UNITED STATES
ATOMIC ENERGY COMMISSION

707787

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

AREA CODE 615
TELEPHONE 483-8611

MAY 4 1970

REPOSITORY Oak Ridge Operations Ofc. Records Holding Area
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Ralph Elson, Director, Contract Division

REQUEST FOR CONTRACT ACTION

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

1. Nature of Action Requested:

   [X] Selection of New Contractor and/or Negotiation of Contract
   Number: AT-(40-1)-3646
   Contractor: U. of Tennessee

   [ ] Modification of Contract
   Number:
   Contractor:

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 CA: $21,349.00

5. AEC Percentage of Est. Total Cost To Be Shown by This CA: 76%

6. Description of Other Changes To Be Covered by This CA:

   Modify contract to provide for the performance of additional research to be completed during the period 4-1-70 - 3-31-71. The AEC support ceiling for the extended period is $21,349. Title to equipment shall vest in the contractor under authority of the AEC Act of 1954.

7. Authority:
   Form AEC-481 (CA) fr J. R. Totter dtd 4-17-70. CONTRACTS - 3646 (Tenn)

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OLE: LM
Medley
5-4-70

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

C. S. Shoup
5-4-70

1033908

APPENDIX "A"

For the Contract Period April 1, 1970 through March 31, 1971.

A-I RESEARCH TO BE PERFORMED BY CONTRACTOR

The Contractor will continue studies of radiation, chemical, and aging effects on viral transformation, and conduct studies of the fundamental mechanisms in virus-cell interactions that lead to conversion toward malignancies. Radiation, certain pyrimidine analogues, and cell aging will be studied in the sensitization of hamster cells to SV40 and adenovirus transformation. Target cell DNA will be studied, and the metabolic changes occurring with infection with SV40 will be investigated including the tumorigenicity of SV40 in the neonatal animal and as influenced by x-ray exposure. Radiation sensitivation will be evaluated and in relation to tumor production.

The Principal Investigator, Dr. Joseph H. Coggin, Jr., expects to devote approximately 40% of his time or effort to the project work during the academic year.

A-II WAYS AND MEANS OF PERFORMANCE

(a) Items Included in Total Estimated Cost:

(1) Salaries and Wages: $13,060.00

Principal Investigator
Research Assistant
Laboratory Assistant
Labor

(2) Employee Benefits: 730.00

(3) Materials and Supplies: 5,500.00

Chemicals, cages and bedding, media and sera, plastic culture ware, and CO_2.

(4) Equipment to be Purchased or Fabricated by the Contractor: 1,100.00

a Equipment Estimated to Cost Less than $1,000:

None

b Equipment Estimated to Cost in Excess of $1,000:

Transfer and Tissue Culture Hood

1033909

(5)	Publications:	$ 295.00
(6)	Travel:	250.00
(7)	Communications:	200.00
(8)	Indirect Cost (54.2% of Salaries and Wages):	7,078.00

(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 Consideration in Proportioning Costs:

None under this paragraph

A-III The total estimated cost of items under A-II (a) above for the contract period stated in this Appendix "A" is $28,213.00; the Commission will pay 76% 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-XXVII. The estimated AEC support cost for the contract period stated in this Appendix "A" is $21,349.00.

APR 21 1970

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

CONTRACT NO. AT-(40-1)-3646

Dear Dr. Coggin:

We have authorization to extend Contract No. AT-(40-1)-3646 through March 31, 1971, with $21,349 new AEC funds. Please advise us whether or not the University is willing to accept the approved financing toward the contract project cost of $29,718. If it is not acceptable, please submit the revised budget, as soon as possible, based on the approved financing. Please have the information submitted and endorsed by the appropriate administrative official of the University.

Sincerely,

ORIGINAL SIGNED BY
C. S. SHOUP

C. S. Shoup, Chief
Research Contracts Branch
Laboratory and University Division

OLE:LM

CC: Hilton A. Smith, Univ. of Tenn.

