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UNITED STATES
ATOMIC ENERGY COMMISSION

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

AREA CODE 615
TELEPHONE 483-8611

November 8, 1972

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:
- Modification of Contract
Number: AT-(40-1)-4155
Contractor: University of Florida

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

Title: "Formation and Repair of Gamma-Ray Induced Nucleic Acid Base Damage in Bacteria and Mammalian Cells"

3. Type of Contract:

- Support Agreement
- Cost Type
- Other

4. Amount of AEC Funds To Be Obligated by this CA: \$46,000

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

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

Modify contract to provide for the performance of additional research during the period 9-1-72 - 8-31-73. Increase AEC Support Ceiling from \$69,209 to \$115,209. Title to equipment shall vest in the contractor under authority of PL 85-934.

7. Authority:

Form AEC-481 (CA) from J. R. Totter dated October 2, 1972.

REPOSITORY Oak Ridge Operations
 COLLECTION Records Holding Area
Documents 1944-1994
 BOX No. H-75-17 Bldg. 2714-H
EVO 4155
 FOLDER Expenditure Statement
 ACR:LM

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

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APPENDIX "A"

UNIVERSITY OF FLORIDA

CONTRACT NO. AT-(40-1)-4155

For the contract period September 1, 1972 through August 31, 1973.

ARTICLE A-I. RESEARCH TO BE PERFORMED BY CONTRACTOR

The Contractor will continue studies of the formation and repair of gamma-ray induced nucleic acid damage in bacteria and in mammalian cells, particularly thymine damage, to include (1) a study of the efficiency of the intracellular formation of damage in E. coli, Chinese hamster ovary cells and human embryonic lung fibroblasts relative to free DNA in solution and mononucleotide mixtures. Estimation will be made of the extent of nucleic acid base damage relative to other types caused by ionizing radiation under in vivo conditions; (2) a study of the molecular mechanism of the removal of gamma-ray damaged thymine from irradiated DNA in vitro, and the contribution of nucleic acid base damage to the biological effects of gamma-irradiation will be assessed in phage system, and (3) a study of the post-irradiation removal of gamma-ray damaged thymine from the DNA of Chinese hamster ovary cells and human embryonic fibroblasts in unsynchronized and synchronized cultures.

The Principal Investigator, Dr. P. A. Cerutti, expects to devote approximately 25% of his time or effort to the project.

ARTICLE A-II. WAYS AND MEANS OF PERFORMANCE

(a) Items Included in Total Estimated Cost:

- | | |
|---|----------|
| (1) <u>Salaries and Wages</u> (Includes Fringe Benefits): | \$19,653 |
| (2) <u>Equipment to be Purchased or Fabricated by the Contractor:</u> | \$ 3,309 |
| <u>a</u> Equipment Estimated to Cost Less than \$1,000: | |
| Vaccum pump and rotary evapo-mix | |
| <u>b</u> Equipment Estimated to Cost in Excess of \$1,000: | |
| Rollacell apparatus and absorbance monitor | |
| (3) <u>Travel:</u> | \$ 1,400 |
| Domestic----- | \$800 |
| Foreign----- | \$600 |

UNIVERSITY OF FLORIDA

CONTRACT NO. AT-(40-1)-4155

- (4) Other Direct Costs: \$11,925
- (5) Indirect Costs (Based on a predetermined rate of 49.42% of Salaries and Wages and Fringe Benefits): \$ 9,713

(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

(2) Items to be Contributed by the Government:

None

(c) Time or Effort of Principal Investigator 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 \$46,000; the Commission will pay 100% of the actual costs of these items incurred during the contract period stated in this Appendix "A", subject to the provisions of Article III and Article B-XXIX. The estimated AEC Support Cost for the contract period stated in this Appendix "A" is \$46,000.

The estimated AEC Support Cost is funded as follows:

- (a) Estimated unexpended balance from the prior periods: \$ 0
- (b) New funds for the current period: \$46,000

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

THE HILLIS MILLER HEALTH CENTER
UNIVERSITY OF FLORIDA

Department of Biochemistry



Phone: 904-392-3361
Gainesville, 32601

November 1, 1972

A. H. Frost, Jr., Chief
Research Contracts, Procedures and Reports Branch
Contract Division
U. S. Atomic Energy Commission
Post Office Box E
Oak Ridge, Tennessee 37830

Dear Dr. Frost:

Enclosed please find a revised budget for the period of September 1, 1972 to August 31, 1973 of contract AT-(40-1)-4155. I am also sending you a copy of a letter to my project director in Washington which I thought might be of interest to you.

Sincerely yours,

A handwritten signature in cursive script that reads "P. Cerutti".

Peter A. Cerutti

A handwritten signature in cursive script that reads "Thomas D. Fontana, for".

Budget Approved: George K. Davis
Director, Sponsored Research

PAC:alf

Enclosures *OK*

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BUDGET

Revised Budget for first year period (September 1, 1972 to August 31, 1973)
of renewed Contract AT-(40-1)-4155

1. Salaries

Name	Title	% of Time	
J. L. Roti Roti, Ph.D.	Postdoctoral Associate	100	\$ 8,650 (no fringe benefits)
F. Roquemore, M.S.	Lab. Technologist II	100	7,371
E. Walker	Dishwasher	50	2,486
Fringe Benefits (9.8%) (includes unemployment compensation) plus \$180 for hospitalization insurance			<u>\$ 1,146</u>
SUBTOTAL			\$19,653

2. Permanent Equipment

1 Forma rollacell apparatus with 3 additional tiers	\$ 1,225
1 Isco absorbance monitor UA 4	1,295
1 Isco drop detector model 6000 F	75
1 Duo-seal vaccum pump	355
1 Buchler rotary evapo-mix	<u>434</u>
SUBTOTAL	\$ 3,384

3. Supplies

Chemicals	\$ 500
Radioisotopes	2,500
Biochemicals	1,500
Tissue-culture media	2,500
Glass and plastic ware	<u>2,500</u>
SUBTOTAL	\$ 9,500

4. Other Expenses

Instrument service contracts	\$ 1,500
Publication costs	<u>850</u>
SUBTOTAL	\$ 2,350

5. Travel

Domestic	\$ 800
Foreign	<u>600</u>
SUBTOTAL	\$ 1,400

TOTAL DIRECT COSTS	\$36,287
INDIRECT COSTS (49.42% of salaries, wages and fringe benefits	\$ 9,713
TOTAL	\$46,000

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FORMATION AND REPAIR OF γ -RAY INDUCED NUCLEIC ACID BASE DAMAGE
IN BACTERIA AND MAMMALIAN CELLS

Peter A. Cerutti

University of Florida
Gainesville, Florida

September 1, 1972 - August 31, 1975

Renewal Proposal for Contract No. AT-(40-1)-4155 of the U. S.
Atomic Energy Commission

C 718

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ABSTRACT

The threat to be at some point exposed to dangerous levels of ionizing radiation remains. Means for the protection to ionizing radiation can only be devised if the major effects of radiation on living organisms are understood on a cellular level. In particular, the type of damage produced in the cell and the mechanisms which allow the cell to survive exposure to a limited radiation dose have to be elucidated. On the other hand, ionizing radiation remains one of the most potent tools in cancer therapy. Progress in radiotherapy will, to a considerable extent, depend on progress in our understanding of the effects of ionizing radiation on normal and cancerous human cells. Beyond the direct relevance to the medical sciences and radio-protection, the study of the action of radiation on living cells has made and undoubtedly will make substantial contributions to basic molecular biology.

