



UNITED STATES
ATOMIC ENERGY COMMISSION

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(R)

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

AREA CODE 615
TELEPHONE 483-8611

September 27, 1973

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: \$45,793

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 September 1, 1973 through August 31, 1974. AEC Support Ceiling will be increased from \$115,209 to \$161,002. Title to the equipment, if any, shall vest in the Contractor under authority of Public Law 85-934.

7. Authority:

Form AEC-481 (CA) from
J. L. Liverman, HQ, dated
September 21, 1973.

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

ACR: LM **E 4378**
bcc: Alice Brown
File
Pink
Reading

RCP&R BR
MEDLEY:ejb
9-27-73
CONTRACTS - 4155 (7la) *file*
C.A.

REPOSITORY *Oak Ridge Operations*
COLLECTION *Records of Oak Ridge Area*
Documents 1944-1994
BOX NO. *H-109-6* *Blkg. 2714-H*
4155 Univ. of Florida
FOLDER *2A* *9-27-73*

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UNITED STATES
ATOMIC ENERGY COMMISSION
WASHINGTON, D.C. 20545

September 6, 1973

Dr. Peter A. Cerutti
Department of Biochemistry
University of Florida
Gainesville, Florida 32601

Dear Dr. Cerutti:

This is to advise you that your renewal proposal entitled "Formation and Repair of Gamma-Ray Induced Nucleic Acid Base Damage in Bacteria and Mammalian Cells, AT(40-1)-4155, has been approved for the year September 1, 1973 to August 31, 1974 at approximately last year's level of research support. You will be contacted by the Oak Ridge Operations Office when funds for the next fiscal year become available after September 1, 1973.

Sincerely,

(S)

George R. Shepherd
Molecular Biologist
Biomedical Programs
Division of Biomedical and
Environmental Research

cc: George K. Davis
Director of Research

Oak Ridge Operations Office ←

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SEP 10 1973

CONTRACTS - 4155 (7la)
C.A.

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SEP 21 1973

Dr. Peter A. Cerutti
Department of Biochemistry
College of Medicine
University of Florida
Gainesville, Florida 32601

Dear Dr. Cerutti:

This is to advise you that the Research Committee has approved renewal of your Research Contract No. AT(40-1)-4155, "Formation and Repair of Gamma-Ray Induced Nucleic Acid Base Damage in Bacteria and Mammalian Cells," for an additional year at approximately last year's level of research support, which includes your unexpended balance of \$207.

You will be contacted by someone from the Oak Ridge Operations Office in the near future regarding negotiation of the renewal contract.

Sincerely,

George R. Shepherd
Molecular Biologist
Biomedical Programs
Division of Biomedical and
Environmental Research

cc: George K. Davis, Director of Research

E 4262

SEP 24 1973

CONTRACTS - 4155 (72a)
C.A.

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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, 1973 - August 31, 1974

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

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E 2561
JUN 6 1973
CONTRACTS - 4155 (Fla)
04758
C.A.

ABSTRACT

During the coming year we plan to continue our studies on the formation and repair of thymine damage induced by γ -rays in the DNA of bacterial and mammalian cells.

The following problems concerning the radiochemical reactivity of thymine in DNA in vitro and in vivo will be investigated. (1) Is there an oxygen effect for production of thymine damage in free E. coli DNA? (2) Is the formation of thymine damage in native DNA preceded by local denaturation of the polymer? (3) What are the molecular reasons for the pronounced protection of the thymine residues to γ -ray induced damage in the DNA in situ in mammalian cells? The radiochemical reactivity of thymine in isolated native and reconstituted HeLa chromatin will be investigated.

A major effort will be made to improve our present methods for the determination of radiation induced thymine damage. If methods of sufficient sensitivity can be developed the molecular mechanism of the excision from the DNA of γ -ray damaged thymine in mammalian cells will be investigated.

OUTLINE

- I. Introduction
- II. Radiochemical Reactivity of Thymine in Free DNA, HeLa Chromatin and DNA in situ in Bacterial and Mammalian Cells.
 - A. Experiments on free E. coli DNA.
 - 1. Comparison of efficiency of formation of thymine damage under aerobic and anoxic conditions.
 - 2. Efficiency of formation of thymine damage in native and denatured E. coli DNA at low doses.
 - B. Experiments with intact E. coli cells.
 - C. Experiments with HeLa Chromatin.
 - 1. Efficiency of formation of thymine damage in native HeLa chromatin.
 - 2. Efficiency of formation of thymine damage in partially and fully reconstituted HeLa chromatin.
- III. Excision of γ -ray Damaged Thymine From the DNA in Mammalian Cells.
 - A. Attempts to develop a more sensitive assay for the determination of thymine damage.
 - B. The molecular mechanism of the excision from the DNA of γ -ray damaged thymine in mammalian cells.
- IV. References.
- V. Supporting Data.
 - A. Personnel.
 - B. Publications resulting from work supported by A.E.C.
 - C. Support received from other federal agencies.
- VI. Budget
- VII. Financial Statement for the Present Contract Period.

1035053

I. Introduction

During the second year of this project we plan to continue our studies on the formation and repair of γ -ray induced thymine damage in bacteria and mammalian cells.