OAK RIDGE OPERATIONS
US AEC

Z 2443

CONTRACTS -3646 (Tenn)

OFFICE ▶	Adm. Ser. Br.	Res. Cont. Br.			
SURNAME ▶	Medley:lbd Medley	CSShoup			
DATE ▶	4/20/70	4-21-70			

Form AEC-318 (Rev. 9-53)

U.S. GOVERNMENT PRINTING OFFICE : 1966—O-214-629

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AECPR 9-4.51

U. S. ATOMIC ENERGY COMMISSION

CONTRACT AUTHORIZATION

1. DATE: APR 17 1970

2. AUTHORIZATION NO.: BM-70-478

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
Department of Microbiology

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

5. ☐ NEW CONTRACT ☒ RENEWAL ☐ OTHER

6. TERM OF CONTRACT: 4/1/70 thru 3/31/71

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

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

9. EQUIPMENT 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 Transformatic In Vitro"

12. HEADQUARTERS TECHNICAL CONTACT
George E. Stapleton

13. FINANCING

A. OPERATING EXPENSES

New AEC Funds	$ 21,349
Estimated AEC Balance From Prior Term, if any	$ -
	$ 21,349
Estimated Contractor Contribution, On Proportionate Sharing Basis, if any	$ *
Estimated Project Cost, For Pertinent Budget Period	$

Budget and Reporting Classification: 06 02
Allotment Transfer: 06-01-91(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.

* To be determined.

15. SCOPE OF WORK

An investigation of enhancement of viral transformation in mammalian cells by x-rays and DNA base-analogs.

CONTRACTS — 3646 (Tenn)
B 3206
APR 20 1970

1033912

April 16, 1970

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

Dear Dr. Coggin:

This is 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 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,

George E. Stapleton
Radiation Biologist, Biology Branch
Division of Biology and Medicine

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

B 3206

APR 20 1970

1033913

THE UNIVERSITY OF TENNESSEE
KNOXVILLE 37916
OFFICE OF THE VICE CHANCELLOR
FOR
GRADUATE STUDIES AND RESEARCH

203 ADMINISTRATION BUILDING

AREA 615
TELEPHONE: 974-3466

April 22, 1970

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

Attention: Mr. Medley

Re: Contract No. AT (40-1)-3646

Gentlemen:

In accord with your telephone conversation of April 20 with Dr. Joseph H. Coggin, Jr., of our Department of Microbiology, this is to advise that the amount of $21,349.54 in AEC funds for the fourth-year support of the above-referenced contract is acceptable to the University. The total project cost for this research has been reduced to $28,213.98. To accomplish this, the items of isotopes, $500, and hamsters and rabbits, $1,000 (in the supplies and materials category) have been deleted; the publication costs have been reduced from $300 to $295.46.

We trust that this meets with your satisfaction.

Sincerely,

Hilton A. Smith

Hilton A. Smith
Vice Chancellor
Graduate Studies and Research

jdk

CC: Dr. Joseph H. Coggin, Jr.

P.S. I have just received a copy of your letter of April 21, addressed to Dr. Coggin, in which you indicate that AEC funds should be provided toward a total project cost of $29,718. It is our intention to provide the isotopes and hamsters and rabbits from other sources.

H.A.S.

B 3331
APR 23 1970
CONTRACTS — 3646 (Tenn)

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1794/1969 175 Distinguished Past... Dynamic Future

Lt U file

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

AREA CODE 615
TELEPHONE 483-8611

January 12, 1970

J. R. Totter, Director, Division of Biology and Medicine, HQ

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

We are submitting for your review and appropriate action the following information concerning the contract which will expire on March 31, 1970:

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, Director
Laboratory and University Division
Oak Ridge Operations

OLE: Medley 1-12-69
LM

Enclosures:
As Listed Above

DC: R. L. Shannon, w/cy Progress Report
C. S. Shoup, w/cy encls.
D. S. Zachry, w/cy Progress Report
Alice Brown

JAN 13 1970

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CONTRACTS - 3646 (Tenn)

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Renewal Proposal

Fourth Year

Contract
AT (40-1) - 3646

University of Tennessee
Department of Microbiology

Principal Investigator

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

December 30, 1969

1033916

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.

The parameters of virus-induced tumor production will continue to be evaluated in a primary cell culture system. Quantitative investigations of fundamental mechanisms involved in virus-cell interaction leading to malignant conversion will be conducted. Radiation, certain pyrimidine analogues and cell aging markedly sensitize hamster embryo cells to SV40 and adenovirus transformation. All three methods employed for sensitizing normal cells to virus transformation are observed to render lesions in the target cell DNA suggesting that a common mechanism may be involved in enhancement. Disclosure of the mechanism(s) for enhancing virus transformation is a primary objective of this study. Metabolic, physical, structural and regulatory changes occurring prior to, during and after infection with SV40 in pre-irradiated hamster cells will continue to be investigated in an orderly fashion. Localized radiation has now been observed to increase the tumorigenicity of SV40 in neonatally infected hamsters when virus is administered in the area of low-level x-ray exposure. The radiation levels employed to increase tumor appearance in hamsters by 50% is comparable to dosages delivered in certain diagnostic x-ray procedures in humans. Radiation type and dosage will be evaluated to determine the usefulness of the radiation sensitization technique to detect oncogenicity among teratogenic agents in hamsters and to increase the tumorigenic potential of weakly oncogenic agents.