There is little doubt that chromosomal DNA represents a major target for the lethal and mutagenic effect of ionizing radiation on living tissues. However, the chemistry of γ -ray induced DNA damage, in particular of damage involving the heterocyclic bases, is largely unknown. Techniques have recently been developed in our laboratory which for the first time allow the study of the formation and post-irradiation repair of nucleic acid base damage induced by ionizing radiation. During the coming three year period, we propose to investigate the radiochemistry and radiobiology of γ -ray induced thymine damage in bacteria and mammalian cells. We will concentrate on three major aspects:

(1) The study of the efficiency of the intracellular formation of thymine damage in E. coli, Chinese hamster ovary cells and human embryonic lung fibroblasts (WI-38) relative to free DNA in solution and mononucleotide mixtures. These studies will allow an estimation of the extent of nucleic acid base damage relative to other types of damage caused by ionizing radiation under in vivo conditions.

(2) The study of the molecular mechanism of the removal of γ -ray damaged thymine from irradiated DNA in vitro. The contribution of nucleic acid base damage to the biological effects of γ -irradiation will be assessed in a well defined phage system.

(3) The study of the post-irradiation removal of γ -ray damaged thymine from the DNA of Chinese hamster ovary cells and human embryonic lung fibroblasts in unsynchronized and synchronized cultures.

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OUTLINE

I. INTRODUCTION

II. SPECIFIC AIMS

- A. Radiochemistry of Thymine in situ in the DNA in E. coli, Chinese Hamster Ovary Cells (CHO) and Human Embryonic Lung Fibroblasts (WI-38).
- B. The Molecular Mechanism of the Removal of γ -Ray Damaged Thymine From Irradiated DNA in vitro. The Biological Effect of Nucleic Acid Base Damage.
 1. Recognition of γ -ray damaged bases in ϕ X174 RF DNA by UV specific endonuclease.
 - a. Preparation of endonuclease and DNA.
 - b. Assay for nuclease binding and single strand scission.
 2. Removal of γ -ray damaged thymine from ϕ X174 RFI* and RF* by exonucleolytic activity.
 - a. Enzyme purification and DNA substrates.
 - b. Characterization of released material.
 3. Action of DNA polymerase I from E. coli on ϕ X174 RF* and endonuclease treated ϕ X174 RFI*.
 4. Infectivity studies with ϕ X174 RFI* and ϕ X174 RF*.
- C. Repair of γ -Ray Induced Thymine Damage in Chinese Hamster Ovary Cells (CHO) and Human Embryonic Lung Fibroblasts (WI-38).
 1. Analysis of the radioactive material released during post-irradiation incubation.
 2. Post-irradiation removal of damaged and undamaged thymine and its derivatives from the DNA as a function of the cell cycle.
 3. The effect of inhibitors of macromolecular synthesis on the process of product removal.

III. REFERENCES

IV. SUPPORTING DATA

- A. Personnel. Biographical Sketches and Selected Personal Publications Related to Present Proposal.

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B. Personnel Contributing to the Program but not Supported by A.E.C.

C. Support Received from Other Federal Agencies.

D. Facilities

V. BUDGET

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I. INTRODUCTION

The impressive progress made in photobiology is to a good part due to the identification of cyclobutane-type pyrimidine dimers as the major lesions produced by UV-light in DNA. The major steps of UV-repair in bacteria have been identified (Setlow, 1966*; Howard-Flanders, 1968*) and the detailed mechanisms leading to the removal of photodimers from DNA are being studied by several laboratories in well defined in vitro systems (Grossman et al, 1968; Heijneker et al, 1971; Nakayama et al, 1971; Carrier and Setlow, 1970; Kaplan et al, 1971a and b). Evidence for the repair of UV-damage in mammalian cells has been obtained (Painter, 1970*). In contrast, progress in our understanding of the action of ionizing radiation on living material has been comparatively slow. Although much of the work in radiobiology has concentrated on studies of the formation and repair of DNA strand breakage, there is no reason to believe that strand breaks are the only type of biologically significant lesions formed in DNA.

We concentrate in our laboratory on studies of the radiochemistry and radiobiology of γ -ray induced base damage in bacterial and mammalian systems. Recent radiochemical studies with synthetic and natural polynucleotides in vitro using our new methods for the determination of the formation of 5-methylene-uracil radicals from thymine and of products of the 6-hydroxy (or hydroperoxy)-5,6-dihydrothymine type suggest that base damage rather than strand breakage are the major lesions produced in DNA by γ -rays (Hariharan and Cerutti, 1971, 1972a; Cerutti et al, unpublished results). However, no reliable data is available so far on the efficiency of the production of base damage in the DNA in situ in bacteria and mammalian cells. In this project (Section A) we propose to determine the efficiency of the production of thymine damage by ^{137}Cs γ -rays in *E. coli*, Chinese hamster ovary cells and human embryonic lung fibroblasts (WI-38). These studies will allow an estimation of the extent of nucleic acid base damage relative to other types of damage caused by ionizing radiation under in vivo conditions.

The post-irradiation removal of damaged thymine from γ -irradiated *M. radiodurans* has been demonstrated (Hariharan and Cerutti, 1971, 1972). However, the molecular mechanism of the post-irradiation repair of nucleic acid base damage caused by ionizing radiation remains largely unknown. Some of the data in the literature suggests that at least part of the steps in bacteria used to repair UV-damage may also occur in the repair of DNA damage introduced by ionizing radiation (see e.g., Emmerson and Howard-Flanders, 1965). However, there is no doubt that significant differences between UV and X-ray repair exist in bacteria (Fangman and Russel, 1971) and mammalian cells (Cleaver, 1969). In this project (Section B) we propose to study, in vitro, whether the enzymes involved in repair of UV damage can remove γ -ray damaged thymine residues from irradiated DNA, and if so, their mechanism of action. These studies will be done with purified enzyme preparations and γ -irradiated ϕX174 RFI DNA. Emphasis will be on the question of whether endonucleolytic incision is necessary for the removal of damaged residues from γ -irradiated DNA (cf. Hariharan and Cerutti, 1972a and b), and on the biochemical analysis of the material which is

* A reference with an asterisk indicates a review.

released from irradiated DNA upon treatment with repair enzymes. The biological effects of nucleic acid base damage will be investigated using irradiated ϕ X174 RFI DNA containing base damaged residues but no strand breakage.

