

U. S. ATOMIC ENERGY COMMISSION
CONTRACT AUTHORIZATION

1. DATE

JUL 15 1975

2. AUTHORIZATION NO.

BER-76-3

3.A. TO

R. J. Hart, Manager
 Oak Ridge Operations Office

3.B. FROM

James L. Liverman, Director ⁵⁹⁶
 Division of Biomedical and ^(R)
 Environmental Research

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

UNIVERSITY OF TENNESSEE
 Knoxville, Tennessee 37916
 Department of Microbiology

4.B. PRINCIPAL INVESTIGATOR(S)

JOSEPH H. COGGIN

707791

5.

 NEW CONTRACT RENEWAL OTHER

6. TERM OF CONTRACT

7/1/75 thru 6/30/76

7. CONTRACT NUMBER

E(40-1)3646

8. RECOMMENDED TYPE OF CONTRACT:

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

9. PROPERTY TITLE TO VEST IN:

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

10. SECURITY CLASSIFICATION:

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

11. PROJECT TITLE "A Comparative Study of Radiation, Chemical, and Aging Effects on
 Viral Transformation"

12. HEADQUARTERS TECHNICAL CONTACT

George E. Stapleton, Radiation Biologist

13. FINANCING

A. OPERATING EXPENSES

New AEC Funds \$ 34,102

Estimated AEC Balance From Prior Term, if any \$ -0-

Total Estimated AEC Support Cost for New Term \$ 34,102

Estimated Contractor Contribution, On Proportionate Sharing Basis, if any \$ -0-

Estimated Project Cost, For Pertinent Budget Period \$ 34,102

Budget and Reporting Classification: RT 03 03

Allotment Transfer: 06-60-91(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.

Please note that this is a terminal year.

15. SCOPE OF WORK

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

REPOSITORY

Oak Ridge Operations Office

COLLECTION

Records & Holding Area
Documents 1944-1994

BOX No.

A-59-3 Bldg. 2714H

FOLDER

Cont. 3686 CA
Univ. of Tenn. 7-1-75
6-30-76

1034072

JUL 15 1975

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

Dear Dr. Coggin:

I wish to advise you that we have approved renewal of your Research Contract No. E(40-1)3646, "A Comparative Study of Radiation, Chemical, and Aging Effects on Viral Transformation," for an additional year. You will be contacted by someone from the Oak Ridge Operations Office in the near future regarding negotiation of the renewal contract.

Your next renewal period comes after the completion of nine years of operation of this contract. For every multiple of three years the Division of Biomedical and Environmental Research requires a comprehensive report of the overall activities of the contract during the past three years. In particular, the following items should be included in this report:

1. The main research accomplishments, with special reference to the originally stated objectives.
2. Plans for the continuation of present objectives and possible new objectives in consideration of past results.
3. Graduate students trained, degrees granted, and post-doctoral tenures completed.
4. Bibliography, with titles of publications associated with this contract.
5. Your opinion as to the present state of knowledge in this area of research, its significance in the fields of biology and medicine, and needed future investigations.

1034073

Dr. Joseph H. Coggin

-2-

JUL 15 1975

6. The present division of federal support for your overall research program.

Six copies of the above-outlined request should be submitted in addition to the usual progress report and renewal proposal. Since the complete proposal will be sent for outside evaluation, submission is requested three months prior to the end of the contract year.

Sincerely,

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

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

bcc: Oak Ridge Operations Office



1034074



UNITED STATES
ENERGY RESEARCH AND DEVELOPMENT ADMINISTRATION

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

AREA CODE 615
TELEPHONE 483-8611

March 26, 1975

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

RENEWAL OF CONTRACT NO. AT-(40-1)- 3646 - The University of Tennessee

We are submitting for your review and appropriate action the following information concerning the contract which will expire on JUN 30 1975

1. Renewal Proposal (4)
2. Progress Report (4)
3. Financial Statement (4)
4. Form SI-SIE-78a (200-Word Summary) (3)

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


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

ACR:LM

Enclosures:
As stated above

bcc: D. S. Zachry, w/Prog. Rpt. (2) & 427
L. Medley, w/encls.
Tickler, w/Ren. Prop. & Fin. Stmt.
Green
Reading

