vitro.

(2) The role of radiation dose (extent and degree of x-ray damage) in stimulating enhancement is under investigation and a titration of radiation exposure on cell viability has been completed. Doses to 750 R were not observed to stimulate post-mitotic exponential killing and we are presently determining the effects of this dose range on transformation frequency.

(3) Periods of active DNA synthesis and dimunition have been defined in x-irradiated embryo cells.

(4) SV40 was not observed to alter the survival plots of lethally irradiated embryo cells in preliminary studies.

C. Specific Studies Planned or on Test.

(1) The titration of radiation dose and cell viability is completed and the effects of increasing radiation exposure on the observed frequency of SV40 transformation is now being

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evaluated. These studies are time consuming and each experiment must be repeated several times to obtain the proper confidence levels. All data are being statistically evaluated by computer analysis to determine interaction levels and limits. (Estimated time to completion of titration is 6 to 8 months).

(2) Chromosome karyologic studies are under way in embryo cells to correlate numerical aberrations induced by exposure to x-ray with transforming frequency. Preliminary results show that no change in ploidy is evident in cells given radiation doses as high as 2500 R.

(3) We are preparing highly purified virus pools of SV40 and enumerating infectious particles by the plaquing technique in an effort to correlate virus multiplicity of infection with transforming frequency in cells pretreated with 150 R of x-ray.

(4) A precise evaluation of the time of virus addition post-irradiation which has the most striking effect on transformation efficiency is planned. Such a study should give considerable insight into the role of DNA repair mechanisms in the transformation phenomenon. Inhibitors of protein, DNA and RNA synthesis will be employed to further characterize repair mechanism and their influence on SV40 transformation.

(5) Having characterized periods of DNA synthesis and suppression post-irradiation, experiments are now planned to correlate infection sequence with cell replication periods in x-irradiated embryo cells.

(6) A Beckman Tm analyzer has been made available for our use and plans are being made to determine alterations in G:C content of cloned SV40 transformed cells which were irradiated prior to infection. The thermal melting patterns of DNA from these transformed cells will be compared with those of DNA from normal cells and SV40 DNA in an effort to detect the presence of the SV40 genome in the neoplastic cell by altered Tm plots. This study is a deviation from the original outline, but will not interfere with planned work outlined above and may produce valuable results.

These studies will occupy the majority of our efforts in the next year. Additionally, however, we will investigate the following parameters in the third year of the study as time and facilities permit.

(a) Evaluate rates of host cell and viral DNA synthesis after treatment with irradiation or IUDR using isotopic procedures already worked out in dttail. Characteiize the types of DNA synthesis occurring in cells during transformation. Methods available from the following sources:

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Black, P. and W. Rowe. 1965l Proc. Nat. Acad. Sci., 54: 1126-1133.

Gershon, D., P. Hausen, L. Sachs and E. Wincour. 1965. Proc. Nat. Acad. Sci., 54: 1584-1592.

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Wincour, E. 1965. Virology 24: 276-288.

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Reich, P., P. Black, S. Weissman. 1966. Proc. Nat. Acad. Sci., 56: 78-85.

Dulbecco, R., L. Hartwell and M. Vogt. 1965. Proc. Nat. Acad. Sci., 53: 403-410.

(b) Compare the effects of specific antimetabolites and inhibitors of DNA synthesis and function on radiation and IUDR-damaged cells in an attempt to define any possible sequence of events leading to transformation.

(c) Evaluate other inhibitors of DNA activity for the enhancement phenomenon.

eg. (1) Ultraviolet irradiation
(2) Mitomycin C
(3) Hydroxylamine
(4) Bromouracil
(5) Bromodeoxyuridine
(6) Colchicine
(7) Cytosine arabinoside ref: Nichols et al. Hereditas 52: 101-118, 1965.
(8) Fluorodeoxyuridine ref: Taylor et al. Proc. Nat Acad. Sci., 55: 1141-1148, 1966.
(9) Other inhibitors of protein transcription or translation.

(d) Evaluate the effects of sublethal irradiation and chemical treatment on other types of cells which demonstrate resistence to SV40 polyoma or adenovirus transformation. eg. Adenovirus transformation of irradiated monkey kidney cells which normally do not demonstrate transformation by adenovirus.