The post-irradiation removal from the DNA of cyclobutane-type photodimers has been demonstrated in human cells (Regan et al, 1968; Setlow et al, 1969; Cleaver and Trosko, 1970) but not in rodent cells (Klimek, 1965, Trosko and Kasschau, 1967) or cells of patients with the hereditary disease Xeroderma pigmentosum. There is considerable indirect evidence in the literature for the occurrence of excision repair in mammalian systems following exposure to ionizing radiation. In particular, X-ray induced repair replication and unscheduled DNA synthesis can best be understood in molecular terms if it is assumed that damaged (and undamaged) residues are first removed from the DNA in early steps of post-irradiation repair (see e.g., Painter, 1970*). However, the actual excision from the DNA of γ -ray damaged residues has not been demonstrated in mammalian cells. A major effort is being made in our laboratory to determine the kinetics and the extent of the removal of damaged thymine from γ -irradiated Chinese hamster ovary cells and human embryonic lung fibroblasts (WI-38) using the experience gained with M. radiodurans. Preliminary results are encouraging and we propose to pursue these studies further (Section C) both in unsynchronized and synchronized cultures.

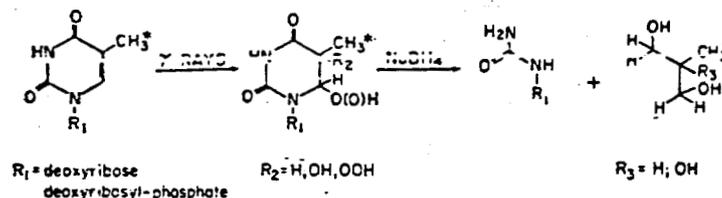
The principal investigator has recently reviewed the state of our knowledge about "DNA Base Damage induced by Ionizing Radiation" in a chapter contributed to a monograph entitled Photochemistry and Photobiology of Nucleic Acids (eds. S. Y. Wang and M. Patrick), Gordon and Breach, Science Publishers, Inc., New York. A copy of this manuscript is included and should give the reviewers a detailed and critical appraisal of the areas dealt with in this proposal.

II. SPECIFIC AIMS

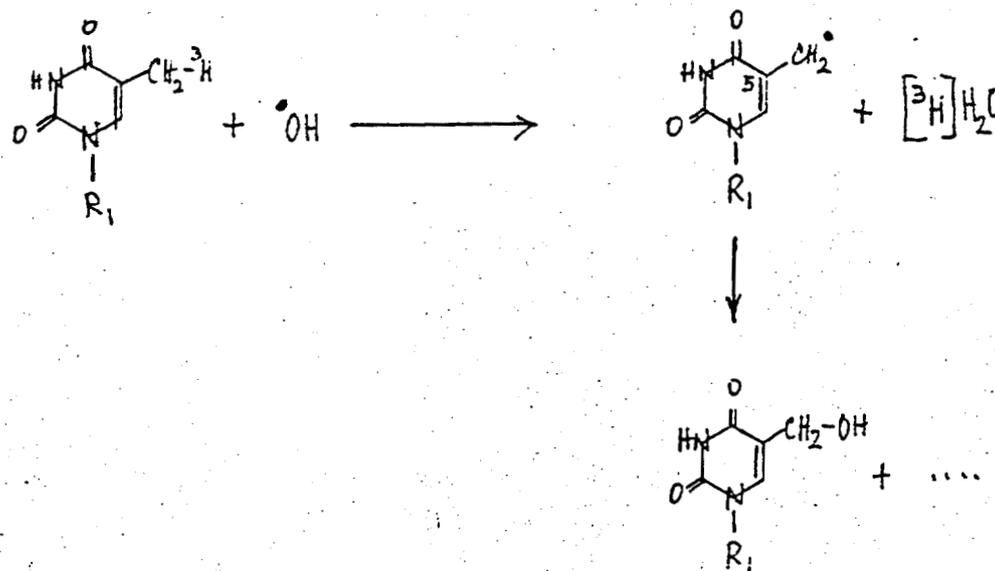
A. Radiochemistry of Thymine in situ in the DNA in E. coli, Chinese Hamster Ovary Cells (CHO) and Human Embryonic Lung Fibroblasts (WI-38).

While the radiochemistry of the free nucleic acid bases in particular of thymine has been studied in some detail, our knowledge of the radiochemical reactions of residues in oligonucleotides, RNA and DNA is very limited. It is not known whether major reactions of the free bases are also major reactions of the residues in a polynucleotide. Furthermore, most of the experiments on polynucleotides reported in the literature were carried out in non-protective salt media and only total base destruction was determined (see e.g., Scholes et al, 1960). No reliable conclusions about the radiochemical behavior of nucleic acids in situ in the cell are therefore possible on the basis of these results.

We have recently developed radiochemical techniques which allow the reliable determination of the formation of products of the 6-hydroxy (or hydroperoxy)-5,6-dihydrothymine type (Hariharan and Cerutti, 1972a) and of 5-methylene-uracil radicals (Cerutti et al, unpublished results) from thymine in γ -irradiated polydeoxynucleotides (Figure 1). These



Principal steps in the reductive assay for the radiolysis products of thymine and its derivatives of the 6-(hydroxy or hydroperoxy)-5,6-dihydrothymine type.



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methods are presently being used in studies of the radiochemical reactivity of thymine in synthetic and natural polydeoxynucleotides in protective and non-protective media (see Final Report, Section IA and IB). We propose to extend these investigations and to study the radiochemical reactivity of thymine in DNA in situ in the living cell (E. coli, CHO and WI-38). The major questions which will be asked are: (1) What is the reactivity of thymine in situ in E. coli relative to free E. coli DNA and relative to a nucleotide mixture of the same composition in vitro? What are the reactivities under protective (e.g., cystine, AET) and non-protective conditions? (2) What is the reactivity of thymine in situ in CHO and WI-38 cells relative to free DNA in solution or a corresponding nucleotide mixture? (3) What is the reactivity of thymine in CHO as a function of the cell cycle? These studies will yield information on the effect of the intracellular milieu on the radiosensitivity of thymine and on the contribution of direct and indirect radiation action to base damage. A comparison of the results obtained for the formation of products of the 6-hydroxy- (or hydroperoxy)-5,6-dihydrothymine type and 5-methylene-uracil radicals may give some clues about the relative accessibility to radicals of the thymine-methyl group and carbon atoms 5 and 6 of the thymine ring in the chromosome. It will be of particular interest to compare differences observed in the radiochemical reactivity of thymine for the different stages in the cell cycle with the known cycle dependent fluctuations in the killing efficiency of ionizing radiation. Procedures used in these experiments: The reductive assay has been modified slightly for the application to high molecular weight DNA. The values obtained are reproducible although relatively high backgrounds are still obtained for the unirradiated controls. Further work is presently being done to improve this situation. In the case of mammalian cells prepurification of the DNA may be necessary (e.g., batch treatment or column chromatography with hydroxyapatite - according to Britten et al, 1970) or extraction of nuclear fraction with phenol followed by treatment with RNase before the reduction with sodium borohydride). For the determination of the formation of 5-methylene-uracil radicals the amount of [^3H]H $_2\text{O}$ from thymine-methyl[^3H] is determined in the acid soluble fraction of the samples (i.e., combined acid soluble fraction of the culture medium and the cell pellet). After neutralization the samples are chromatographed on Dowex 1 (OH $^-$) - Dowex 50 (H $^+$) double columns. The [^3H]H $_2\text{O}$ is eluted in the exclusion volume of the column. For the computation of the amount of 5-methylene-uracil radicals formed from the amount of [^3H]H $_2\text{O}$ released corrections are made for the isotope effect and the probability of the release of hydrogen instead of tritium. The radioactivity in the column exclusion volume has been identified as [^3H]H $_2\text{O}$ by vapor phase chromatography in experiments on free DNA in vitro. The assay is very reliable and gives low backgrounds for unirradiated samples.

