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

5798
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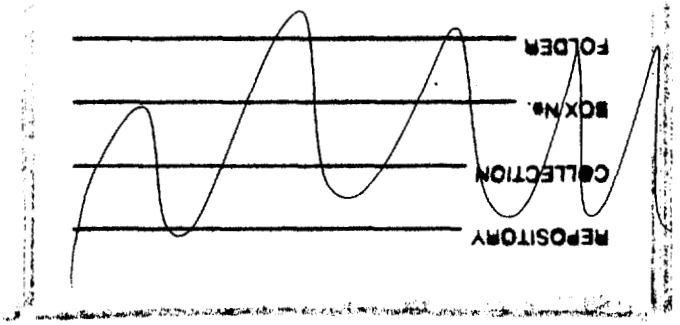


January 6, 1972 707793

REPOSITORY Oak Ridge Operations Off.
Records Holding Area
COLLECTION Documents 1944-1994
BOX No. A-59-3 Bldg. 2714-H
FOLDER Cont 3646 Tenn CA 2-14-72

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

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

We are submitting for your review and appropriate action the following information concerning the contract which will expire on March 31, 1972:

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 Divis
Oak Ridge Operations

OLE:OHO

Enclosures:
As Listed Above

BC: D. S. Zachry, w/2 cys. Progress Report & Form AEC-427 — with Encl
C. S. Shoup, w/cys. Encl.
Alice Brown, w/o Encl.

Adm. Ser. Br.
1/11/72
Osborne
1-11-72

CONTRACTS — 3646 (Tenn)
C. A.

1034114

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

AREA CODE 615
TELEPHONE 483-8611

FEB 14 1972

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:

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

   [X] Modification of Contract
   Number: **AT-(40-1)-3646**
   Contractor: **The 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 Transformations"**

3. Type of Contract:

   [X] Support Agreement [ ] Cost Type [ ] Other

4. Amount of AEC Funds To Be Obligated by this CA: **$29,062**

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

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

   **Modify the contract to provide for the performance of additional research to be conducted during the period April 1, 1972 through March 31, 1973. Increase the AEC Support Ceiling from $47,652 to $76,714. Title to equipment shall vest in the Contractor under authority of PL 85-934.**

7. Authority:

   **Form AEC-481 (CA) frm J. R. Totter, HQ, dtd 2/2/72**

CONTRACTS—3646(Ten...

Herman M. Roth, Director
Laboratory and University Division

OLA

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2-11-72

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APPENDIX "A"

For the Contract Period April 1, 1972 through March 31, 1973.

ARTICLE A-I RESEARCH TO BE PERFORMED BY CONTRACTOR

The Contractor will continue studies of radiation, chemical and aging effects on viral transformation, including a study of oncogenic virus transformation of primary mammalian cells and its stimulation by ionizing radiation, with emphasis on the molecular processes leading to cell transformation. Description will be made of the specific molecular association between viral DNA and the transformed cell DNA and to determine mechanisms by which radiation or radiomimetic agents promote this association.

The Principal Investigator, Dr. J. H. Coggin, Jr., expects to devote approximately 30% 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: | $ 9,100 |
| | 2 Research Assistants<br>Laboratory Aide<br>Labor | |
| (2) | Supplies and Materials: | 8,000 |
| | Chemicals, isotopes, animals, media and sera, and tissue culture plastic ware. | |
| (3) | Equipment to be Purchased or Fabricated by the Contractor: | 2,625 |
| | a Equipment Estimated to Cost Less than $1,000: | |
| | Gradient prep unit, centrifuge head, tissue homogenizer and prep equipment. | |
| | b Equipment Estimated to Cost in Excess of $1,000: | |
| | Fraction Collector with drop counter | |
| (4) | Travel: | 500 |
| (5) | Publications: | 300 |
| (6) | Animal Room Costs: | 2,400 |
| (7) | Reprints, Equipment Repair, and Service Contract Costs: | 550 |

(8) Indirect Costs(Fixed at 61.4% of Salaries & Wages): $5,587

(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:

All costs of the Principal Investigator including salary, related overhead and fringe benefits.

(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

ARTICLE A-III The total estimated cost of items under A-II (a) above for the contract period stated in this Appendix "A" is $29,062; the Commission will pay 100% of the actual costs of these items incurred during the contract period stated in this Appendix "A", subject to the provisions of Article III and Article B-XXVII. The estimated AEC Support Cost for the contract period stated in this Appendix "A" is $29,062.

# PROJECT AUTHORIZATION

RM-72-398

3.A. TO
Robert J. Hart, Manager
Oak Ridge Operations Office

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

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
4-1-72 thru 3-31-73

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 Transforma

12. HEADQUARTERS TECHNICAL CONTACT
George E. Stapleton

13. FINANCING

A. OPERATING EXPENSES

| | |
|---|---|
| New AEC Funds | $ 29,062 |
| Estimated AEC Balance From Prior Term, if any | $ |
| | $ 29,062 |
| Estimated Contractor Contribution, On Proportionate Sharing Basis, if any | $ |
| Estimated Project Cost, For Pertinent Budget Period | $ 29,062 |

Budget and Reporting Classification: 06 01 03
Allotment Transfer: 06-21-91(24)

B. PLANT AND CAPITAL EQUIPMENT ..... $
Budget and Reporting Classification:
Allotment Transfer:

14. SPECIAL PROVISIONS AND INSTRUCTIONS:

The technical aspects of the proposed work have been reviewed and are approved. A need currently exists for the results of the research or other work that is to be undertaken. None of the AEC funds shall be used to confer a fellowship.