4. Scientific Background.

A. Summary of Recent Literature - (See previous renewal application for older reviews)

Investigations of the mechanism of virus-induced cellular transformation *in vitro* have increased by a factor of at least ten in the past two years. Dulbecco's recent review (1) entitled "Cell Transformation by Viruses" clearly outlines the accomplishments made to date and define the problems that lie ahead in this most

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important area of cancer research. At this point (2 years, 9 months) into our own study of virus-induced tumors, it is important to review summarily, pertinent new facts about the small, DNA-containing tumor viruses SV40, polyoma and certain adenoviruses and to briefly reconsider what is known of how these agents interact with cells to produce tumorous foci *in vitro* and cancer *in vivo*. Having done this, certain adjustments have been made in our research plan devised some three and one-half years ago and our future efforts can be realistically concentrated on those problems most in need of exploitation.

SV40, Polyoma and Adenoviruses:

SV40 and polyoma are among the smallest of the DNA-containing animal viruses. The molecular weight of their DNA is 3.2×10^6 daltons, enough genetic information to code for six to ten small proteins. Both viruses exhibit several "forms" of DNA in infected cells and both seem to have ring-shaped or circular DNA (polyoma virus has also yielded supercoiled DNA: see Crawford reference 2). Adenovirus DNA is believed to be a linear structure with a molecular weight of 23×10^6 daltons (3).

The guanosine + cytosine ratios of polyoma, SV40 and the oncogenic adenoviruses are 48%, 41% and 47-49%, respectively (2). Mammalian cell DNA usually possesses a G+C ratio of 42-43%.

Viral DNA of the circular type can be separated from the cellular, linear DNA by sedimentation velocity centrifugation technique (2, 4, 5).

Transformation in vitro:

The studies of transformation induced by SV40 and polyoma virus conducted in the past three years have clarified certain points concerning the mechanism of transformation and have set the stage for future investigations. Certain facts are now in evidence to facilitate our work:

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1. SV40, polyoma and certain adenovirus cause either stable or an abortive type of transformed cell clones following infection (1).

2. Only non-permissive cells are susceptible to normal virus transformation, most probably by limiting the expression of late virus functions which result in virion maturation and cell death. Permissive cells can only be transformed by defective or mutant virions incapable of late viral synthesis or maturation and the induction of cell death.

3. In certain cell lines a rather high frequency (40-50%) of transformation can be achieved (3T3-mouse skin fibroblasts) when a high multiplicity of input virus is used but the frequency of transformation observed for diploid or normal primary cells is very low (0.2 - 0.5%). Abortive transformation is frequently and readily observed in cell lines exposed to virus (eg. BHK-21 cells) but most of the cell foci cease growing as transformed cells after a few cell generations with only a small fraction becoming stable transformants (6, 7). It is unclear why many clones show abortive transformation, with only a few becoming stable but the state of the viral DNA in the cell seems to hold the key to the explanation (1).

4. Numerous workers have reported the recovery of infectious virus from stable cell lines transformed by SV40 which for generations had shown no mature virions (1). Such is not the case for polyoma transformed cells (1). Fusion of transformed SV40 cells with producer or permissive cells employing Sendai virus has been the most popular technique (8-11). These and other studies have shown that certain transformed lines which do not apparently contain infectious virus for a number of passages can be stimulated to yield infectious virus when heterokaryon genetic information is added to the cell environment to code for cell functions required for virus synthesis or maturation. Rescued SV40 is more efficient in transforming the mouse cell

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line, 3T3, than was the parental virus (10). It is important to discover why one cell clone is transformed in a stable fashion and many others revert to "normal" cells after exhibiting unrestricted growth characters for a number of generations. Primary hamster cells sensitized to SV40 and adenovirus 31 transformation by x-ray pretreatment were uniformly stable transformants possessing specific T antigen and S antigen as were non-x-ray treated cells (12).