(e) The role of low levels of virus multiplication in cells infected with oncogenic viruses can now be investigated. The long "latent" period in other systems and the low number of transformants obtained presents a problem in being able to detect the presence

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of small numbers of virus particles harbored intracellularly. In the new system where transformation occurs in a high proportion of the infected cells and more rapidly than in other systems (7-10 days as compared with 18-60 days reported for other systems) cells can be "analyzed" for intracellular virus multiplication employing sonication and plaquing techniques.

(f) Investigate the possibility of SV40 rescue of lethally irradiated cells. The premise for this study would be that since incorporation of viral genome may lead to rapid transformation cells exposed to sublethal irradiation, it might also lead to transformation and ultimately "rescue" in cells given lethal irradiation.

(g) Determine levels of interferon production in normal and irradiated cells to evaluate the role of radiation in possibly inhibiting interferon production thereby leading to rapid transformation. Ref: Todaro and Green.Proc. Nat. Acad. Sci., 54: 752-756, 1965.

(h) Evaluate the effects of low level irradiation on the ability of non-oncogenic or poorly oncogenic viruses to achieve transformation eg. Adenovirus type 7 cultured in sublethally irradiated hamster cells.

(i) Evaluate the effects of cell synchronization at the time of irradiation on the enhancement phenomenon in the hope of defining the phase of cell division which might be involved in the the initial step in viral transformation.

(j) Evaluate the effects of radiation and specific chemical treatments (mutagens) on the reversion of virus-induced neoplastic tissue to the nommal state as expressed phenotypically. eg. reinstitute oriented growth, or contact inlibition in neoplastic cells.

(k) Test for possible synergistic effects of radiation and chemical treatment on the enhancement phenomenon. eg. IUDR + x-irradiation.

D. Development of Oncogenic Virus Research Program.

This study, currently in progress and sponsored by AEC, is an integral part of a developing program of virus and cancer research at this institution. These *in vitro* investigations of viral oncogenesis complement *in vivo* studies of virus tumorigenesis in progress in our laboratory which are sponsored by the NIH. A significant change has been effected in the rapport between this department and the Oak Ridge National Laboratories since the initiation of this grant and this program offers a vital outlet for graduate training of Oak Ridge personnel interested in oncology. Further, the program affords students in the radiation biology program at

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this institution a research outlet. Three graduate students supported by the radiation biology training program are currently working or planning to actively participate in this research program.

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6. Scientific Personnel. Principal Investigator.

Joseph H. Coggin, Jr; Soc. Sec. No. 424-44-4749. Ph. D, Assistant Professor of Microbiology. Dr. Coggin will spend 40 percent time on this project for the entire year.

(a) Experience. Consultant Virologist to Biophysical Separation Laboratory. Virologist and Assistant Professor. 1966-Present. University of Tennessee. Lecturer in virology and molecular biology at graduate and undergraduate level. Principal Investigator-AEC contract No. AT(40-1)3646 Principal Investigator NIH Grant-CA-10429-01. National Cancer Institute.

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 Principal Investigator, 1965. NIH Grant CA-07525. Responsabilities included supervision and planning of research for laboratory 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 in 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.

(b) Pertinent Publications.

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

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

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

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Metabolism of 6-mercaptopurine by Resistant Escherichia coli cells. 1966. J. H. Coggin, M. Loosemore and W. R. Martin. J. Bacteriol., 92, 446-454.

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

Submitted for Publication.

Radiation Responses of Normal and SV40 Transformed Hamster Cells. J. H. Coggin and R. E. Kouri. 1967. Proc. Soc. Expt. Biol. Med.

Enhanced SV40 Transformation of Hamster Cells in vitro By Irradiation, Thymidine Analogue Exposure and Aging. 1967. J. H. Coggin. J. Virology.

Metabolic Studies in Anucleate Escherichia coli cells. 1967. D. J. Silverman and J. H. Coggin. J. Bacteriol.

Growth Responses of Escherichia coli Cultures Producing Anucleate Cells. 1967. J. Bacteriol.

6. A Junior Investigators.

Richard E. Kouri. M. S. Graduate Student. Radiation Biology Trainee. 75 percent time to project.