Please keep us informed as to any problems encountered in your negotiations, as well as the date of execution of this contract and the amount of funds obligated. If the budget as negotiated differs substantially from that in the proposal, please forward a copy of the revised budget to Headquarters.

If not already submitted, a 200-word summary of the proposed work should be forwarded by the contractor as soon as possible after negotiation of the contract.

15. SCOPE OF WORK

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

816
CONTRACTS — 3643 (Tenn.)
FEB 8 1972

1034118

FEB 3 1972

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

Dear Dr. Coggin:

This is to advise you that we have approved renewal of your Research Contract No. AT(40-1)3646, "A Comperative Study of Radiation, Chemical and Aging Effects on Viral Transformations," for an additional year at the level of research support requested. You will be contacted by someone from the Oak Ridge Operations Office in the near future regarding negotiation of the renewal contract.

As per our telephone conversation, please acknowledge your desire to withdraw the supplemental request we are now holding as an approved and unfunded proposal.

Your next renewal period comes after the completion of three years of operation of this contract. For every multiple of three years the Division of Biology and Medicine 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.

D 516

FEB 8 1972

1034119

(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 K. Stapleton
Radiation Biologist, Biology Branch
Division of Biology and Medicine

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

816

BMB BMB

Stapleton/bel Edington

1-31-72 2- -72

FEB 3 1972

1034120

FINANCIAL STATEMENT

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

| | | |
|---|---|---|
| 1. | Total actual project cost to date for the current period | $13,524.74 |
| 2. | Estimated total cost for remainder of period | 8,142.24 |
| 3. | Total acutal and estimated cost chargeable to AEC for current period based on percentage of cost agreed upon as contained in A-III of Appendix "A" to contract | ~~13,524.74~~ 21,666.73 Indirect 1,168.00 |
| 4. | Accumulated costs chargeable to AEC (include costs reported in certified statement for preceding period(s) and the costs stated in Item 3 above) | ~~91,402~~.94 47,779.2 |
| 5. | Accumulated AEC Support Ceiling as stated in Article III of contract | ~~26,303.00~~ 47,[illegible]2 |
| 6. | Total estimated AEC funds remaining under contract (subtract Item 4 from Item 5) which may be used to reduce amount of new funds required from AEC for proposed renewal period | $ ~~527.98~~ Credit 0-- Projected 127.25 |

1034121

SI-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 Affects 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: ____ NO. OF GRADUATE STUDENT MAN-YEARS: 5

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 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 object 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 susceptibility to weakly oncogenic agents.

Incorporated into above section

| | PROGRAM CATEGORY NO. |
|---|---|
| BUDGET | |
| PRIMARY | |
| SECONDARY | |

Joseph H Coggin Jr
Signature of Principal Investigator

DATE: Dec 30 1972

INVESTIGATOR – DO NOT USE THIS SPACE

1034122

FINANCIAL REPORT AND SUMMARY

Project Director Dr. J. H. Coggin Date November 23, 1971

Contract or Grant No. AT-40-1-3646 Account No 141012-6216P

Name of Grant A E C

Period budgeted herewith 4-1-71 - 11-23-71 Total period of budget 4-1-71 - 3-31-72

Amount budgeted herewith $21,135.00

| Catagories | Budget | Expended | Encumberances | Salaries Projected | Estimated Free Balance |
|---|---|---|---|---|---|
| Salaries | 9,100.00 | 6,157.78 | 750.20 | 2,720.00 | -527.98 |
| Consultants & Honorariums | -0- | - | - | - | - |
| Supplies | 8,850.00 | 2,058.18 | 4,169.10 | | 2,622.72 |
| Stipends | -0- | - | - | | - |
| Tuition | -0- | - | - | | - |
| Equipment | 2,685.00 | - | 304.00 | | 2,381.00 |
| Travel | 500.00 | - | 85.48 | | 414.52 |
| Other | -0- | - | - | | |
| TOTALS | 21,135.00 | 8,215.96 | 5,308.78 | 2,720.00 | ~~$-4,889.76*~~ 4,840.26 * |

* This apparent free balance is schedule for expenditure to cover a large plasticware order pending award through purchasing and centrifuge equipment. We anticipate an actual $527.98 deficit in salaries. We requested supplemental funds earlier this year to cover a full time technician and this proposal is still pending.

Note: Above report does not reflect #5,168.00 in indirect cost.

1034123

Renewal Proposal

Sixth 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, 197.

1. Title of Project

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

2. Institution

University of Tennessee
Department of Microbiology
Knoxville, Tennessee 37916

Telephone: 615 - 974 - 3441

3. Project Abstract

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

techniques. The mechanism by which irradiation potentiates the viral transforma-tion process to initiate cellular retrogression is a prime concern in our present study plan.

4. Scientific Background (See previous applications for consideration of the older literature, please)

A. Status of information regarding SV40 integration into cellular DNA

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

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

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

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

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

Added to this list one would now include the observations that different transformed cell lines may possess different amounts of virus DNA (genome equivalents) associated with their chromosomal DNA (2-4, 10). Only transformed cell lines containing T antigen were observed to actively synthesize viral RNA suggesting that S or surface antigen is not a direct virus specific function (11).