Radiation Enhanced Transformation:

Stoker (13) reported that the frequency of stable transformants could be increased among BHK-21 cells infected with polyoma virus by exposure of the cells to x-irradiation. In 1966, I reported to the A.E.C. that primary hamster embryo cells of several types were markedly sensitized to SV40 transformation by exposure to low-level x-ray prior to infection (published November 1967). Similar enhancement of transforming efficiency was observed for embryo cells pretreated with 5-iododeoxy-uridine prior to infection. Delay in infection after x-ray treatment lowered the enhancement factor indicating that post-irradiation repair mechanisms might limit the availability of attachment sites for the transforming virus genome (12). Pollock and Todaro (14) reported that x-ray treatment prior to or after infection of the 3T3 mouse cell line or human skin cells with SV40 increased cell susceptibility to transformation. Since x-rays and radiomimetic chemicals cause breaks in cellular DNA, the results suggest that virus integration might occur at the site of DNA lesions. SV40 and certain adenoviruses clearly cause chromosome aberrations in cells which they infect either through direct interaction with the cellular DNA or by lysozomal destruction and subsequent DNA damage (15). Dulbecco (1) suggests that single strand breaks would occur at a rate of 1.1×10^{-6} breaks per rad (R) for DNA with a molecular weight of 10^6 (16). Repair activity would increase the size of the break site and would require a molecular weight of about 10^7 for the site. Such a potential integration site induced by x-ray could not be specific and

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radiation-stimulated integration must be possible at many places. We have completed radiation titration studies and their influence on transforming efficiency and numbers and distributions of chromosome "lesions" (see ORO-3646-8). Post-irradiation survival for hamster embryo cells at increasing x-ray exposures, periods of DNA synthesis and DNA profile studies have also been reported (17).

State of Virus DNA in Transformed Cells:

Stoker (8) cited four lines of evidence to suggest the presence of the virus genome in stable, transformed cells.

a) Covalently linked to the chromosomal DNA is a fraction of DNA which hybridizes with purified viral nucleic acid and contains identical base sequences to those of the infecting virus (4, 5).

b) Rapidly labelled RNA (mRNA) which hybridizes with purified viral DNA is found in the transformed cell (18-19).

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

d) Fusion studies and mitomycin C treatment can result in virus "release" from stably transformed SV40 tumor cells (8, 9, 10).

Added to this list one would now include the observations that different transformed cell lines possess different amounts of virus DNA (genome equivalents) associated with their chromosomal DNA (4, 5, 20). Only transformed cell lines containing T antigen were observed to actively synthesize viral RNA suggesting that S or surface antigen is not a direct virus specific function (21).

Integration of virus DNA in the cellular DNA (nuclear DNA) has only been shown to occur to date in the SV40 transformed 3T3 mouse cell, strain SV3T3 (1). It is not known whether such integration or fixation occurs in other transformed lines or in primary cells transformed cells although this might generally be the case (20). The problem is to identify the site or sites of insertion and to establish the uniformity of the mechanism involved. Certain chromosomes seem to be involved in at least two systems examined to date (22, 7).

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Functions of Viral Genes:

In lytic infection, three to four of the supposed ten virus genes are transcribed in the early phase of infection as mRNA (prior to virus DNA replication). About one-third of the virus DNA is apparently transcribed in the transformed cell but only part of the mRNA is identical to that transcribed in early infection. Irradiation of viral particles suggest that only two to three viral genes seem required for transformation. Late functions in virus maturation occupy about 3 to 4 viral genes and these genes seem to be unimportant in transformation. These and other findings suggest that, at most, only one or two virus genes seem to be intimately involved in inducing transformation (1). One additional virus gene seems related to the induction of cellular DNA synthesis occurring at the same time as virus DNA replication is initiated (1).