 RCP&R BR
MEDLEY:ejb
3/26/75



1975

1034075

Renewal Proposal

Ninth Year

Contract

AT(40-1)3646

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

Sponsored By

The East Tennessee Cancer Research Center

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

Principal Investigator

Professor of Microbiology

April 1, 1975

1500

MAR 25 1975

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1. *Title of Project*

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

2. *Institution*

The University of Tennessee
Department of Microbiology
Knoxville, Tennessee 37916

Telephone: 615-974-2356

1034077

3. Project Abstract.

The focus of this proposal is to conclude ongoing efforts to discover how tumor viruses regulate and control the expression of embryo associated cellular products as an integral activity in transforming normal cells. AEC (ERDA) has supplied long-term, responsive and badly needed support for these and previous studies, and the agency is largely responsible for being the mainstay in our progress in the area of embryonic antigen expression in cancer. This is to be the last year of the project. We plan to have the major answers sought for the past 18 months in this project by the end of the ninth year. These studies are feasible because exposure of normal target cells to sublethal X-irradiation greatly sensitizes the cells to viral oncogenesis. We have observed that coordinate with the induction of neoplastic characteristics in viral transformed cells, embryonic moieties (antigens abbreviated EA) appear in the plasma membrane of the tumor cells and act as autoantigens. Radiation (25 to 350R) alone does not cause transformation of normal hamster or human cells *in vitro*, however, these levels of exposure do promote 50 to 1000-fold increases in viral oncogenesis induced by oncoviruses. We have successfully demonstrated that early embryo tissue of the hamster, mouse, and human possess antigens (EA) which are identical to those occurring on viral transformed cells. During the terminal stages of embryonic or fetal development *in vivo* these antigenic entities are masked by biochemical processes associated with membrane maturation and subsequently reside cryptically in adult cell membrane. Viral oncogenesis serves to cause defective biosynthesis and results in the reexpression of EA. The central problem in our current work is to discover (1) whether we can successfully use embryonic markers expressed at the cell surface to reliably detect the earliest stages of viral induced neoplastic traits in the tumor cells, (2) to determine the biochemical events related to membrane maturation involved in the control and expression of EA in fetal development and in cancer induction and (3) to determine how low-level radiation serves to potentiate this virus-host cell interaction. If we are successful in developing a reliable assay for fetal marker expression (EA) associated with the very early events in neoplasia, a valuable tool for detecting radiation potentiated oncogenesis will be available for understanding and analyzing the cancer induction process.

4. Scientific Background.

A. Historical Survey of This Contract.

This project was initiated nine years ago in an effort to discover whether exposure to sublethal levels of radiation, pyrimidine analogues, or cell "aging" processes *in vitro* served to potentiate oncovirus oncogenesis. This program, which has generated some 28 publications in major journals, requires a brief historical review and accounting. Since the initiation of this work we have reported that radiation and radiomimetic chemicals markedly potentiate viral oncogenesis in primary cell culture systems *in vitro* and in animals treated locally with X-ray before infection with tumor viruses. Recall that in this work the target cells, not the transforming virus, are presensitized by irradiation.

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

Four years ago, as a "spin-off" from previous work, we discovered that embryonic antigens were generally expressed in virally and chemically induced tumor cells. This new observation from rodent model system studies was quickly recognized to relate to human cancer since studies in many laboratories now show quite unequivocally that human cancers carry histologically related, cross-reacting antigens. Many of these antigenic determinants are recognized to be embryonic in character and the possibility stands that all are embryonic antigens (EA). We have reviewed the central problems associated with retrogenic expression in neoplasia and reprints of several reviews* are included in the 1975 Progress Report for your perusal. Over 60 publications by other workers have appeared in the literature to date which relate to our report that oncovirus tumors carry EA.

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

Proposed Mechanisms by Which Autochthonous Neoplasms Escape Immune Rejection. Cancer Res., 34:2092, 1974.

An Evaluation of the Isotopic Antiglobulin Assay and the Cytostatic Assay for Detecting SV40 Tumor Immunity. Isr. J. Med. Sci., 10:904, 1974.

Eighteen months ago, in our proposal for an additional three years support from the AEC to study the relationship of radiation damage to viral oncogenesis and retrogenesis in neoplasia, we outlined a series of new objectives aimed at discovering the interrelatedness of EA expression with radiation-stimulated oncogenesis. Our central theme was then and continues to be to develop immunologic and molecular tools for discerning the *earliest events* in viral oncogenesis, potentiated by radiation, which can be used as "markers" of neoplastic conversion leading to the malignant state. We have worked hard these past 18 months to develop reliable assays for embryonic antigens and we feel that we are on the right track and that our ideas were still correct and experimentally testable. Funds made available for this work through the ERDA support only graduate student research.