Our laboratory is familiar with the procedures for the growth and thymidine labelling of E. coli, CHO and WI-38 cells.

B. The Molecular Mechanism of the Removal of γ -Ray Damaged Thymine from Irradiated DNA in vitro. The Biological Effect of Nucleic Acid Base Damage.

As mentioned in the INTRODUCTION radiobiology is far behind photobiology in the elucidation of the molecular mechanism of excision repair of damaged residues from DNA. While the lethal and mutagenic effects of

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cyclobutane-type photodimers has been demonstrated the contribution of γ -ray induced base damage to the biological effects of radiation has not been clearly assessed (see review on "DNA Base Damage Induced by Ionizing Radiation" contributed by the principal investigator). We feel that substantial progress could be made on these problems by combining our methodology for the determination of γ -ray induced thymine damage in polynucleotides with procedures which presently are being developed in Dr. L. Grossman's laboratory (Brandeis University) for the study of the incision step in UV-repair and procedures used by Dr. T. Blok (Vrije Universiteit, Amsterdam) using Coliphage ϕ X174 RFI DNA.

In our studies we plan to irradiate ϕ X174 RFI DNA with ^{137}Cs γ -rays and to separate molecules with no strand breaks but which are expected to contain base damaged residues (RFI*) from molecules containing both strand breaks and base damage (RF*). Since damage other than strand breakage appears to contribute substantially to the biological effects of γ -radiation on ϕ X174 RF DNA (T. Blok, personal communication), the preparation of RFI* appears feasible. RFI* (and RF*) will be used in in vitro studies on the mechanism of the removal of γ -ray damaged residues using the purified UV-endo- and UV-exonuclease from M. luteus. Furthermore, a correlation of the extent of base damage in RFI* with the residual infectivity of E. coli spheroplasts will allow an estimation of the killing efficiency of γ -ray induced base damage.

1. Recognition of γ -ray damaged bases in ϕ X174 RF DNA by UV specific endonuclease.

Kushner et al (1970), Setlow et al (1970), and Nakayama et al (1971) have shown that a purified endonuclease from M. luteus can in vitro recognize cyclobutane type photodimers in DNA and make a single strand scission on the 5' side of the dimer. The observation that E. coli K12 uvr⁻, lacking UV-endonuclease activity, exhibited increased radiation sensitivity, suggests the involvement of an endonucleolytic activity also in the repair of damage introduced by ionizing radiation (see e.g., Grossman et al, 1968). Our own work with M. radiodurans, on the other hand, suggested that an incision step may not be necessary for removal from the DNA of γ -ray damaged thymine at least during the early phase of post-irradiation repair (Hariharan and Cerutti, 1972a and b). It will be interesting, therefore, to test whether the UV-endonuclease from M. luteus can recognize γ -ray damaged bases in ϕ X174 RFI* DNA.

a. Preparation of the endonuclease and DNA. The UV-endonuclease will be purified from M. luteus using the procedure of Kushner and Grossman (1971). [^3H]methyl-thymine labelled ϕ X174 RFI DNA will be isolated from ϕ X174 infected E. coli using procedures from this laboratory (J. Swinehart, personal communication), and those of Lindquist and Shinsheimer (1968). After irradiation with γ -rays, the RF molecules which contain only damaged bases (RFI*) will be separated from RF* containing both strand breaks and base damage by CsCl-ethidium bromide density gradient centrifugation in the dark.

b. Assay for nuclease binding and single strand scission. A new assay for the determination of the binding of UV-endonuclease to UV-

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irradiated DNA, and the subsequent introduction of single strand breaks, has been developed in L. Grossman's laboratory (L. Grossman, personal communication) and will be used in our experiments. To assay for binding, Mg^{++} is omitted from the reaction mixture containing endonuclease and RFI*. This prevents the nuclease from causing strand scission but still enables it to bind to the DNA. The binding of the nuclease to the RFI* can be detected by passing the reaction mixture through a nitrocellulose filter, which retains the DNA-enzyme complex, but not free RFI*. The amount of DNA-enzyme complex formed will be related to the amount of damaged thymine in RFI*, as measured by the reductive assay of Hariharan and Cerutti (1972a). An estimate for total base destruction will be obtained using acid hydrolysis of the DNA followed by paper chromatographic separation of the residual bases according to Scholes et al (1960). To determine whether the UV-endonuclease introduces single strand breaks in RFI*, this DNA will be treated with endonuclease under conditions which allow the enzyme to "nick" UV irradiated DNA. The nuclease treated RFI* will then be denatured and filtered through a nitrocellulose filter under conditions under which only single stranded DNA is retained. The amount of material retained is a measure of the number of single strand breaks introduced, and this will be related to the amount of damaged thymine determined by the reductive assay and to the extent of total base destruction. Similar studies may be carried out with endonuclease II from E. coli described by Friedberg and Goldthwait (1968).

2. Removal of γ -ray damaged thymine from ϕ X174 RFI* and RF* by exonucleolytic activity.

In addition to a UV specific endonuclease, Kaplan and Grossman (1971) have also isolated a UV specific exonuclease from M. luteus. They have shown that the purified enzyme can efficiently degrade UV irradiated DNA, while other exonucleases, such as snake venom phosphodiesterase, were strongly inhibited under similar conditions (Grossman et al, 1968). In this project we plan to study the action of UV-endonuclease as well as other exonucleases on RF* and UV-endonuclease treated RFI*. Special emphasis will be placed on the characterization of the material released from the DNA.

a. Enzyme purification and DNA substrates. The UV specific exonuclease will be purified from M. luteus according to Kaplan and Grossman (1971). RF* [prepared as in 1(a)] will be tested directly as a substrate since it contains radiation induced single strand breaks. RFI* will be tested after treatment with UV-endonuclease or DNase I. A comparison of the action of the UV-exonuclease on the RF* (which contains γ -ray induced breaks) and endonuclease treated RFI* may yield valuable information on the chemistry of radiation induced strand breaks. The characterization of the products released from the two DNA's will provide information about the selectivity of the endonucleolytic incision step and the distribution of damaged thymidine residues in the irradiated DNA. In addition, several of the commercially available nucleases (e.g., snake venom phosphodiesterase) will be tested on RF* and RFI* (after endonuclease treatment), and their ability to degrade these irradiated DNA's compared to that of the UV specific exonuclease.

b. Characterization of released material. The radioactive material removed from the DNA by exonucleolytic digestion will be tested

for acid solubility and analyzed by chromatography on cellulose acetate, DEAE paper, and by column chromatography. The products will also be analyzed for their content of damaged thymine using the reductive assay. End group analysis (i.e., whether 5'-PO₄, 5'-OH, 3'-OH) will be carried out using specific phosphatases and kinases.