5. Scientific Scope

This research effort is designed to investigate the precise conditions and parameters of enhanced virus transformation of normal cells stimulated by radiation, radiomimetic chemicals and associated natural processes (cell aging). As before, the program involves a quantitative examination of radiation or chemically induced alterations in cells which sensitize them to the transforming potential of SV40 and adenoviruses. An important result to be simultaneously gleaned from the present approach will be information about the mechanism for virus transformation of cells under conditions of normal infection. Previously we have emphasized the importance of using normal cells for these studies. Cells which possess none of the "unit characters" of tumor progression including loss of contact inhibition, changes in cell and culture morphology, antigenic alterations, karologic changes, growth characters and malignancy. Researchers in other laboratories employ cell systems which have already undergone certain spontaneous changes to study virus transformation (eg. 3T3 cells). These changes make the

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established cells useful for the study of certain aspects of transformation but always with qualification since they are not normal cells. A system has been developed in this laboratory which permits the study of transformation mechanisms using hamster cells from primary tissue with no unit character of the transformed cell prior to infection with the oncogenic agent. We have recently demonstrated that radiation stimulates SV40 tumorigenicity at the cellular level in hamsters paralleling our in vitro findings.

A. Rationale

Stoker, Todaro and Green and others 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 aging the cells to a degenerate stage prior to infection with oncogenic viruses. We have extended this observation, for all three types of treatment, to normal hamster embryo lung and kidney cells in culture and Pollock and Todaro to 3T3 mouse cells and human skin cells.

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 vrial 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 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

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and by carefully controling culture conditions 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 and partially completed 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. Specific studies planned or on test.

In addition to studies being completed as outlined in the attached progress report (ORO-3646-8) the following parameters will be examined or brought to a conclusion.

(1) A precise evaluation of the time of virus addition post-irradiation which has the most striking effect on transformation efficiency is one-third completed. Such a study will give considerable insight into the role of DNA repair mechanisms in the transformation phenomenon.

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inhibitors of protein, DNA and RNA synthesis will be employed to further characterize repair mechanism and their influence on SV40 transformation.

(2) 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.

(3) Conduct hybridization experiments to determine the number of viral genome equivalent present in the various cell clone types appearing in the SV40 transformed hamster cell population (Table II, p 19 ORO-3646-8).

(4) Evaluate rates and changes in host cell and viral RNA synthesis after treatment with irradiation or IUDR using isotopic procedures already worked out in detail. Characterize the types of DNA and RNA synthesis occurring in cells during transformation for the variant clones developed (see p. 19, Table II ORO-3646-8).

(5) 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.

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

eg. (1) Ultraviolet irradiation
(2) Mitomycin C
(3) Hydroxylamine
(4) Bromouracil
(5) Cytosine arabinoside
(6) Fluorodeoxyuridine
(7) Other inhibitors of protein transcription of translation

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(7) Continue the evaluation 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.

(8) Continue studies on the role of low levels of virus multiplication in cells infected with oncogenic viruses (SV40,adenoviruses 31 and 7) The long "latent" period in other systems and the low number of transformants obtained presents a problem in being able to detect the presence 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.

(9) Determine quantitatively the histone content of normal and SV40 transformed cells.

(10) Continue to evaluate the effects of cell synchronization at the time of iradiation on the enhancement phenomenon in the hope of defining the phase of cell division which might be involved in the initial step in viral transformation.

(11) Continue to evaluate the effects of radiation and specific chemical treatments (mutagens) on the reversion of virus-induced neoplastic tissue to the normal state as expressed phenotypically. eg. reinstitute oriented growth, or contact inhibition in neoplastic cells.

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

C. 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 this institution a research outlet.

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References

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1033929

6. Scientific Personnel.

Principal Investigator:

Joseph H. Coggin, Jr. - 40% time on project.
Social Security No. - [redacted]
Business Address - University of Tennessee, Department of Microbiology
Knoxville, Tennessee 37916

Telephone - 615-974-5466
Born - [redacted]

Education: [redacted]

Current Memberships:

American Society for Microbiology
American Association for Advancement of Science
Tissue Culture Association
Sigma Xi
Torch Professional Club

Experience:

Section Chief of Transplantation Antigen Study MAN Program and Consultant Virologist to Biophysical Separation Laboratory, 1967 to present. Consultant virologist East Tennessee Children's Hospital and Ft. Sanders Hospital, 1966 to present.

Virologist and Associate 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-10429-02. 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. Responsibilities included supervision and planning of research for laboratory personnel in a biochemical investigation of 6-mercaptopurine degradation by leukemic cells and *Escherichia coli*.

1033930

Experience (continued)

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

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

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

Pertinent Publications

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

Induction of Tumor-Specific Transplantation Immunity in Hamsters with Cell Fractions from Adenovirus and SV40 Tumor Cells. Proc. Soc. Expt. Biol. Med. 132, No. 1, 328-336. 1969. J. H. Coggin, Jr., L. H. Elrod, K. R. Ambrose, and N. G. Anderson.