Integration of virus DNA in the cellular DNA (nuclear DNA) has only been shown to occur to date in the SV40 transformed 3T3 mouse cell, strain SV 3T3 (4, 12). Aloni, et al (15, 16) warned against the use of SV40 DNA preparations to conduct hybridization studies with certain monkey cell lines transformed by SV40.

Cellular DNA packaged in virions with SV40 DNA can produce confusing data. It is not known whether such integration or fixation occurs in other transformed lines or in primary cells transformed cells although this might generally be the case (10). The problem is to identify the site or sites of insertion and to establish the uniformity of the mechanism involved. Certain chromosomes seem to be involved in at least two systems examined to date (13, 14).

In lytic infection, three to four of the supposed ten 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 (12). One additional virus gene seems related to the induction of cellular DNA synthesis occurring at the same time as virus DNA replication is initiated (12).

Several recent reports have been most enlightening regarding the number of viral equivalents per genome. Employing complementary (c) RNA produced in vitro from SV40 or polyoma DNA template, Westphal and Dulbecco (3) examined a number of polyoma and SV40 transformed tumor lines to determine the number of equivalents of SV40 per cell type. Results using the cRNA:DNA hybridization technique indicated that each line of tumor cell contained a different number of "viral equivalents" ranging from 5 to 60 equivalents within the nucleus of the cells. These workers asserted that no biological significance could be attributed to a cell line containing 60 equivalents of virus rather than 5 with

respect to being "more" transformed. Tai and O'Brien (10) confirmed that a large number of viral equivalents existed in SV40 tumor cells using a cRNA:DNA hybridization technique at 24° for 18 hours in formamide.

Gelb, Kohne, and Martin (personal communication, 17) have devised a DNA: DNA hybridization technique which is sufficiently sensitive to detect one molecule or less of viral DNA per genome of the transformed cell using hydroxyapatite rather than DNA trapped on nitrocellulose filters. The high number of viral equivalents per genome reported above for different SV40 tumor lines employing the cRNA:DNA technique seems to be erroneous. Results using this technique (RNA:DNA) depend on the "fidelity and completeness" of SV40 DNA transcription by E. coli polymerase and the method of calculating the number of viral equivalents (17). Background reactions between SV40 cRNA and normal cell DNA were subtracted routinely to obtain the reported results. Aloni, et al (15) observed the purified form I SV40 DNA reacted with normal cell DNA from several species. Gelb et al point out that $^{3}H$-labelled green monkey DNA in which the SV40 is prepared was one-tenth as efficient as labelled SV40 DNA in reacting with SV40 DNA trapped on filters; a fact suggesting the unlikely prospect that thousands of copies of viral DNA sequences were present in monkey kidney DNA. Employing proper viral DNA with no homology for monkey kidney or hamster or mouse cell DNA, DNA:DNA hybridization studies were conducted and hybrids were trapped on hydroxyapatite after the technique of Gelb, et al. The percentage of viral DNA ($^{32}P$) reannealed with homologous viral DNA or test DNA is plotted from results obtained by sampling the reactant DNA in solution at 60° and noting the nucleic acid concentration, Co (O.D. at 260 mμ) for a given reaction time, t: Cot = OD x hours/2. The Cot 1/2 obtained from seven experiments hybridizing SV40 DNA with $^{32}P$ SV40 was $3.6 \times 10^{-4}$ with extremely high reproducibility.

The effect of test preparations of normal and transformed cell DNA on the reassociation of known equivalent concentrations of $^{32}P$-SV40 DNA are used to determine the concentrations of SV40 DNA sequences in the preparations. Results using the technique of Gelb, Kohne and Martin indicate that SV40 transformed clones actually contain an average of one SV40 genome per cell. In preliminary efforts with two SV40 transformed cell lines we have observed a similar result (0.9 to 1.4 SV40 equivalents per genome). We are currently employing the hydroxyapatite method for all determinations for DNA:DNA association experiments described in Section 5.