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

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

Papovaviruses are the best characterized tumor viruses particularly when we consider the wealth of information now available regarding the molecular interactions between these viruses and their transformed cell hosts. These viruses have been particularly amenable to study because they possess a small DNA genome which has a limited gene complement and many of the unique viral coded proteins have been identified. Viral genetic information responsible for tumor induction seems restricted to one or two genes. Intranuclear tumor or T antigen is now recognized to be coded for from the viral genome since T antigen expression occurs in cells lytically infected with SV40 and only mutations in the viral genome effect the synthesis of this antigen (see Robb, J.A., J. Virol., 12:1187, 1973). Recent reports have shown that at least *one cellular gene* is required for maintaining some manifestation of the transformed state. These characteristics of transformation include atypical cellular DNA synthesis, excess cell saturation density, lack of growth factor requirements, and agglutinin

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

binding receptors on tumor cells. Thus, at least one viral gene and one cellular gene are essential for regulating the *induction* of transformation. In the work we are doing we seek to add a quantitative parameter to indicate transformation, specifically by developing a reliable assay for EA expression using objective assays. Portugal and his colleagues (J. Virol., 12:1616, 1973) recently showed that a number of new isoaccepting tRNA species (leucyl-, isoleucyl-, phenylalanyl-, and threonyl- tRNA's) thought originally to be associated with viral gene activity are now recognized to be *identical* to tRNA alterations accompanying *differentiation* of developing normal cells.

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

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

Background Current (Since July, 1974)

A number of very exciting observations have signalled the importance of the work covered by this grant. These data fall into three groups: (1) information relating to the regulatory interactions of SV40 with cells transformed by the virus, (2) development of improved methods for the isolation of tumor antigens and for their characterization *in vitro*, and (3) the universality of fetal antigens on neoplasms.

1) Information concerning SV40 gene function in transformed cells.

It is generally acknowledged, from the work of several laboratories, that 1-2% of the mRNA transcribed in SV40 transformed cells is derived from SV40 DNA segments A, H, I and B of the SV40 genome covalently linked to host cell chromosome. These DNA fragments (produced by nuclease treatment) represent all the *early* genes of SV40 which function to yield mRNA prior to viral DNA synthesis in permissively infected cells. Since *early* SV40 gene expression (not late cells on genome fragments C, D, E, K, F, J and G) is transcribed in cells transformed by SV40, T antigen is clearly associated with early gene function and the transcription of A, H, I and B fragments (early genes). SV40 T antigen appears in both abortively and permissively infected cells prior to viral DNA synthesis and is an infallible marker for SV40 in transformed cells. Further, it is generally accepted that SV40 TSTA expression is linked to T antigen expression; more accurately stated, cells which exhibit SV40 TSTA in the plasma membrane also contain T antigen in the nucleus (TSTA+ T- SV40 transformed cells have yet to be documented to exist). The inference is clear that T antigen and TSTA are products of early SV40 gene function from viral DNA segments A, H, I or B. Two laboratories

have recently published data which suggest that T antigen expression may not be a direct product of SV40 gene transcription and translation.

Khoury and his associates at the NCI (personal communication) have recently reported that the human virus BK isolated from the urine of a renal transplant patient and thought to be a divergent evolutionary relative of SV40 from the rhesus monkey induces "SV40" T antigen in human cells which is indistinguishable from true T antigen induced in WI38 human cells transformed by SV40. When DNA-DNA hybridization was performed between BK DNA and SV40 DNA ~ 20% sequence homology was detected with sheared DNA's. Analysis of the areas of homology between BK virus and SV40-both viruses induced T antigen, a product of DNA fragments A, H, I or B in SV40-clearly showed that the annealing homology was exhibited in *late* gene function *other than* those present in excision fragment A, H, I or B. One is faced with the real possibility that T antigen expression is not directly coded for by the SV40 genome and the possibility exists that T antigen is a *host cell* product, the expression of which is *regulated* by the genome in some way.

Similarly, Sambrook (personal communication) has recently demonstrated that mRNA's transcribed from the A, H, I and B fragments or SV40 (early function DNA) do not yield *in vitro* protein "translates" that represent T antigen. T antibody was used to probe for T antigen in the *in vitro* translational products of early SV40 mRNA.