Frederick Miller. Graduate Student. A. E. C. Supported. 85 percent time to project.

Kathleen R. Ambrose. Graduate Student. 20 percent time to project.

7. Other Personnel.

Laboratory Assistants. Four Undergraduate Students. 20 hours per week, each. Work-Study Students.

Animal Room Worker-one graduate student, 40 hours per week. Work Study-NIH sponsored.

Laboratory Aide-Miss Linda Mullins. 25 hours per week. A. E. C. Sponsored.

8. Other Financial Assistance.

Mr. R. E. Kouri is supported by a U. S. Public Health Service traineeship through the Radiation Biology Training Program.

Miss K. R. Ambrose is sponsored as laboratory assistant by NIH Grant No. CA-10429-01. Since some of her duties on this project overlap similar duties for the A. E. C. Program (preparation of virus pools for both projects, etc.,), it is estimated that she contriuutes 20 percent of her time toward A. E. C. related work.

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Most of the Laboratory Assistants listed in (7.) above are devoting full-time (20 hours each per week) to AEC related projects and are sponsored by funds available through the U. S. Office of Education under the Work-Study Program.

9. Premises, Facilities, Equipment and Materials Furnished by Contractor.

The Department of Microbiology has a 6000 square foot laboratory facility devoted to research purposes in Hesler Biology Building. This area has been newly constructed with matching funds from the Universtiy and the National Science Foundation. 2500 square feet of this area are given to virologic research including new tissue culture handling facilities, animal isolation facilities, virus research laboratories, constant temperature rooms, walk-in cold rooms and incubators, instrumentation room, media preparation area and glassware washing facility.

The virology laboratory is newly equipped with tissue culture microscopes, centrifuges, CO_2 incubator, water baths, fluorescent microscope, radiation scalers, serology equipment, and essential glassware. An excellent science library facility with necessary current journals are conveniently available in the biology building.

Funds made available in the past year ($57,175.00) through the AEC, NIH, NSF, NASA and University have enabled us to obtain the following items for virologic research: Beckman DB-G spectrophotometer DU-2 spectrophotometer, LS-100 liquid scintillation system, recording spectrophotometers,Tm thermal melting analyzer, CO_2 incubators, Revco Ultracool storage box, tissue culture incubators, a new 4°C walk-in facility, Ultracentrifuge, 4 refrigerated centrifuges and fraction collection devices. The University has provided many small items of equipment as they have been needed over the past year.

Our research capability has been significantly increased in one year's time with the availability of this new instrumentation, enabling us to carry out the planned research more adequately than before and at a considerable saving of time. The spectrophotometer originally requested for the second year of the research program, is no longer needed. Instead, we badly need the following items which are either not available or are in short supply:

1-Mettler Analytical Balance Model H6T- None Available
1-Mettler P1200 Top loading Balance- None Available
1-Nikon Phase Tissue Culture Scope- Only one available and in constant use
1-PR2 Refrigerated Centrifuge. One instrument obtained this past year from AEC for preparing cells for culture is not sufficient for the current work load and delays are created frequently.

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10. Budget. (Renewed Proposal)
Second Year: From March 15, 1967 to March 14, 1968

	Estimated Requirements Second Year
1. Salaries and Wages:	
Principal Investigator, Joseph H. Coggin, Assistant Professor. 40% time for 12 months ($14,375. yearly including summer).	$5,750.00
Graduate Assistant, Mr. Frederick Miller, 85% time for 12 months.	2,800.00
Lab Assistant, Miss Linda Mullins, 50% time	1,200.00
Animal Maintenance Helper, Mr. Roy Tyler, 20% time	800.00
Social Security and Retirement (10% of Salaries and Wages excluding Coggin)	480.00
2. Supplies and Materials:	
Chemicals	1,400.00
Isotopes	1,250.00
Cages and Racks	1,000.00
Animals, food and bedding	750.00
Media and Serum	1,500.00
Expendibles (culture dishes, tubes, gas)	1,000.00
Tissues (Monkey kidney cells, etc.)	1,750.00
3. Equipment:	
Mettler Analytical Balance 4T6 Model	950.00
Mettler Top Loading Balance Model PL 1200	550.00
International PR 2 Centrifuge	2,980.00
Sorvall Omnimixer Blender	450.00
2 Tissue Culture Roller Drum Assembly (Lobline)	450.00
4. Publications	125.00
5. Travel (Dr. Coggin, two annual national meetings)	500.00
6. Other-telephone and mailing costs	195.00
7. Indirect Charges-50.4% salaries and wages overhead rate,* (excluding salary for J. H. Coggin which is part of the University's contribution)	2,419.20
Total Project Cost	$28,299.20
Percentage and amount to be contributed by the University of Tennessee-24.56% (Principal Investigator's salary plus $1200 in supplies category)	6,950.00
Percentage and Amount Requested from AEC-75.44%	21,349.20