3. Action of DNA polymerase I from *E. coli* on RF* and endonuclease treated RFI*.

Kelly et al (1969) have demonstrated that DNA polymerase I from *E. coli* is able to excise pyrimidine dimers and insert new nucleotides into the DNA chain in the 5' and 3' direction. Heijneker et al (1971) were able to restore transforming activity of UV-inactivated *B. subtilis* DNA using *E. coli* DNA polymerase I in conjunction with UV-endonuclease and DNA-ligase. We will test DNA polymerase I for similar activities using γ -irradiated DNA.

The polymerase will be purified from *E. coli* using the procedure of Englund (1971). Both RF* and endonuclease treated RFI* will be tested as substrates for the enzyme under conditions favoring either its nuclease or polymerizing activity. Excision of damaged bases due to the exonucleolytic activity of the polymerase will be tested for and the products analyzed as in 2(b). Replacement of the γ -ray damaged by undamaged nucleotides will be tested for under polymerizing conditions. It will be interesting to attempt reconstitution of RFI molecules from RF* molecules using the combined action of the polymerase and several other "repair enzymes". Reconstitution will be determined both by sedimentation analysis and determination of infectivity of the treated RF*.

4. Infectivity studies with RFI* and RF*.

RFI* will be used to infect *E. coli* K12 uvr⁻ spheroplasts and the spheroplasts from the wild type strain, using the procedure of Guthrie and Sinsheimer (1963). The efficiency of infection will be compared to that of unirradiated RFI. The relative efficiency of infection of RFI* and RFI will provide a direct test of the effect on viability of γ -ray induced base damage in the absence of strand breakage. Since *E. coli* uvr⁻ apparently lacks a UV specific endonuclease, comparing the relative efficiency of infection of RFI* in wild type and uvr⁻ strains of *E. coli* will provide *in vivo* information for the role of the UV specific endonuclease in the repair of γ -ray damaged bases.

C. Repair of γ -Ray Induced Thymine Damage in Chinese Hamster Ovary Cells (CHO) and Embryonic Human Lung Fibroblasts (WI-38).

A major effort will be made in this program to demonstrate the removal of damaged thymine from γ -irradiated CHO and WI-38 cells. The following goals are set: (1) unambiguous chemical characterization of the material released from the DNA during post-irradiation incubation, (2) determination of the extent and kinetics of product removal, (3) elucidation of the relation between post-irradiation degradation (removal of undamaged residues) and removal of damaged residues, (4) investigation of product removal as a function of the cell cycle in CHO, (5) study of the effect of inhibitors of macromolecular synthesis on the process of product

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removal.

A considerable amount of preliminary work has been and is being done in our laboratory mostly using CHO. We have become familiar with and have established optimal conditions for the growth of large amounts of CHO and WI-38 cells in monolayers, for the thymidine-methyl[³H] labelling of the cells, for the irradiation of the cultures in large roller bottles, for the chromatographic analysis of the radioactive material released into the culture medium and the acid soluble fraction of the cells, etc. (see also "Final Report", Section III).

1. Analysis of the radioactive material released during post-irradiation incubation.

Cells are prelabelled in their DNA with thymidine-methyl[³H], irradiated and incubated under growth conditions for various lengths of time. The total radioactive material released into the culture medium is chromatographed on sephadex and the fractions are further analyzed by DEAE-chromatography and the reductive assay for products of the 6-hydroxy (or hydroperoxy)-5,6-dihydrothymine type. The acid soluble material of the culture medium and the cell pellet is chromatographed separately on Dowex 50 (H⁺) ion-exchange columns. Irradiated and unirradiated ¹⁴C-thymine and ¹⁴C-thymidine are cochromatographed as internal markers. Conditions have been found which allow the separation of thymidylic acid, thymidine and thymine and radiation products. The material which is eluted from the ion-exchange columns is further analyzed by the reductive assay and thin-layer chromatography.

2. Post-irradiation removal of damaged and undamaged thymine and its derivatives from the DNA as a function of the cell cycle.

The radiosensitivity of mammalian cells is known to vary with the cell cycle state (Terasima and Tolmach, 1961; Sinclair and Morton, 1963). The exact pattern of radiosensitivity is dependent upon a given cell line. It has so far not been possible to relate the changes in radiosensitivity with the production or repair of a particular type of DNA damage. The rejoining of single strand breaks does not show any dependency on cell cycle stage. Unscheduled DNA synthesis occurs throughout the cell cycle, except in S stage, where it is undefined. Repair replication is difficult to measure during S, it occurs in G₁ (Brent and Wheatly, 1971) but Richold and Arlett claim that it does not occur in G₂ (1972). The evidence for the latter is not very good because of the large amount of S contamination in the G₂ population. If there is, in fact, a lack of repair replication in G₂ following irradiation, then repair replication would correlate qualitatively as far as G₁ and G₂ are concerned, with the cell cycle dependence of radiosensitivity. There are no reliable data regarding the cell-stage dependence on the repair of double-strand cuts. In this project we plan to study the removal of radiation damaged thymine from the DNA in CHO cells as a function of the cell cycle.

Because a large number of cells are required, CHO cells will be synchronized by a combination of colcemide blocking and "shake-off" techniques (Terasima and Tolmach, 1961; Stubblefield and Klevecz, 1965).

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This method will give a fairly "clean" M-stage population with a high yield. The cells will be irradiated in G₁, S, G₂ and M and analyzed for the extent of removal of damaged thymine. Depending on the outcome of the above experiments, analogous experiments will be done with embryonic human lung fibroblasts (WI-38) and cell lines which have a pattern of cell cycle radiosensitivity different from that of CHO (as e.g., mouse L cells) (Whitmore et al, 1967). If the repair of base damaged plays an important role in the survival of cells, then the pattern of base damage repair during the cell cycle should correlate inversely with the pattern of cell cycle radiosensitivity. It will be interesting to compare the results obtained in these experiments with those of the studies of the radiosensitivity of thymine as a function of the cell cycle proposed under Section A of this proposal.

3. The effect of inhibitors of macromolecular synthesis on the process of product removal.

Effect of metabolic inhibition on the repair of base damage in synchronized cells: if a repair process is identified which correlates with the variation of radiosensitivity during the cell cycle, the repair process should be sensitive to agents which block progress through the cell cycle. The role of protein, RNA and DNA synthesis in the ability of cells to repair γ -ray induced base damaged as related to changes in their radioresistance as cells pass from one stage to the next will be studied. These experiments will help clarify the role of repair of γ -ray damaged thymine in cell survival following γ -irradiation. It is recognized that the increased radioresistance may be due to a mixture of repair ability and increased protection.

a. Blocking protein and RNA synthesis: Cells will be treated at various times before the onset of a radioresistant phase using cycloheximide. This will measure the time at which the process(es) leading to increased repair ability and/or radioresistance became insensitive to the blocking agent. This determines the time at which new protein synthesis is needed for the increased repair ability or radioresistance to occur (cf. Terasima and Yasukawa, 1967; Schneiderman et al, 1971; Doida and Okada, 1972). Analogously, actinomycin-d will be used to determine if new RNA synthesis is necessary for increased ability to repair γ -ray induced thymine damage (cf. Tobey et al, 1966; Doida and Okada, 1972).

b. Blocking DNA synthesis: Since it is known that DNA synthesis is necessary for the increased resistance during S of hamster V79 cells (Sinclair, 1968), the effect of stopping DNA synthesis by excess thymine or fluorodeoxyuridine on the ability of cells to repair γ -ray induced thymine damage will be determined.