The significance of these data in relationship to the work of this contract is that mounting evidence is building that SV40 neoantigens may frequently represent the modulated products of host DNA expression under specific virus control. A major focus of this contract is to determine whether virally induced TSTA's are products of viral gene information or of cellular gene information.

2) Embryonic antigen expression in rat and mouse tumors.

Baldwin and his co-workers have become extremely interested in embryonic antigens in the past two years. All of their work has involved the use of DAB and MCA hepatomas of the rat (Reviewed in *Transpl. Rev.* 20:77, 1974). These hepatomas have been carried for over 9 years as subcutaneous transplant lines. Using a fluorescent antibody technique and the microcytotoxicity assay, Baldwin has reported the following findings, to be true in the rat:

(1) Multiparous (MP) rats yield highly cytotoxic effector cells for all hepatomas tested *in vitro*. Antibody detected by immunofluorescence was also present in multiparous rat serum which bound to rat hepatomas.

(2) Baldwin's group has reportedly isolated a "cell sap" component which can be separated into two antigenic components; one said to be embryonic antigen because it desensitized MP effector cells for cytotoxic action against hepatoma cells which carry individually specific TSTA's and EA's as well. Another fraction, said to be the TSTA, which is only capable of inactivating the cytotoxicity of specifically tumor immune effector cells and not MP effector cells was also isolated in crude form. EA extract (from hepatoma cells) does not reportedly inactivate effector cells sensitized to tumor cells (EA⁺ TSTA⁺). Baldwin interprets (*Nature* 252:751, 1974) these data, for chemically induced hepatomas, to mean that EA on rat tumors are either not immunogenic in activating CMI *in vivo* or else do not provide good target antigens for rejection *in vivo*. Individually specific transplantation antigens are conversely considered to be "tumor rejection" antigens. This latter point is, in this investigator's view (J. H. Coggin, Jr.) patently ridiculous since almost every malignant

1034082

neoplasm that ever appeared in man or beast contained such a "tumor rejection antigen whilst the cancer proved progressively lethal. The *in vivo* reality is, of course that there are no true, functional tumor rejection antigens on malignant neoplasms that kill their host. There are antigens, of course, that activate the effector arm of the cell mediated response system but such activation is ineffectual. The realistic tumor immunologist searches today to determine the means to alter the immunogenicity of these TSTA's or EA's to render either of both functional so that they can effectively induce true rejection reactions. Other facets of Baldwin's work which warrant discussion relate to the findings that immunization with irradiated fetus does not result in tumor protection. Aware of the success of Wells, Grant, and others in detecting immunity induced by immunization with fetus with other chemically induced rat tumors, Baldwin feels that either EA's are not able to serve as effective rejection antigens or conversely that they do, but that they are poor target antigens for cell mediated tumor destruction *in vivo*.

Again, Baldwin could not transfer immunity to rat hepatomas with multiparous (MP) effector cells (unwashed); MP effector cells were, however, highly cytotoxic for hepatoma cells *in vitro*. This incongruity is most alarming and a conspicuous discrepancy in Baldwin's model system if true. MP effector cells kill hepatoma cells *in vitro* but not *in vivo*. The hepatoma cells have good EA targets *in vitro* but are insensitive *in vivo*. His group did not test primiparous effector cells which effectively destroy tumor cells nicely when adoptively transferred as shown in our laboratory and in other laboratories. These effector cells are not inactivated with circulating EA found in high concentration in MP serum and are highly cytotoxic *in vitro* and *in vivo* unlike MP effector cells. Baldwin's interpretation of his results in this regard are not supported by his own *in vitro* data on the cytotoxicity of MP effector cells.

To our very great pleasure Baldwin and his colleagues have confirmed our observation that soluble tumor associated antigens can inactivate or desensitize effector cells from MP or tumor resistant donors and prevent their cytotoxicity for tumor cells *in vitro* (Nature 252:751, 1974). Such observations add to our original suggestion in 1973 that EA may play a major role in tumor progression *in vivo* (Cancer Research 34:2092, 1974).

(3) Expression of EA in human tumors.