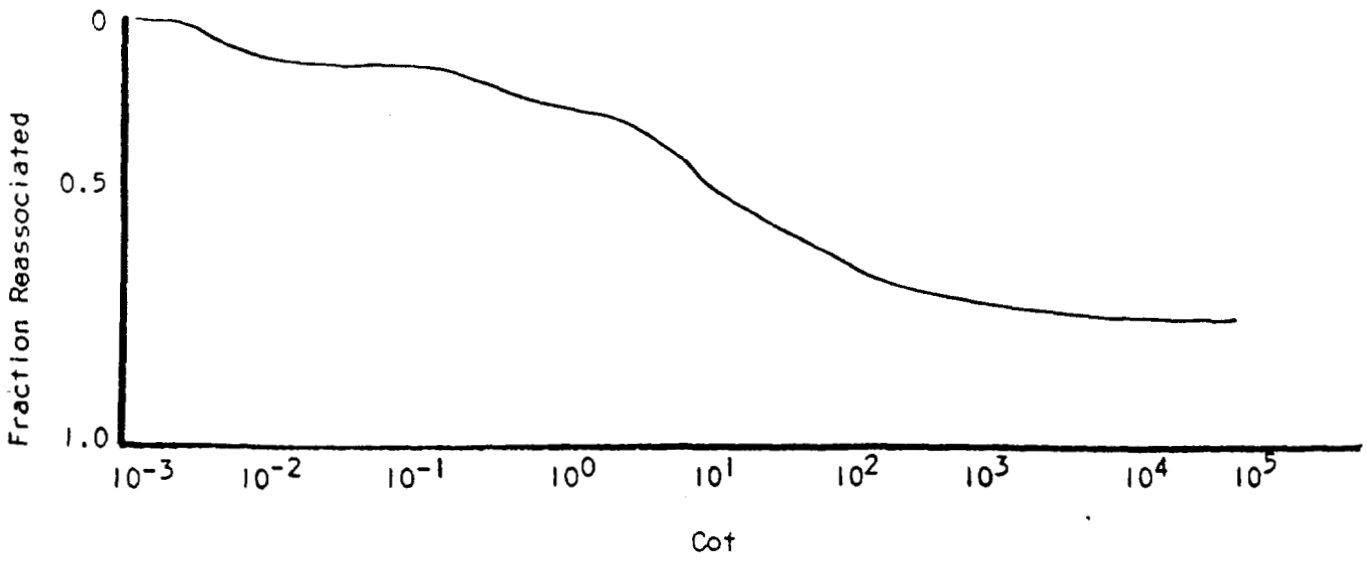
Martin (18) examined the possibility that SV40, polyoma or adenovirus 12 might alter the normal pattern of RNA synthesis in cells transformed by these agents. As in most differentiated mammalian cells, only a small fraction (5%) of the cellular DNA is transcribed. Employing competition hybridization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

B. Unique DNA - Techniques and Significance

Until very recently the traditional techniques for conducting hybridization with RNA actually measured only a special class of RNA representing transcripts of related gene families. Britten and Kohne (19) have shown that about 70% of the DNA of the mouse consists of genes having but a single copy per haploid genome.

These unique RNA transcripts require 500 times as long to find their complement in RNA:DNA hybridization as do RNA copies from the highly repetitious sequences (20). Britten and Kohne can obtain reassociation of the single copy genes by incubating high concentrations of sheared DNA over long periods of time. A relation exists between genome size (repetitious to unique) and the rate of reassociation or annealing for the DNA as a function of Cot: [Cot = product of time of incubation (sec.) and concentration (moles nucleotide per liter)]. The precision of gene repetition is imperfect and members of a family of repeated DNA are closely related rather than identical.

It would be of interest to know whether the same results obtained by Martin (18) for repetitive DNA transcription could have been obtained with unique DNA copies which constitute the majority of the DNA. Reason and a large body of evidence suggests that all cells of a given metazoan species in the same state of ploidy contain the same complement of DNA (20). It is important that information be rapidly collected to show whether, in fact, tumor cells transcribe the same unique DNA sequences as do their progenitor tissues. We are currently seeking to discover if, indeed, the SV40 genome(s) present in transformed cells is (are) located among the unique DNA sequences. Such studies are plausible after techniques described by Britten and Kohne (19), McCarthy, et al (20) and Gelb, et al (17). Nuclei are collected from the desired tissues and the DNA extracted. The DNA is sheared or depurinated into small fragments, dissociated by heating and held in formamide (40-48%) at 37°C (21). Samples are removed at regular intervals, diluted to reduce the formamide concentration to less than 1%; the annealed DNA is subsequently trapped on hydroxyapatite, eluted and quantitated at 270 mμ. Results are plotted as shown below:




Redundant gene sequences reassociate at a rate reflecting their extent of repetition and the total plot reflects the sum of all the families of DNA (frequency spectrum); a given Cot "cut" can be selected by collecting a sample fraction from any segment of the reassociation plot. These fractions, especially those representing unique DNA, can subsequently be examined for homology with RNA or DNA.

For some eight years, researchers have conducted a large number of investigations employing hybridization techniques. McCarthy and Church (22) have recently reviewed many aspects of the proper conduct of these techniques and has suggested a marked lack of specificity which can be obtained using high RNA:DNA ratios, elevated temperatures, and other conditions which introduce ambiguity into competition experiments. A new stringency assay for conducting reliable competition hybridization experiments has been developed by McCarthy and his colleagues (personal communication) and these methods will be employed for our studies.

C. Fetal Expression in Cancer Cells

A growing list of human cancers are known to possess fetal components not expressed in homologous adult tissues (colonic, hepatic and lung cancers 23, 24, 25). We recently demonstrated (26, ORO-3646-11) that hamster, mouse and human fetal cells contain surface membrane antigens cross-reactive with SV40, adenovirus 31 and certain chemically-induced tumor specific transplantation antigens (TSTA) in syngeneic hamsters. Fetal cells also induced a specific antibody reactive with the TSTA present in the tumor cell membrane, termed cytostatic or C antibody. The role of C antibody in tumor progression or rejection has been characterized in our laboratory under a research program supported in part by the A. E. C. (27, 28, 29). Soluble extracts of 72 different mouse tumors have recently been examined and found to be cross-reactive with antiserum produced in rabbits against mouse embryo cells confirming, indirectly, that a parallel situation to that found in hamsters exists in the mouse (30). Recently P. Dierlam of our laboratory has shown that fetal membranes are masked in the latter stages of gestation by a three-fold increase in sialic acid content and these membranes (14 day fetal cells) do not induce transplantation immunity. SV40 transformed kidney tumor cells were observed to have one-third less sialic acid than normal adult kidney cells. These and other findings suggest the following summarial facts regarding fetal antigen and cancer antigens:

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

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

(c) SV40 produces the same TSTA in mouse, hamster and human cells transformed by the virus and embryo cells from all three species of fetus have antigens cross-reactive with this transplantation antigen.