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- Englund, P. T., In: Procedures in Nucleic Acid Chemistry, Harper and Rowe, New York, Vol. 2, p. 864 (1971).
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* Review articles

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IV. SUPPORTING DATA

A. Personnel. Biographical sketches; responsibility in the proposed project, per cent of time devoted to project and selected personal publications related to present proposal.

1. Peter A. Cerutti

NAME: Peter A. Cerutti, M. D., Ph. D.
TITLE: Professor and Chairman
BIRTHDATE/BIRTHPLACE: [REDACTED] Zurich, Switzerland
PRESENT NATIONALITY: USA
SEX: Male
SOCIAL SECURITY NUMBER: [REDACTED]

EDUCATION:

Institution	Degree	Year	Specialty
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]

HONORS:

First Prize, University of Zurich, 1957, for Medical Research.
Fellowship of "Swiss Foundation for Chemistry and Pharmacy", 1957-60
Fellowship of Swiss National Science Foundation, 1960-63.
Member of "Honorary Society of Sigma Xi

RESEARCH AND/OR PROFESSIONAL EXPERIENCE:

- 1971 - Professor and Chairman, Department of Biochemistry, J. Hillis Miller Health Center, University of Florida, Gainesville, Florida.
- 1966 - 1970 Assistant Professor, Department of Biochemical Sciences, Princeton University, Princeton, New Jersey.
- 1964 - 1966 Research Associate, NIAMD and NIH (with Drs. Nirenberg, Witkop and Udenfried), Bethesda, Maryland.

RESPONSIBILITY IN THE PROPOSED PROJECT AND PER CENT TIME DEVOTED TO IT:

Principal Investigator; 25% of time devoted to project

SELECTED PERSONAL PUBLICATIONS IN AREAS RELATED TO PRESENT PROPOSAL

P. Cerutti und H. Schmid - Photoreaktionen von Methanol mit N-Heterocyclen (1. Mitteilung) *Helv. Chim. Acta* 45, 1992 (1962).

P. Cerutti und H. Schmid - Photoreaktionen von Methanol mit N-Heterocyclen (2. Mitteilung) *Helv. Chim. Acta* 47, 203 (1963).

P. Cerutti, K. Ikeda and B. Witkop - The Selective Photoreduction of Uridine in Polynucleotides. *J. Am. Chem. Soc.* 87, 2505 (1965).

H. Goeth, P. Cerutti und H. Schmid - Photoreaktionen von Acridine und Acridinabkoemmlingen sowie von Arylketonen mit Methanol. *Helv, Chim. Acta* 48, 1395 (1965).

F. Rottman and P. A. Cerutti - The Template Activity of Uridylic Acid - Dihydrouridylic Acid Copolymers. *Proc. Natl. Acad. Sci.* 55, 960 (1966).

C. Ballé, P. Cerutti and B. Witkop - Selective Photoreduction of Nucleotides and Nucleic Acids. II. Mechanism of the Two-Step Reduction of Thymine. *J. Am. Chem. Soc.* 88, 3946 (1966).

O. Yonemitsu, P. Cerutti and B. Witkop - Photoreductions and Photocyclizations of Tryptophan. *J. Am. Chem. Soc.* 88, 3941 (1966).

P. Cerutti and N. Miller - The Selective Reduction of Yeast Transfer Ribonucleic Acid with Sodium Borohydride. *J. Mol. Biol.* 26, 55 (1967).

P. Cerutti, Y. Kondo, W. B. Landis and B. Witkop - Photoreduction of Uridine and Reduction of Dihydrouridine with Sodium Borohydride. *J. Am. Chem. Soc.* 90, 771 (1968).

N. Miller and P. Cerutti - The Structure of the Photohydration Products of Cytidine and Uridine. *Proc. Natl. Acad. Sci. USA* 59, 34 (1968).

P. Cerutti, J. W. Holt and N. Miller - Detection and Determination of 5, 6-Dihydrouridine and 4-Thiouridine in Transfer Ribonucleic Acid from Different Sources. *J. Mol. Biol.* 34, 505 (1968).

M. Pleiss, H. Ochiai and P. Cerutti - Photochemically Induced Transition of 4-Thiouridine to Uridine and Cytidine in *E. coli* Transfer RNA. *Biophys. Res. Commun.* 34, 70 (1969).

A. M. Bobst, P. A. Cerutti and F. Rottman - The Structure of Poly 2'-O-Methyladenylic Acid at Acidic and Neutral pH. *J. Am. Chem. Soc.* 91, 1246 (1969).

A. M. Bobst, F. Rottman and P. A. Cerutti - Role of the Ribose 2'-hydroxyl Groups for the Stabilization of the Ordered Structures of RNA. *J. Am. Chem. Soc.* 91, 4603 (1969).

P. A. Cerutti, N. Miller, M. G. Pleiss, J. F. Remsen and W. J. Ramsay - Photohydration of Uridine in the RNA of Coliphage R17. I. Reduction Assay for Uridine Photohydration. *Proc. Natl. Acad. Sci.* 64, 731 (1969).

A. M. Bobst, F. Rottman and P. A. Cerutti - The Effect of the Methylation of 2'-Hydroxyl Groups in Polyadenylic Acid on its Structure in Weakly Acidic and Neutral Solutions and on its Capability to Form Ordered Complexes with Polyuridylic Acid. *J. Mol. Biol.* 46, 221 (1969).

J. F. Remsen, N. Miller and P. A. Cerutti - Photohydration of Uridine in the RNA of Coliphage R17. II. The Relation Between Ultraviolet Inactivation and Uridine Photohydration. Proc. Natl. Acad. Sci. USA 65, 460 (1970).

P. V. Hariharan and P. A. Cerutti - Repair of Radiation Damaged Thymine in Micrococcus radiodurans. Nature New Biology 229, 247 (1971).

J. F. Remsen, M. Mattern, N. Miller and P. A. Cerutti - Photohydration of Uridine in the Ribonucleic Acid of Coliphage R17. Lethality of Uridine Photohydrates and Nonlethality of Cyclobutane-type Photodimers. Biochemistry 10, 524 (1971).

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J. Swinehart, A. Bobst and P. Cerutti - The Effect of Saturated Pyrimidine Bases on RNA Conformation. FEBS Letters 21, 56 (1972).

P. V. Hariharan and P. A. Cerutti - Formation and Repair of γ -ray Induced Thymine Damage in Micrococcus radiodurans. J. Mol. Biol. 66, 65 (1972).

M. Mattern, R. Binder and P. A. Cerutti - Cytidine Photohydration in R17-RNA. J. Mol. Biol. 66, 201 (1972),

P. Cerutti - Photochemie der Nukleinsäuren, Houben-Weyl Band IV/3 Kapitel I \bar{A} , Organische Photochemie (E. Müller, ed.), Georg Thieme Verlag, Stuttgart; in press (1972).