Characterization of Tumor Specific Transplantation Immunity in Diffusion Chambers *in vitro*. Proc. Soc. Expt. Biol. Med., 132, No. 2, Dec. 1969. K. R. Ambrose and J. H. Coggin, Jr.

A Rapid *In Vivo* Assay for SV40 Tumor Immunity In Hamsters. Proc. Soc. Expt. Biol. Med. 130, 246-252. 1969. J. H. Coggin, Jr. and K. R. Ambrose.

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

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

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

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

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

1033931

Pertinent Publications (continued)

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

Submitted

Radiation Stimulated SV40 Oncogenesis *In Vivo*.
Submitted to Science December 1969. J. H. Coggin, Jr. and S. E. Harwood.

Surface Changes in Hamster Embryo Cells Transformed by SV40 and Adenovirus.
Submitted to Proc. Soc. Expt. Biol. Med., November 1969. J. H. Coggin, Jr. and K. R. Ambrose.

Nucleic Acid Profiles in Variant SV40 Transformed Hamster Cell Clones.
Submitted to J. Virology October, 1969. J. H. Coggin, Jr. and S. E. Harwood.

Radiation Chromosome Damage and SV40 Transformation.
Submitted to Proc. Soc. Expt. Biol. Med. December, 1969. J.H. Coggin, Jr.

Role of Antibody in Tumor Rejection.
To be submitted to J. Immunology January, 1970. J. H. Coggin, Jr., K. R. Ambrose and N. G. Anderson.

Associate Investigators:

S. E. Harwood, M.S. Graduate Student NDEA Predoctoral Candidate. 100% time on project.

G. W. Fortner, M.S. Graduate Student NDEA Predoctoral Fellow. 100% time on project.

J. M. Minchey, B.A. Graduate Student NSF Premasters Fellowship. 50% time on project.

Mrs. Peggy Dierlam, B.A. Research Assistant - 100% time on project.

Mrs. Patti Warrington, B.A. Laboratory Assistant - 100% time on project.

7. Other Personnel:

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

8. Other Financial Assistance

AEC alone sponsors our study of virus transformation described here. An NIH (NCI) grant to study mechanisms of immunity to tumor transplantation antigen in hamsters has been in effect for two and one-half years (CA-10429-02). This

Other Financial Assistance (continued)

grant is being concluded in lieu of a new contract with Union Carbide (MAN Program) under subcontract No. 3379 to study "Immune Reactions in Virus-Induced Tumor Rejection" (Effective dates November 1, 1969 to July 1, 1970 - $17,500)

The University provides approximately $6,000.00 for animal room supplies and caretakers and $1200 in supplies to the project.

Mr. John Minchey is sponsored by an NSF premasters fellowship
Mr. G. W. Fortner is sponsored by an NSF predoctoral fellowship
Miss S. E. Harwood is sponsored by an NDEA predoctoral fellowship
Mrs. Peggy Dierlam is supported by the AEC as a laboratory assistant
4 part-time (15 hours per week each) laboratory aides are sponsored by the U. S. Office of Education, Work-Study Program.
Miss K. R. Ambrose devotes 10% time to the project in cell preparation procedures and is sponsored by subcontract 3379.

9. Premises, Facilities, Equipment and Materials:

These remain essentially the same as in previous years. Our research facilities have recently been expanded from 6000 square feet to 15,500 square feet. Of this newly expanded area two new rooms have been added to virologic research increasing the total research area to 5000 square feet exclusive of animal facilities. Some $75,000 have been expended in the past 15 months to generally upgrade our research area providing a new scintillation system, spectrophotometers, centrifuges, cold rooms,wash area facilities and animal room improvements. Electron microscopy facilities are available to us through the MAN Program, ORNL.