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

D. Unique Macromolecular Synthesis in Tumor Cells

Cassingena and Tournier (33) have isolated a "repressor-like" extract from SV40 transformed cells which inhibits the replication of SV40 in permissive cells (30% inhibition). The extract entered the cells only when combined with poly-L-lysine. Extracts from control tissues or from heterologous tumors were not similarly active. The componen from SV40 tumors was nuclease insensitive and protease sensitive. "Repressor" was found in small quantities in productive or permissive cells (monkey) but appear late in the intectious cycle; in abortively infected cells more repressor was evident and appeared quite early following viral adsorption and penetration. These data suggested that unintected cells might contain a factor which counteracts the "repressor" and indeed, such a factor was observed. This factor, termed FBR, could enhance virus plaque formatic in permissive cells indicating its ability to neutralize "repressor" in normal cells. The "repressor" does not interfere with viral adsorption or penetration nor prevent viral DNA synthesis. In recent discussions (October, 1971) with Cassingena I was disappointed to learn that little new information regarding the isolation or characterization of the repressor had been obtained and that the repressor could not consistently be isolated in active form. He indicated that the cell source was unreliable and that present efforts in their laboratory centered on trying to repeat their initial observations with

repressor from a different type of permissive cell. When the detailed method for isolation becomes available it will be interesting to determine whether hamster, mouse and human fetal tissues have a similar repressor which increases and decreases coordinate with surface antigen (SV40 TSTA) appearance during differentiation.

Many new studies have been reported in the past year on the transcription of viral information in transformed cells prior to and following SV40 infection. Sokol and Carp (34) have confirmed the initial observations of Martin and Byrne (35) that polycistronic RNA transcripts of SV40 DNA are present in the nuclei of productively infected monkey cells (larger than DNA of SV40) late in infection. These large 32-50S RNA transcripts are degraded or fragmented before passing into the cytoplasm (28S). Some evidence suggests that transformed cells have RNA larger than the 28S cytoplasmic SV40 RNA which is synonymous with RNA transcribed from the viral genome in productive infection. Characterization of this SV40 RNA in transformed cells, derived from the integrated SV40 genome is of major concern to us in our study of the similarities and identity between certain fetal RNA species and SV40 RNA produced in tumor cells.

Late viral functions in cells transformed by SV40 are curtailed. Gerhard Sauer (36) confirmed that SV40 DNA synthesis was essential to "late" SV40 mRNA transcription as is now known to be true for DNA viruses in general. Surprisingly, Sauer observed that late viral mRNA synthesis in SV40 transformed cells does <u>not</u> require DNA synthesis (ara-C inhibition failed to halt late viral transcript production). These findings show that the "normal" regulatory pattern of viral RNA synthesis evident in reproductive infection is not operational when the viral genome becomes integrated into the host DNA. Jaenisch, et al (37) and Bourgaux et al (38) have shown that the intact ring is the normal replicating DNA molecule in SV40 reproduction. Perhaps linear insertion of SV40 genome into the host genetic apparatus leads to augmentation in normal trans-

criptional control of SV40 RNA synthesis in transformed cells. The implications of this control change in relation to the transforming properties of the virus and tumor induction are unclear at present. It should be noted that although late viral mRNA synthesis can proceed in certain tumor clones late protein synthesis (capsid production) still does not occur suggesting yet another defect in normal control in SV40 transformed cells, this at the translational level. Lindberg and Darnell also observed that lengths of viral RNA in the nucleus of the transformed cells were longer than the lengths of a normal viral genome (39). They proposed that several viral equivalents might be integrated at a given site accounting for these poly-viral RNA transcripts. Evidence by Gelb, et al in the previous section clearly showed that most SV40 transformed tumor lines contained approximately 0.5 to 2 genome equivalents per cell. Several investigators have reported that SV40 DNA replication proceedes via circular concatenate formation. Perhaps such structures are opened and inserted into cellular DNA during transformation. Only by working with cloned derivatives of these lines with uniform amounts of viral DNA per cell (if that is the case) can this problem be solved. Nevertheless, the consistent data forthcoming from these studies enables subsequent investigation to be forewarned of the heterogenicity and fragmentation of viral RNA in tumor cells and such information is particularly useful to our work.

Koprowski (40) recently reviewed the usefulness of cell hybrid studies in characterizing the association between the viral genome and the cellular genome. Wever et al (41) proposed the following sequence of virus production in heterokaryons formed between permissive cells and non-virus producing, transformed tumor cells. After fusion with Sendai virus, SV40 DNA appeared in the tumor cell nucleus followed by the appearance of mature SV40 virions in the nucleus. Virus remained confined to the nucleus for 40 hours then was released to infect the permissive cell nucleus.

Afterward, virus production proceeded normally with virus release into the cytoplasm after six days.