P. Cerutti - Base Damage in Deoxyribonucleic Acid Induced by Ionizing Radiation, Chapter VI in Photochemistry and Photobiology of Nucleic Acids (S. Y. Wang and M. Patrick, eds.) Gordon and Breach, Science Publishers, Inc., New York, to be published.

P. Cerutti - Deoxycytidine Photohydration in DNA, Chapter IC in Photochemistry and Photobiology of Nucleic Acids (S. Y. Wang and M. Patrick, eds.), Gordon and Breach, Science Publishers, Inc., New York, to be published.

2. Joyce F. Rensen

NAME: Joyce F. Rensen, Ph. D.
 TITLE: Associate Scientist and Instructor
 BIRTHDATE/BIRTHPLACE: [REDACTED] Trenton, New Jersey
 PRESENT NATIONALITY: USA
 SEX: Female
 SOCIAL SECURITY NUMBER: [REDACTED]

EDUCATION:

Institution	Degree	Year	Specialty
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]

HONORS:

Phi Beta Kappa

RESEARCH AND/OR PROFESSIONAL EXPERIENCE:

1971 - Associate Scientist and Instructor, Department of Biochemistry, University of Florida, Gainesville, Florida.
 1969 - 1971 PHS Postdoctoral Fellow (Dr. P. A. Cerutti, sponsor), Department of Biochemical Sciences, Princeton University, Princeton, New Jersey.
 1960 - 1964 Research Information Scientist - Squibb Institute for Medical Research, New Brunswick, New Jersey.
 1957 - 1958 Technician in Plant Physiology, Boyce Thompson Institute for Plant Research, Yonkers, New York.

RESPONSIBILITY IN THE PROPOSED PROJECT AND PER CENT TIME DEVOTED TO IT:

Dr. J. F. Rensen will carry major responsibility for our work on the molecular mechanism of the repair of γ -ray induced base damage in Coliphage ϕ X174 RFI DNA (see Section B of proposal). Dr. Rensen, who is a part-time Instructor in Biochemistry, will devote 60% of her time to the project.

SELECTED PERSONAL PUBLICATIONS IN AREAS RELATED TO PRESENT PROPOSAL

J. F. Rensen and F. F. Davis - The Presence of New Minor Mononucleotides in E. coli B DNA. To be submitted.

P. A. Cerutti, N. Miller, M. G. Pleiss, J. F. Rensen and W. J. Ramsay - Photohydration of Uridine in the RNA of Coliphage R17. I. Reduction Assay for Uridine Photohydration. Proc. Natl. Acad. Sci. 64, 731 (1969).

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J. F. Remsen, N. Miller and P. A. Cerutti - Photohydration of Uridine in the RNA of Coliphages R17. II. The Relation Between Ultraviolet (Inactivation) and Uridine Photohydration. Proc. Natl. Acad. Sci. US 65, 460 (1970).

J. F. Remsen, M. Mattern, N. Miller and P. A. Cerutti - Photohydration of Uridine in the Ribonucleic Acid of Coliphage R17. Lethality of Uridine Photohydrates and Nonlethality of Cyclobutane-type Photodimers. Biochemistry 10, 524 (1971).

J. F. Remsen and P. A. Cerutti - Ultraviolet Inactivation and Miscoding of Irradiated R17-RNA in vitro. Submitted for publication.

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3. Joseph L. Roti Roti

NAME: Joseph Lee Roti Roti, Ph. D.
 TITLE: Postdoctoral Associate
 BIRTHDATE/BIRTHPLACE: [REDACTED] Newport, Rhode Island
 PRESENT NATIONALITY: USA
 SEX: Male
 SOCIAL SECURITY NUMBER: [REDACTED]

EDUCATION:

Institution	Degree	Year	Specialty
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]

HONORS:

AEC Health Physics Fellowship, 1965-68.
 Phi Kappa Phi, June 1965

RESEARCH AND/OR PROFESSIONAL EXPERIENCE:

1971 - Postdoctoral Associate with Dr. P. A. Cerutti, Department of Biochemistry, University of Florida, Gainesville, Florida.

1963 and 1964 (summers) Research Assistant with Dr. Robert Brown, Department of Biology, Michigan Technological University, Houghton, Michigan.

RESPONSIBILITY IN THE PROPOSED PROJECT AND PER CENT TIME DEVOTED TO IT.

Dr. J. Roti Roti will work on the problems outlined under Section A and Section C(2) and C(3) of the proposal, i.e., on the studies of the cell-cycle dependence of the radiosensitivity of thymine in situ in CHO - and WI-38 cells and the repair of thymine base damage as a function of the cell cycle in CHO cells. He will devote 100% of his time to the project.

SELECTED PERSONAL PUBLICATIONS IN AREAS RELATED TO PRESENT PROPOSAL.

J. L. Roti Roti and S. Okada - A Mathematical Model of the Cell Cycle of L5178Y. Cell and Tissue Kinetics, submitted for publication.

D. F. Liberman and J. L. Roti Roti - Effects of Drugs on L5178Y Cells. I. Influence of Chloramphenicol on the Cell Cycle. Exptl. Cell Res., submitted for publication.

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D. F. Liberman, J. L. Roti Roti and C. S. Lange - Effects of Drugs on L5178Y Cells. II. Cell Cycle Stage Specificity of Chloramphenicol Toxicity. *Excerpt. Cell Res.*, submitted for publication.

J. L. Roti Roti, S. Okada, and H. Eberle - Protein Synthesis During the Cell Cycle of L5178Y, submitted for publication.

J. L. Roti Roti, S. Okada and H. Eberle - Effects of Actinomycin-D on G₁ Protein Synthesis in L5178Y Cells, manuscript in preparation.

J. L. Roti Roti and R. B. Painter - Equations for Estimating DNA Chain Growth and Replication Size Using an Equilibrium Density Gradient Method, manuscript in preparation.

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4. Gaylordia S. Warren

NAME: Gaylordia S. Warren, B. S.
 TITLE: Research Assistant
 BIRTHDATE/BIRTHPLACE: [REDACTED], El Paso, Texas
 PRESENT NATIONALITY: USA
 SEX: Female
 SOCIAL SECURITY NUMBER: [REDACTED]

EDUCATION:

Institution	Degree	Year	Specialty
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]

RESEARCH AND/OR PROFESSIONAL EXPERIENCE:

1971 - Research Assistant, Department of Biochemistry, University of Florida, Gainesville, Florida.
 1968 - 1971 Principal Research Assistant, Virological Laboratory, Colorado State University, Fort Collins, Colorado.
 1965 - 1968 Research Technician, Parke-Davis and Co.

RESPONSIBILITY IN THE PROPOSED PROJECT AND PER CENT TIME DEVOTED TO IT.

Miss G. Warren will be responsible for the culturing of our mammalian cell cultures and will participate in the experiments on the radiobiology of CHO and WI-38 cells.

SELECTED PERSONAL PUBLICATIONS IN AREAS RELATED TO PRESENT PROPOSAL

A. K. Eugster, G. S. Warren and J. Storz - Enterovirusinfektion und Immunität vor und nach der Geburt bei Kühen und Kälbern. Zeitschr. Vet Med. B17, 40, 409 (1970).