C. Granatek and her colleagues at M. D. Anderson have recently reported (Gordon Conference, 1975) that a variety of human tumors (leukemias - AML, ALL, CLL; lymphomas, melanomas and carcinomas) carry fetal antigens that induce inhibitory antibody in the mouse FCFU assay. Blocking reactions could be demonstrated with serum from patients with progressive colon adenocarcinomas using the passive FCFU test (In press, J. Immunol., 1975). At least four manuscripts reviewed by this investigator for several journals have demonstrated that the cross-reacting antigens detected between tumors of common histologic type in *in vitro* assays are of fetal or embryonal origin. These results should appear in the literature in the next six months.

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

5. Scientific Scope.

A. Overall Objectives.

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

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

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

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

B. Specific Projects.

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

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

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

*An Evaluation of the Isotopic Antiglobulin Assay and the Cytostatic Assay for Detecting SV40 Tumor Immunity. Isr. J. Med. Sci., 10:904, 1974.

- b. Employing purification procedures developed in our laboratory, fetal phase-specific mRNA's will be isolated and tested for their identity to "tumor-specific" mRNA. The competitive hybridization procedure is being used.
- c. The role of cyclic AMP concentration, synthesis and degradation in retrogenesis induced by SV40 virus will be investigated.
- d. The "masking" of fetal antigen in the normal developing hamster fetus will be evaluated. Such studies provide not only specific parameters to measure when viruses stimulate retrogressive or dedifferentiative changes in normal cells leading to neoplasia but also are more suitable for evaluating subtle changes in fetal development which radiation exposure might introduce.

(2) Immunological Studies.

These studies are ongoing and are being conducted with the basic format described last year with certain specific revisions. We have developed a suitable quantitative system for detecting extremely low quantities of EA at the surface of tumor cells during transformation using the isotopic antiglobulin test (ITA), a modified radioimmunoassay.

IAT Procedure:

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

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

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

Absorption

Absorption of sera prior to use in the isotopic antiglobulin assay was performed in a standard fashion (Ting and Herberman, 1971). Cells to be used for absorption were removed from tissue culture flasks by trypsinization (0.25% trypsin - 0.1 EDTA), suspended and maintained overnight in spinner culture flasks. Aliquots containing the indicated number of cells were centrifuged at 800 x g for 10 min, the supernatant was aspirated, and 0.2 ml of the serum to be absorbed was added. After incubation for 60 min at 37°C and 5% CO₂ with frequent agitation, the samples were centrifuged first at 800 x g then the supernatant was aspirated and centrifuged at 2,000 x g for 10 min and this supernatant was employed in the isotopic antiglobulin test. Single-cell suspensions of various fetuses of 10-15 days gestation are prepared by extensive mincing, then used for absorption as described above.

Serum fractionation and iodination

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

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

Experimental Design:

Fourteen day fetal cells (EA⁻) in primary culture are exposed to radiation levels between 0 R and 150R and infected with SV40 virus (1 PFU/cell). Uninfected cells and non-infected, non-irradiated cells serve as controls. High titer SV40 antiserum is added following virus adsorption. Sample plates are washed at selected times post-infection (24, 28, and 72 hours post-infection) and target cells are harvested by aspiration and exposed to either fetal antibody or IgG from hyperimmune serum from hamsters immunized against SV40 tumors. Normal serum and/or virgin serum serves as control sera. Following adsorption and washing highly specific ¹²⁵I-tagged anti-hamster IgG is added, incubated with target cells and washed. The wells are collected as described and scored. Immunofluorescence studies run in parallel are being conducted as described last year. These data reflect the changes in cell surface or plasma membrane indicating the unmasking of fetal antigen. Challenge studies with graded doses of these cells reflect relative oncogenic potential of the cell population.

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

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

U. S. DEPARTMENT OF HEALTH, EDUCATION & WELFARE

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

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

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

HONORS

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

MAJOR RESEARCH INTEREST

Tumor Immunology

ROLE IN PROPOSED PROJECT

Principle Investigator

RESEARCH SUPPORT

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

RESEARCH AND/OR PROFESSIONAL EXPERIENCE

1973 - present	Professor of Microbiology
1967 - present	Section Chief of Transplantation Antigen Study and Consultant Virologist to Biophysical Separation Laboratory, Molecular Anatomy Program, ORNL.
1966 - present	Virologist, University of Tennessee, Knoxville. Lecturer in Virology and Molecular Biology at graduate and undergraduate level. Principle investigator, AEC Contract No. AT(40-1) 3646. Principle Investigator NIH Grant 16278 (National Cancer Institute). Principal Investigator NCI Tumor Transplantation Antigen Study FS-7 (ORNL).
1965 - 1966	Senior Research Virologist. Virus and Cell Biology Division, Merck Institute for Therapeutic Research (Merck, Sharp, and Dohme Laboratories). Planned and supervised research in Cancer Virology and Immunology in conjunction with Dr. Maurice Hilleman. The program involved experimentation into problems of tumor antigen assay and purification, the viral etiology of cancer, recovery of viruses from transformed cells, and immune mechanisms operative in hamster infected with oncogenic viruses.