*50.4% of salaries and Wages Overhead Rate. This is a new tentative overhead rate based on fiscal year 1966 operation. Formal application in support of this rate has been made to the Department of Defense (DCAA), and approval is expected in the near future.

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The items listed in the equipment category cannot be supplied by the University during 1968-69 fiscal year.

The amount of funds to be requested for the third year of the program cannot be accurately estimated at the present time but should approximate the amount requested for the second year.

II. Authentication.

Principal Investigator

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

For the University

Original Signed By
HILTON A. SMITH

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

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SI-SIE-78A; REV. 6-64

NOT FOR PUBLICATION OR PUBLICATION REFERENCE

NOTICE OF RESEARCH PROJECT
SCIENCE INFORMATION EXCHANGE
SMITHSONIAN INSTITUTION

UNITED STATES ATOMIC ENERGY COMMISSION

SIE NO.

AGENCY NO.

SUPPORTING AGENCY: Division of Research

TITLE OF PROJECT: A Comparative Study of Radiation, Chemical and Aging Effects on Viral Transformation In Vitro.

Give names, departments, and official titles of PRINCIPAL INVESTIGATORS and ALL OTHER PROFESSIONAL PERSONNEL engaged on the project.

Dr. Joseph H. Coggin, Jr., Assistant Professor (Principal Investigator)
Department of Microbiology

NAME AND ADDRESS OF INSTITUTION:

University of Tennessee, Knoxville, Tennessee 37916

SUMMARY OF PROPOSED WORK – (200 words or less.) – In the Science Information Exchange summaries of work in progress are exchanged with government and private agencies supporting research, and are forwarded to investigators who request such information. Your summary is to be used for these purposes.

A study of SV40 transformation was initiated in this laboratory eleven months ago in an effort to define the sequence of molecular events which result in the conversion of normal hamster embryo cells to neoplastic cells in vitro. A unique system has been developed for this purpose. SV40 virus transforms normal hamster embryo cells in culture within an efficiency range of 0.1 to 0.4%. If the cells were subcultured until they became senescent (15 to 17 passages in vitro or 87 days) and then infected with SV40 virus, the efficiency of transformation increased to 14.3%. Pretreatment of young, embryo cells with 150R of x-ray just prior to infection with SV40 resulted in rapid transformation of surviving cells with an efficiency of 27.8%. Similar results were obtained by pretreatment of cells with a radioimmetic thymidine analogue. The similarities between the reported biological effects (chromosome damage) of x-irradiation, thymidine analogue exposure and aging suggested that a common mechanism in enhancing the viral transformation "process" may be operating for all three treatments.

The experimental approach selected for this program seeks to clarify the role of x-irradiation, DNA antagonists (5-iododeoxyuridine) and aging in potentiating SV40 transformation of hamster cells in vitro. Our first efforts have been directed to a definitive examination of the parameters of the irradiated cell test system which exhibits enhanced transforming efficiency. A titration of x-ray exposure on embryonal cell survival has been completed and experiments are presently underway to evaluate the effects of increasing radiation exposure on the frequency of transformation. A tissue culture method has been developed for performing all studies with cells from primary explant, avoiding the use of subcultured cells and eliminating many selection events. Periods of active DNA synthesis post-irradiation have been defined and studies are now in progress to correlate these periods with sensitivity to SV40 transformation. An evaluation of several DNA antagonists for potential to enhance SV40 transformation has been completed.

SIGNATURE OF PRINCIPAL INVESTIGATOR Joseph H Coggin Jr

PROFESSIONAL SCHOOL (medical, graduate, etc.) Graduate School - Liberal Arts

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