Hirai (42) et al recently demonstrated that the SV40 genome becomes integrated into the hamster genome within 15 hours after infection paralleling cellular DNA synthesis and T antigen synthesis. Numerous studies (e.g., 43, 44) have shown the circular SV40 or polyoma genome (form I) can induce cellular DNA synthesis and this may indeed be pre-requisite to successful viral integration. Rabovsky has recently written an excellent review of the problems of viral insertion into the chromosomes of mammalian cells (45) and many articles have appeared confirming the intimate association of the viral DNA with the host DNA (e.g., 46, 47).

E. Surface Changes Associated with Viral Transformed Tumors

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

Sheppard (52) recently showed that dibutyryl cAMP treatment of SV40 tumor cells reinstated "normal contact inhibition" in cell culture and removal of the $(But)_2$ c-AMP resulted in cell overgrowth typical of tumor cells. The explanation for this restoratic of normal cell behavior when treated with a derivative of the ubiquitous cAMP, important in cellular control in some as yet obscure way, is not known. Viral induced alterations in surface membrane structure or in the normal metabolic or regulatory

activities of the plasma membrane of the cell warrant intensive investigation and afford the best approach to understanding the specific mechanisms involved in neoplastic conversion.

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5. Scientific Scope

The research effort is designed to investigate the precise conditions and parameters of enhanced virus transformation of normal cells stimulated by radiation, radiomimetic chemicals and associated natural processes (cell aging). As before, the program involves a quantitative examination of radiation or chemically-induced alterations in cells which sensitize them to the transforming potential of SV40 and adenoviruses. An important result to be simultaneously gleaned from the present approach will be information about the mechanism for virus transformation of cells under conditions of normal infection. Previously we have emphasized the importance of using normal 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 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.

We now seek to describe the specific molecular association between viral DNA and transformed cell DNA and to determine the mechanism by which radiation or radiomimetic agents promote this association. Finally, we have overwhelming evidence that the result of the interaction between oncogenic virus and target cell

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

A. Rationale

The present and proposed research is designed to investigate the precise mechanism by which radiation, radiomimetic chemicals and cell aging processes potentiate neoplastic transformation stimulated by DNA-containing oncogenic agents. Huebner and Todaro (Proc. Natl. Acad. Sci., 64, 1969) have proposed that all malignant conversion is the result of genetic expression of RNA tumor viruses equipped with "virogenes" to permit the production of a virus particle (C-type virus) containing "oncogenes" for promoting malignant changes in cells. The RNA tumor viruses are suggested to have the genetic capacity to promote or code for the synthesis of a reverse transcriptional enzyme capable of forming a DNA-copy of the RNA genome. It is also feasible that the true primordal virus is the vertically transmitted, DNA oncogene which may be an innate genetic component of the cell, occasionally activated by transcription to an RNA copy or C-type particle (Huebner, et. al., Proc. Natl. Acad. Sci., 67, 1970). There is growing support that a reverse transcriptase enzyme, in fact, exists in the RNA virion and in the infected or transformed cells harboring or releasing C-type particles. Thus, RNA viruses could theoretically reside as a DNA-template (copy) in the chromosomal complement of the cell whether or not that cell is malignant. Certainly not all cells have demonstrable C-particles. Carcinogenes, DNA viruses and physical mutagens are, by inference, believed to function indirectly in inducing malignancy by stimulating the expression of oncogene

information stored in normal cells as cellular genetic information.

This theory cannot readily be confirmed at the present time since any neoplastic cell could potentially be stimulated to express only oncogene information and not virogene information and hence would not be expected to have detectable viral specific proteins or capsid material. That is, there is no experimental way at present to establish that all cancer is not the total result of the activation of cryptic "oncogene" information innate or stored in the cellular DNA and transmitted vertically to progeny. This theory will and, in fact has, promoted intensive research to show that RNA viruses can undergo reverse transcription to a DNA state and secondly, that all normal cells have segments of DNA which are homologous with C-type viral RNA.

Our effort for five years in this A. E. C. sponsored program has been oriented to define the mechanism by which DNA-viruses produce a consistent form of neoplasia in several species of cell transformed by exposure to these agents; consistent in that the pathology of the virus tumors are identical in a given tissue system and that the neoantigens expressed by the tumor are common or identical for all transformed cell types; even across species barriers (tumor specific transplantation antigen-TSTA, and T antigen). Secondly, we have sought to account for the observation that low-level radiation and other factors which produce lesions in the target cell DNA enhance the transformation process.

That our research objectives are still meaningful in consideration of the potential validity of the "oncogene hypothesis" for malignancy should be evident from the following considerations: the mechamism by which the C-type viral DNA "copy", if it truly exists, in nature is inserted into the host chromosome(s) apparatus must be described. Mounting evidence has clearly demonstrated that

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

Further, we have observed that low-level radiation and subtoxic chemical exposure potentiate the oncogenic qualities of SV40 and adenovirus both _in vitro_ and _in vivo_ presumably by damaging the cellular DNA (in either a physical sense producing a gap or by impairing a regulatory gene) and facilitating insertion of the viral genome into host cell genetic material. This mechanism must be understood because of the widespread application of radiation in medicine and its growing use in industry and because of the strong association between certain forms of neoplasia and DNA-containing herpes viruses. We have carefully prepared ourselves to conduct the proper experiments to answer these questions and have prepared the necessary biological reagents to proceed.