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- K. R. Ambrose, N. G. Anderson, and J. H. Coggin, Jr. Cytostatic Activity and SV40 Tumor Immunity in Hamsters. *Nature*, 233:321-327, 1971.
- Norman G. Anderson and J. H. Coggin, Jr. Retrogenesis: Problems and Prospects. *In: Embryonic and Fetal Antigens in Cancer*, II, 361-368, 1972.

- E. G. Rogan, M. P. Schafer, N. G. Anderson, and J. H. Coggin, Jr. Cyclic AMP Levels in the Developing Hamster Fetus: A Correlation with the Phasing of Fetal Antigen in Membrane Maturation. *Differentiation*, 1:199-204, 1972.
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- N. G. Anderson, D. W. Holladay, J. E. Caton, E. L. Candler, P. J. Dierlam, J. W. Eveleigh, F. L. Ball, J. W. Holleman, J. P. Breillatt, and J. H. Coggin, Jr. Searching for Human Tumor Antigens. *Cancer Res.*, 34:2066-2076, 1974.
- Joseph H. Coggin, Jr. and Norman G. Anderson. Embryonic and Fetal Antigens in Cancer Cells. *In: Developmental Aspects of Carcinogenesis and Immunity: The Thirty-Second Symposium of the Society for Developmental Biology.* (Ed.) Thomas King, Academic Press (New York) pp. 173-185, 1974.
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- Kathleen R. Ambrose and Joseph H. Coggin, Jr. Horizontally Transmissible Hamster Lymphosarcoma. *J. Natl. Cancer Inst.*, 1974. In the press.
- W. H. Hannon, K. R. Ambrose, and J. H. Coggin, Jr. Forssman Antigen and Phase Specific Fetal Antigens: An Evaluation of Their Role in SV40 Tumor Immunity. *Proc. Soc. Exp. Biol. Med.*, 1974. In the press.

NAME TITLE BIRTHDATE (Mo., Day, Yr.)

AMBROSE, Kathleen R.

Research Associate

[REDACTED]

PLACE OF BIRTH

PRESENT NATIONALITY

SEX

[REDACTED]

USA

Female

EDUCATION - INSTITUTION & LOCATION

DEGREE

YEAR CONFERRED

SCIENTIFIC FIELD

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

HONORS

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

MAJOR RESEARCH INTEREST

ROLE IN PROPOSED PROJECT

Tumor Immunology, Viral Oncology

Supervision of tumor immunology experimental studies

RESEARCH SUPPORT

None from this contract

RESEARCH AND/OR PROFESSIONAL EXPERIENCE

1970 - present Research Associate, Department of Microbiology, University of Tennessee, Knoxville, Tennessee

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

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

PUBLICATIONS:

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

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

Coggin, J. H., Jr., L. H. Elrod, K. R. Ambrose, and N. G. Anderson. Induction of Tumor-Specific Transplantation Immunity in Hamsters with Cell Fractions from Adenovirus and SV40 Tumor Cells. Proc. Soc. Exp. Biol. Med., 132(1):328, 1969.

In addition, this contract supports two predoctoral trainees, who devote their full research time to the objectives of this proposal. Current AEC assistantships are held by Cleo Babelay and Mary Joyce Rogers.

7. *Other Personnel.*

2 Laboratory Aides - Dennis Ragsdale - 50% time

Donald Edwards - 50% time

8. *Other Financial Assistance:*

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

A new contract, NCI CP 43325, has been in progress since July, 1974, and is funded at the present level of \$170,000 per year. The objectives of this research are to develop immunotherapeutic techniques for controlling human cancer. A new grant, CA 16278, sponsors studies on the source of fetal antigens and the relationship of EA to other normal cell antigens.

The University provides \$1500 in supplies and services to the ERDA project.