1033933

10. Budget

Fourth Year: March 31, 1970 to April 1, 1971

	Estimated Requirements Fourth Year
1. Salaries and Wages:	
Principal Investigator, Joseph H. Coggin Associate Professor, 40% time for 9 months ($14,400 base 9 month salary ----------	$ 5,760.00
Graduate Students Assistant, Mrs. Peggy Dierlam. 85% time 12 months ----------	3,300.00
Laboratory Assistant, Mrs. Patti Warrington, 100%	3,000.00
Laboratory glassware washers (3 part-time students) Animal Caretaker (25% time) ----------	1,000.00
Social Security and Retirement (10% of Salaries and Wages) -----	730.00
Salaries Subtotal $13,790.00	
2. Supplies and Materials:	
Chemicals	1,000.00
~~Isotopes~~	~~500.00~~
Cages and Bedding	1,500.00
~~Hamsters and Rabbits~~	~~1,000.00~~
Media and Sera	1,500.00
Expendibles (plastic culture ware, CO_2, etc.)	1,500.00
Supplies Subtotal ~~$7,000.00~~ 5,560.00	
3. Equipment:	
Baker Transfer & Tissue Culture Hood	1,100.00
4. Publication Costs:	~~300.00~~ 295.00
5. Travel (one annual Society meeting; one branch meeting	250.00
6. Other - Telephone, Reprints, and Postage	200.00

1033934

7. Indirect charges - 54.2% Salaries and Wages. This is the approved overhead rate based on fiscal year 1967 operations audited by Dept. of Defense (DCAA), Atlanta $ 7,078.~~52~~

Total Project Costs $~~29,718.52~~ 28,213

Percentage and amount to be contributed by the University of Tennessee - (Principal Investigators salary plus $1,200.00 in the supply category)	~~23.1%~~ 24	6,864.~~98~~
Percentage and amount requested from the Atomic Energy Commission -	76.~~9~~%	21,349.00 ~~$22,853.54~~

It is anticipated that a similar rate of funding would be required for a fifth and sixth year.

Changes made in accordance with contractor's letter of 4-22-70

L.M.

1033935

II. Authentication

Principal Investigator

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

For the University

Original Signed By
HILTON A. SMITH

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

1033936

THE UNIVERSITY OF TENNESSEE
KNOXVILLE 37916
DEPARTMENT OF MICROBIOLOGY

December 31, 1969

Herman M. Roth, Director
Laboratory and University Divisions
Oak Ridge Operations
P. O. Box E
Oak Ridge, Tennessee 37830

Dear Mr. Roth:

Please find attached a preliminary expenditure statement for contract AEC AT (40-1) - 3646 through December 22, 1969.

Sincerely yours,

Roy Dean
Administrative Assistant

RD:pda
Enc.

1033937

SIE-78a

PUBLICATION BY AEC AUTHORIZED ☐

SCIENCE INFORMATION EXCHANGE

SMITHSONIAN INSTITUTION

U.S. ATOMIC ENERGY COMMISSION

AEC CONTRACT NO. AT(40-1)-3646

SUPPORTING DIV. OR OFFICE:

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

University of Tennessee
Department of Microbiology
Knoxville, Tennessee 37916

TITLE OF PROJECT:

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

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

Joseph H. Coggin, Jr., Ph.D., Associate Professor
Department of Microbiology

NO. OF GRADUATE STUDENTS ON PROJECT: 5 NO. OF GRADUATE STUDENT MAN-YEARS:

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

The parameters of virus-induced tumor production will continue to be evaluated in a primary cell culture system. Quantitative investigations of fundamental mechanisms involved in virus-cell interaction leading to malignant conversion will be conducted. Radiation, certain pyrimidine analogues and cell aging markedly sensitize hamster embryo cells to SV40 and adenovirus transformation. All three methods employed for sensitizing normal cells to virus transformation are observed to reader lesions in the target cell DNA suggesting that a common mechanism may be involved in enhancement. Disclosure of the mechanism(s) for enhancing virus transformation is a primary objectiv of this study. Metabolic, physical, structural and regulatory changes occurring prior to, during and after infection with SV40 in pre-irradiated hamster cells will continue to be investigated in an orderly fashion. Localized radiation has now been observed to increase the tumorigenicity of SV40 in neonatally infected hamsters when virus is administered in the area of low-level x-ray exposure. The radiation levels employed to increase tumor appearance in hamsters by 50% is comparable to dosages delivered in certain diagnostic x-ray procedures in humans. Radiation type and dosage will be evaluated to determine the usefulness of the radiation sensitization technique to detect oncogenicity among teratogenic agents in hamsters and to increase the tumorigenic potential of weakly oncogenic agents.

RESULTS TO DATE:

	PROGRAM CATEGORY NO.
BUDGET	
PRIMARY	
SECONDARY	

Joseph H Coggin Jr

Signature of Principal Investigator

DATE: 12-25-69

INVESTIGATOR – DO NOT USE THIS SPACE

1033938