Finally, and perhaps most importantly, we have recently shown that an identity exists between membrane components expressed in the fetuses of the hamster, mouse and human during the normal course of fetal development and tumor antigens present as specific transplantation antigens on SV40 and adenovirus 31 hamster tumor cell membranes, chemically-induced hamster tumor cells, and in mouse spleen cells infected with Rauscher leukemia virus. Other investigators have subsequently shown similar antigens in mouse lymphomas, chemically-induced rat sarcomas and in other tumors. These observations provide strong evidence that both chemical and biological carcinogenic agents act to promote the expression of cellular products in adult cells which are normally restricted to fetal life. At least four major types of human malignancy are now known to possess fetal antigens, presently we are seeking to establish that classes of RNA, unique to fetal tissues or absent in these tissues during gestation are also uniquely present, or conversely absent, in SV40-transformed cells when compared to the RNA present in normal cells. These studies are being conducted with RNA and DNA from individual tissues of hamsters representing normal target cells for SV40 transformation, in vitro derived transformed clones from those normal tissues, and fetal tissues giving rise to the adult target cells employing stringent competetion hybridization procedures. The techniques developed for this work are in use in our laboratory and will afford not only a clarification of the significance and character of fetal expression in cancer in our hamster and mouse model systems, but, more importantly, will outline the parameters for a meaningful molecular study of fetal antigens occurring in several forms of human malignancy.

B. Specific studies planned or on test

Particular emphasis in the coming year will be given to the conduct of a molecular and biochemical examination of SV40 transformed cell clones, fetal tissues and radiation-sensitized SV40 tumor clones in an effort to:

(1) discover whether radiation pretreatment of hamster target cells results in the insertion of several or many copies of the SV40 genome into the host DNA compared to the one to two copies of viral genome observed in SV40 transformed clones not sensitized by pre-irradiation.

(2) discover the location of the SV40 genome(s) in the DNA of the various clone phenotypes; specifically, is the SV40 genome incorporated within the repetitious or non-repetitious (unique) DNA of the host cell in each of these tumor clones?

(3) determine if the RNA uniquely present or absent in SV40 tumor cells is identical to that uniquely present or absent in 10 - 12 day fetal tissue by RNA competition experiments performed under conditions of stringency.

(4) characterize the quantitative changes in sialio-compounds in the membranes of radiation sensitized, virus-infected target cells undergoing transformation to correlate the neoplastic changes (development of neoantigens and loss of contact inhibition) observed in membranes of premalignant cells with recently observed maturational changes noted in fetal membranes.

In previous work sponsored by this contract we have characterized temporal and biological factors which affect the usual interaction between several oncogenic agents and target cells transformed by these viruses, and the enhancement of this transformation by radiation. The parameters examined to date include radiation dosage, virus concentration, radiation repair effects, radiation effects on macromolecular synthesis in the target cells, radiation stimulated oncogenesis in vivo, etc.

We are now focusing our total effort on describing the physical association between the viral genome and the cellular genome in an effort to specifically locate the viral genome, to monitor its genetic expression in situ, and to dis-

(d) Do SV40 transformed cells treated with x-ray prior to infection with SV40 have more viral equivalents of DNA per cell genome equivalent than non-irradiated transformed clones and, if so, among what class of cell DNA (unique or repetitious) are these integrated segments observed?

We have preliminary data which suggests that indeed, radiation sensitized clones do contain an increased number of SV40 equivalents per genome compared to the small number of equivalents observed in clone #1 in the above table (0.9 - 1.4). Particular care will be taken to avoid the technical problems encountered by other investigators which have led to erroneous conclusions regarding the number of viral equivalents per cell as discussed in a previous section of this proposal.

(2) Immunologic studies completed in our laboratory (ORO-3646-11, ORO-3646-12) have established that fetal cells have membrane antigens identical or cross-reactive with virus-induced neoantigens on several types of tumors. We now wish to determine whether SV40 transformational processes result from a specific activation of fetal genes repressed in the latter stages of differentiation and not expressed in the normal adult cell, or rather, reflect specific viral interference with normal patterns of membrane synthesis. The result of the latter effect could, among other possibilities, prevent the normal deposition or arrangement of sialic acid residues in tumor membranes, exposing substructure membranes. These basement membranes might normally be "exposed" during embryogenesis and fetal development.

Several approaches are being used to resolve these questions. First, the chemical composition of membranes of each tumor clone, adult tissue and embryo tissues have been prepared and are being analyzed exhaustively for comparative purposes. Secondly, we are seeking to determine whether one oncogenic effect of SV40 transformation of hamster embryo cells in culture is to cause the cell to maintain an embryonic antigen display in the transformed cell-membrane, preventing normal (chemical and antigenic) maturation of the cell to an adult "state". Third, we are comparing the RNA present in embryo or fetal tissues with that present in normal cells by the very sensitive, RNA competition assays developed recently in Dr. Brian McCarthy's laboratory by Doctors Shearer, Church and McCarthy (Problems in Biology: RNA in Development, ed. E. W. Hanly, Univ. Utah Press, 1969 p. 285-313). This technique, termed the stringency competition assay, for measuring RNA specificity for the DNA template again avoids many of the discrepancies and artifact reported to date in competition assays employing a variety of other conditions. Having defined the differences in the sequences of RNA transcribed in fetal and adult cells we intend to set about comparing those RNA sequences unique to fetal cells to those uniquely present in SV40 transformed cells by competition hybridization experiments. Should we

discover that tumor cells are, in fact, devoid of RNA classes or sequences present in normal adult tissues we shall alternatively attempt to determine if these RNAs are also absent in normal fetal tissues displaying the cross-reactive tumor neoantigens.