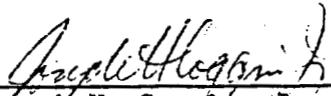
9. *Premises, Facilities, Equipment, and Materials.*

The Virology-Immunology Laboratory available for this project in the Department of Microbiology, University of Tennessee, Knoxville, consists of three contiguous, large laboratories occupying some 3500 square feet of useable space. In addition, 1200 square feet of support space (washroom, prep area, storage, etc.) is available. Animal facilities have been upgraded and all hamsters are kept in two containment units separated completely from other rodents (1500 square feet). A separate animal holding room (500 square feet) is available for breeding the large numbers of pregnant hamsters needed for fetal tissue. The largest supplier of pregnant rodents in the country cannot provide sufficient inbred hamsters or mice on a time-mated basis, hence we breed our own animals at a considerable cost savings. We currently have space to accommodate 6-7,000 hamsters in new facilities and some 3-500 breeders. Mice will be housed at Oak Ridge National Laboratory in excellent barrier facilities made available through the Molecular Anatomy Program, ORNL, on a "pay as needed basis".

We have excellent tissue culture facilities containing some 60 linear feet of Laminar flow work surface in eight units, walk-in incubators and essential cold storage and freezer space. We badly need to acquire an additional laminar flow hood (\$2900) because of the large work load and for the use of the graduate assistants. All major items of equipment for conducting the work including Cytofluorograf, Cytograf, scintillation systems, preparative ultra-centrifuges and the like are available in our own laboratory. Additional facilities or equipment we require from the Molecular Anatomy Program are available to our personnel on a continuing basis. Tissue culture roller assemblies for large scale tissue production, purchased under the previous Interagency Agreement (AEC-NCI) are available.

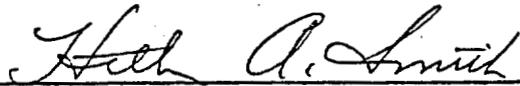
11. Authentication

Principal Investigator



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

For The University



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

10.

COST ESTIMATE

BUDGET

June, 1975 to March 31, 1976

AEC Contract AT-(40-1)3646

ESTIMATED REQUIREMENTS

	<u>Ninth Year</u>
1. Salaries and Wages	<u>\$6,854.00</u>
Principal Investigator - 15% time	N/C
Two Graduate Students - 50% time	6,637.00
Fringe Benefits	217.00
2. Equipment	<u>3,760.00</u>
Hoods and Animal Equipment for Breeding	3,760.00
3. Travel	<u>1,000.00</u>
4. Other Direct Costs	<u>18,220.00</u>
Supplies	13,043.00
Publications	240.00
Other	4,937.00
5. Indirect Costs	<u>4,268.00</u>
64.3% of \$6,637.00	
TOTAL PROJECT COSTS	<u><u>\$34,102.00</u></u>

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

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.)

Department of Microbiology
The University of Tennessee
Knoxville, Tennessee 37916

TITLE OF PROJECT:

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

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

Joseph H. Coggin, Jr., Principal Investigator - 1.5 M.Y.

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

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

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

RESULTS TO DATE:

Available data indicate that radiation induced trauma to the cellular DNA potentiates the integration of the viral genome into target cells. Following integration, viral regulatory activation suppress normal patterns of host membrane synthesis and cease the expression of cryptic embryonic antigens.

	PROGRAM CATEGORY NO.
BUDGET	
PRIMARY	
SECONDARY	


Signature of Principal Investigator

DATE: 3-19-75

INVESTIGATOR - DO NOT USE THIS SPACE

1034095

UNIVERSITY-TYPE CONTRACTOR'S RECOMMENDATION FOR DISPOSITION OF SCIENTIFIC AND TECHNICAL DOCUMENT

(See Instructions on Reverse Side)

1. AEC REPORT NO.

ORO-3646-25

2. TITLE

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

3. TYPE OF DOCUMENT (Check one):

- a. Scientific and technical report
- b. Conference paper not to be published in a journal:
 - Title of conference _____
 - Date of conference _____
 - Exact location of conference _____
 - Sponsoring organization _____
- c. Other (Specify) _____

4. RECOMMENDED ANNOUNCEMENT AND DISTRIBUTION (Check one):

- a. AEC's normal announcement and distribution procedures may be followed.
- b. Make available only within AEC and to AEC contractors and other U.S. Government agencies and their contractors.
- c. Make no announcement or distribution .