J. Storz and G. S. Warren - Effect of Antimetabolites and Actinomycin D on Replication of HADEN, a Bovine Parvovirus. Archiv für die Gesamte Virusforschung 30, 271-274 (1970).

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B. Personnel Contributing to the Program But for Whom No Support is Requested from A.E.C.

1. P. V. Hariharan, Ph. D. Associate Scientist and Instructor
in Biochemistry
2. Miss J. Swinehart Graduate Student
3. Mr. M. Mattern Graduate Student

C. Support Received from Other Federal Agencies.

Support is obtained from the National Institutes of Health, Institute for General Medical Sciences (Grant Number 5 R01 GM 18617-02) for a project entitled "Structure and Function of Ribonucleic Acid" in the amount of \$34,953 (direct costs) for the current year.

D. Facilities

Laboratories with facilities to accommodate 10 persons are available to our research group on the third floor of the Medical Science Building in the J. Hillis Miller Health Center. The laboratories are equipped with all the instruments needed for this research project, including centrifuges (Beckman Model L2-65B), Sorvall, etc.), scintillation counters (Beckman L250), ¹³⁵Cs-Source, special "Schoffel" high intensity monochromator, Laminar flow hoods, CO₂-incubators, roller apparatus for large scale production of cells, etc. Communal facilities include cold room, constant temperature room, chromatography room, etc. Library facilities are located within the Department of Biochemistry and the Health Center. Secretarial service is available in the Department of Biochemistry.

V. BUDGET

Budget for three-year period of September 1, 1972 until August 31, 1975

A. Budget for first year (September 1, 1972 to August 31, 1973)

1. Salaries:

Name	Title	% of Time	
J. F. Remson, Ph.D.	Associate Scientist	60	\$ 8,000
J. L. Roti Roti, Ph.D.	Postdoctoral Associate	100	8,700 (no fringe benefits)
G. S. Warren, B.S.	Research Assistant	100	9,900
	Fringe Benefits (9.8%)		
	(includes unemployment compensation)		<u>\$ 1,754</u>
	SUBTOTAL		\$28,354

2. Permanent Equipment:

Puffer Hubbard refrigerator	\$ 1,150
2 Duo-seal vacuum pumps (2-stage, Welch 1402B)	710
3 Cornwall continuous propellers	70
Accessories for Zeiss-microscope - (special mechanical stage, \$289; reticulocyte disc, \$45; eye-piece micrometer, \$37; stage micrometer, \$36)	407
Buchler rotary evapo-mix	<u>436</u>
SUBTOTAL	\$ 2,773

3. Supplies:

Chemicals	\$ 1,000
Radioisotopes	3,000
Biochemicals	1,500
Tissue-culture media	3,000
Glass and plastic ware	3,000
Unspecified	<u>300</u>
SUBTOTAL	\$11,800

4. Other Expenses:

Instrument service contracts	\$ 2,000
Publication costs	<u>1,000</u>
SUBTOTAL	\$ 3,000

5. Travel:

Domestic	\$ 1,000
Foreign	<u>600</u>
SUBTOTAL	\$ 1,600

TOTAL DIRECT COSTS	\$47,527
INDIRECT COSTS (47.67% of salaries, wages and fringe benefits)	\$13,516
TOTAL	\$61,043

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B. Budget for second year (September 1, 1973 to August 31, 1974)

1. Salaries:

Name	Title	% of Time	
J. F. Remsen, Ph.D.	Associate Scientist	60	\$ 8,800
J. L. Roti Roti, Ph.D.	Postdoctoral Associate	100	9,570 (no fringe benefits)
G. S. Warren, B.S.	Research Assistant	100	10,890
	Fringe Benefits (9.8%)		
	(includes unemployment compensation)		<u>\$ 1,930</u>
	SUBTOTAL		\$31,190

2. Permanent Equipment:

For unspecified needs \$ 1,000

3. Supplies:

Chemicals	\$ 1,000
Radioisotopes	3,000
Biochemicals	1,500
Tissue-culture media	3,000
Glass and plastic ware	3,000
Unspecified	<u>300</u>
SUBTOTAL	\$11,800

4. Other Expenses:

Instrument service contracts	\$ 2,000
Publication costs	<u>1,000</u>
SUBTOTAL	\$ 3,000

5. Travel:

Domestic \$ 1,000

TOTAL DIRECT COSTS	\$47,990
INDIRECT COSTS (47.67% of salaries, wages and fringe benefits)	\$14,868
TOTAL	\$62,858

C. Budget for third year (September 1, 1974 to August 31, 1975)

1. Salaries:

Name	Title	% of Time	
J. F. Remsen, Ph.D.	Associate Scientist	60	\$ 9,680
J. L. Roti Roti, Ph.D.	Postdoctoral Associate	100	10,527 (no fringe benefits)
G. S. Warren, B.S.	Research Assistant	100	11,979
	Fringe Benefits		<u>\$ 2,123</u>
	(includes unemployment compensation)		
	SUBTOTAL		\$34,309

2. Permanent Equipment:

For unspecified needs	\$ 1,000
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3. Supplies:

Chemicals	\$ 1,000
Radioisotopes	3,000
Biochemicals	1,500
Tissue-culture media	3,000
Glass and plastic ware	3,000
Unspecified	<u>300</u>
SUBTOTAL	\$11,800

4. Other Expenses:

Instrument service contracts	\$ 2,000
Publication costs	<u>1,000</u>
SUBTOTAL	\$ 3,000

5. Travel:

Domestic	\$ 1,000
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TOTAL DIRECT COSTS	\$51,109
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INDIRECT COSTS (47.67% of salaries, wages and fringe benefits)	\$16,355
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TOTAL	\$67,464
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Budget Justification:**Salaries:**

All the personnel for whom support is requested are highly trained in the area in which they will participate in the project. Dr. J. Remsen has been very successful in our laboratory in her work on the photochemistry and photobiology of Coliphage R17 and will have major responsibility for the proposed work on the repair of Coliphage ϕ X174-DNA (Section B). Dr. J. Roti Roti, who has obtained his training in Drs. S. Okada's and M. Eberle's laboratory (U. of Rochester), is a specialist in cell cycle analysis in mammalian cells. He will be able to use his experience in the studies on the cell cycle dependence of the γ -ray damaged thymine in CHO and WI-38 cells. Miss Warren is thoroughly familiar with all techniques used in the culturing of mammalian cells and is a most valuable member of our staff.

The increase in salaries relative to the previous period is due to the contribution (60%) requested to the salary of Dr. Remsen. Dr. Remsen is a part-time Instructor in the Department of Biochemistry.

Supplies:

A major increase in funds for supplies is requested relative to previous years mostly due to our expansion into work on mammalian cells in tissue culture. Additionally, relatively large amounts of radioactively labelled compounds are needed for our assays of γ -ray damaged thymine which are based on radiochemical procedures.

Foreign Travel:

A \$600 travel allowance is requested for 1973. These funds will be used for the attendance by the principal investigator of the International Congress of Biochemistry in Stockholm in July of 1973.