(3) We wish to determine whether or not an array of female hormones are capable of suppressing (a) normal transformation of adult hamster target cells by SV40 and (b) radiation-potentiated transformation of adult hamster cells by SV40. The rationale for these experiments is found in our recent observation that adult, female hamsters, but not males, can augment or discriminate against the expression of fetal antigens in her tissues. The establishment of this fact can readily be determined in vitro in the proposed experiment. It is significant here to note that the overall incidences of many forms of human neoplasia is significantly lower in females than in males and this experiment should facilitate an explanation for this fact.

c. Scientific Personnel

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Current Memberships:

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

Experience:

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

Viroiogist and Associate Professor, University of Tennessee, 1966 to present. 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. Principal Investigator - NCI Tumor Transplantation Antigen Study FS-7, ORNL.

Senior Research Virologist. 1965 - 1966. Virus and Cell Biology Division, Merck Institute for Therapeutic Research (Merck, Sharpe and Dohme Laboratories) - Planned and supervised research in cancer virology and immunology in conjunction with Dr. Maurice Hilleman. The program involved experimentation into problems of tumor antigen assay and purification, the viral etiology of cancer, recovery of viruses from transformed cells and immune mechanisms operative in hamsters infected with oncogenic viruses.

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

Acting 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.

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:

Tumor Immunity in Hamsters Immunized with Fetal Tissues. J. Immunol. 107, 526-533. 1971 J. H. Coggin, K. R. Ambrose, B. B. Bellomy and N. G. Anderson.

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

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

Associate Investigators:

E. R. Rogan, Ph. D., Research Associate. 100% time on project. Supported by National Cancer Institute Contract FS-7.

M. Schafer, M. S., Laboratory Technician. Supported by National Cancer Institute Contract FS-7. 100% time on project.

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

W. A. Rutala, B. S. Graduate Student. 50% time on project

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

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.

A new contract with Union Carbide (MAN Program) under subcontract No. 3379 to study "Immune Reactions in Virus-Induced Tumor Rejection" has been in progress since July 1970 and is funded at the present level of $106,000 per year. This program is sponsored by The National Cancer Institute as a segment of contract FS-7. The objectives of this research are to develop immunotherapeutic techniques for controlling human cancer.

The University provides $1200 in supplies and services to the AEC project

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 $25,000 have been expenced in the past 24 months to generally upgrade our research area privided spectrophotometers, wash area facilities and animal room improvements. Additionally, a new transfer hood work station and special equipment

for hybridization studies were obtained. An electron microscope (RCA-EMU-4) has recently been given to me for the conduct of research in my laboratory from the National Institute of Allergy and Infectious Diseases. Additionally, the MAN Program recently provided me with $7,000 in special equipment to make the preliminary hybridization studies feasible.

10. BUDGET

April 1, 1972 to March 31, 1973

| | |
|---|---|
| 1. Salaries and Wages: | |
| Two Graduate Assistants - 50% time at $3,300 each | $6,600.00 |
| Laboratory Aide (Glassware washer) - 50% time | 1,500.00 |
| Student Assistants | 1,000.00 |
| fringe benefits | -0- |
| Salaries and Wages Subtotal - $9,100.00 | |
| Overhead - Indirect Costs - 61.4% of Salaries and Wages | 5,587.40 |
| 2. Supplies and Materials: | |
| Chemicals | 1,500.00 |
| Isotopes | 1,500.00 |
| Animals | 1,000.00 |
| Media and Sera | 1,500.00 |
| Expendable (tissue culture plastic ware) | 2,500.00 |
| Supplies Subtotal - $8,000.00 | |
| 3. Equipment: | |
| Fraction collector with drop counter | 1,250.00 |
| Gradient prep unit | 550.00 |
| Replacement H50 centrifuge head - less trade-in | 400.00 |
| Tissue Homogenizer and prep equipment | 425.00 |
| Equipment Subtotal - $2,625.00 | |
| 4. Publication Costs | 300.00 |
| 5. Travel - Principal Investigator | 500.00 |
| 6. Other - Reprints, repair costs, share of service contract, animal room housing costs* - 100 cages per month at $2.00 per cage x 12 months | 550.00<br>2,400.00 |
| Requested AEC Contribution | $29,062.40 |

University Contribution:

The University proposes to release the time of the principal investigator during the academic year so that 33% of his effort will be devoted to this research program. This contribution includes salary, overhead, fringe benefits and, in addition, generally constitutes $1,200.00 in expendable supplies and services. This represents a substantial cost sharing by the University of Tennessee.

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*This new charge will be incurred this coming year for the first time as part of a required contribution from the contract agency. This represents a diminished University contribution to this contract effective 3-31-72 and these costs are necessary to cover the expense of compliance with AALAC requirements for housing.

11. 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