5. REASON FOR RECOMMENDED RESTRICTIONS:

6. SUBMITTED BY: NAME AND POSITION (Please print or type)

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

Organization

University of Tennessee
Knoxville, Tennessee 37916

Signature



Date

April 1, 1975

FOR AEC USE ONLY

7. AEC CONTRACT ADMINISTRATOR'S COMMENTS, IF ANY, ON ABOVE ANNOUNCEMENT AND DISTRIBUTION RECOMMENDATION:

8. PATENT CLEARANCE:

- a. AEC patent clearance has been granted by responsible AEC patent group.
- b. Report has been sent to responsible AEC patent group for clearance.
- c. Patent clearance not required.

(1)

FINANCIAL STATEMENT

(1) Total actual project cost to date for the current period	\$ <u>27,190.02</u>
(2) Estimated total cost for remainder of period	\$ <u>4,271.94</u>
(3) Total actual and estimated cost chargeable to ERDA for current period based on percentage of cost agreed upon as contained in A-III of Appendix "A" to Contract	\$ <u>31,461.96</u>
(4) Accumulated costs chargeable to ERDA (include costs reported in certified statement for preceding period(s) and the costs stated in Item (3) above)	\$ <u>145,144.00</u>
(5) Accumulated ERDA Support Ceiling as stated in Article III of Contract	\$ <u>145,144.00</u>
(6) Total estimated ERDA funds remaining under Contract (subtract Item (4) from (5) which may be used to reduce amount of new funds required from ERDA for proposed renewal period	\$ <u>None</u>

Cont. 3646

Renewal proposal was submitted in April 1974. Since that time Norm Anderson left the NAFN Program and N.C.T. money turned over to Coggin, U. T. to carry on subcontract No. 3379 work with ORNH.

Mr. Coggin stated that all ORN facilities, hamsters etc. mentioned in proposal had to do with Carbid seed work and that no part was being furnished under Cont. 3646. Salary of person involved at ORNH paid by N.C.T. funds.

Per telex
with Coggin

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

For the contract period: July 1, 1975 through June 30, 1976

ARTICLE A-I. RESEARCH TO BE PERFORMED BY CONTRACTOR

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

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

ARTICLE A-II. WAYS AND MEANS OF PERFORMANCE

(a) Items included in total estimated cost:

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

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

None

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

Hoods and Animal Equipment



UNITED STATES
ENERGY RESEARCH AND DEVELOPMENT ADMINISTRATION

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

AREA CODE 615
TELEPHONE 483-8611

AUG 4 1975

Charles W. Hill, Chief Counsel

REQUEST FOR CONTRACT ACTION

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

1. Nature of Action Requested:

- () Selection of New Contractor and/or Negotiation of Contract
Number:
Contractor:
- (XX) Modification of Contract
Number: E-(40-1)-3646
Contractor: University of Tennessee

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

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

3. Type of Contract:

- (XX) Support Agreement () Cost Type () Other

4. Amount of ERDA Funds To Be Obligated by this CA: \$34,102

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

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

Modify contract to provide for the performance of additional research during the period July 1, 1975 through June 30, 1976. ERDA Support Ceiling will be increased from \$145,144 to \$179,246. Title to the equipment, if any, shall vest in the Contractor under authority of Public Law 25-934.

7. Authority:

Form AEC-481 (CA) from
J. L. Liverman, D-BER, HQ,
dated July 15, 1975

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

ACR:IM



cc: File
Alice Brown
Reading
Green

PROCUREMENT & CONTRACTS
3646-JW 5706
CA

RCP&R Branch
Contract Div.
IMedley:arb
8-1075

- (3) Travel: \$ 1,000
Domestic-----\$1,000
Foreign-----\$ 0
- (4) Other direct costs: \$18,437
- (5) Indirect costs (based on a predetermined rate of 64.3 % of salaries and wages): \$ 4,268

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

(1) Items to be contributed by the Contractor:

None

(2) Items to be contributed by the Government:

None

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

All costs including salary, fringe benefits and related overhead.

ARTICLE A-III. The total estimated cost of items under A-II (a) above for the contract period stated in this Appendix "A" is \$ 34,102; ERDA will pay 100 % of the actual costs of these items incurred during the contract period stated in this Appendix "A", subject to the provisions of Article III and Article B-XXIX. The estimated ERDA Support Cost for the contract period stated in this Appendix "A" is \$ 34,102.