The following questions concerning the efficiency of formation of thymine damage will be studied. (1) Is there an oxygen effect for the formation of thymine damage in free DNA and in DNA in situ in E. coli? (2) Does the formation of thymine damage in native DNA require local denaturation? (3) What is the radiochemical reactivity of thymine in native mammalian chromatin? How much radioprotection is supplied by the histones, how much by the acidic chromosomal proteins? Does the radiosensitivity of thymine differ for native and fully reconstituted chromatin? In addition to the obvious radiobiological interest in these studies with mammalian chromatin information may be obtained about the structural similarity or dissimilarity of native and reconstituted chromatin.

As discussed in the "Progress Report" we have demonstrated the release from the DNA of γ -ray damaged thymine from Chinese hamster ovary cells during postirradiation incubation. (See section IIC). Large amounts of cells and high levels of radioactivity were needed in these experiments mostly due to the limited sensitivity of the reductive assay. Improved methods for the detection of γ -ray damaged residues have to be developed before a detailed study of the molecular steps of the repair process becomes feasible. A major effort in this direction will be made during the coming year. If successful, studies on the cell cycle dependence of the excision of γ -ray damaged residues, on the effect of metabolic inhibitors, on the excision process in cells with a possible defect in DNA repair, etc. will become possible.

II. Radiochemical Reactivity of Thymine in Free DNA, HeLa Chromatin and DNA in situ in Bacterial and Mammalian Cells.

A. Experiments on free E. coli DNA.

1. Efficiency of formation of thymine damage by γ -rays under aerobic and anoxic conditions: An oxygen enhancement factor of 2.4 was observed for the formation of $[^3\text{H}]\text{H}_2\text{O}$ from thymine-methyl $[^3\text{H}]$ in the DNA in situ in CHO cells (Roti Roti and Cerutti, 1973). This result is difficult to interpret from a chemical point of view. It will therefore, be of interest to investigate whether an oxygen effect can be observed in vitro for the formation of thymine damage in free E. coli DNA under protective and non-protective conditions both for the formation of products of the 6-(hydroxy or hydroperoxy)-5,6-dihydro-thymine-type and of $[^3\text{H}]\text{H}_2\text{O}$ from thymine-methyl $[^3\text{H}]$. It should be pointed out in this connection that the oxygen effect observed for DNA single strand breakage in bacteria and mammalian cells has recently

been shown to be due to differences in the efficiency of repair rather than of formation of breaks under anoxic relative to aerobic conditions (Dean et al, 1969; Town et al, 1972).

2. Efficiency of formation of thymine damage in native and denatured E. coli DNA: In our previous studies a significantly lower initial efficiency for the formation of thymine damage was observed for native E. coli DNA than for single stranded ϕ X174-DNA (Swinehart, Lin and Cerutti, 1973). At doses above about 20 Krads where partial denaturation of the native structure has undoubtedly occurred due to strand breakage and disruption of hydrogen bonds the efficiency of product formation is close to that for single stranded DNA. The lowest doses used in these experiments were 10 Krads. The question arises whether local disruption of the helix always precedes formation of base damage. An indication that the nucleic acid bases may be strongly protected in a native double stranded helix has also recently been obtained by Ward and his collaborators (Ward and Kuo, 1970). On the other hand the presence of endonuclease sensitive sites in γ -irradiated break-free PM2-DNA has been observed by Carrier and Setlow (1973) and by Brent (1973) and it has usually been assumed that these sites correspond to base damaged residues.

A series of low dose experiments is, therefore, planned with double stranded circular DNA from Pseudomonas phage PM-2. The number of single strand breaks produced will be compared to the amount of thymine damage at doses below 5 Krads. Single strand breakage in PM-2-DNA can readily be determined by the filter assay of Vander Schans et al (1973) and by alkaline sucrose gradient sedimentation while thymine damage will be determined with our standard assays. It will be particularly interesting to compare at low dose the efficiency of the formation of products of the 6-(hydroxy or hydroperoxy)-5,6-dihydrothymine type which occurs in the center of the double stranded helix with the reaction of the thymine-methyl group (i.e., formation of [3 H]H₂O from thymine-methyl[3 H]) taking place at the helix periphery.

B. Experiments with intact E. coli cells: Comparison of efficiency of formation of thymine damage under aerobic and anoxic conditions.

As mentioned in the preceding section the chemical basis for the oxygen effect for the formation of thymine damage in CHO cells is not understood, in particular since it has been shown that the damage is mostly caused by OH-radicals (Roti Roti and Cerutti, 1973). We plan to extend these studies to E. coli and hope to obtain clues about the basis of the oxygen effect.

C. Experiments with HeLa chromatin.

The factors responsible for the much lower radiosensitivity of DNA in situ in the cell relative to free DNA in solution have not been clearly identified. Even under very strongly protective conditions

thymine in free DNA was approximately twice as reactive as in intracellular DNA (Swinehart, Lin and Cerutti, 1973). The availability of procedures for the isolation of mammalian chromatin in a form in which it retains at least part of its functional integrity allows an approach to this problem. We plan to compare the radiochemical reactivity of thymine in native chromatin to partially and fully reconstituted chromatin. Part of these studies will be carried out in collaboration with Dr. G. Stein of our department.

1. Efficiency of formation of thymine damage in native HeLa chromatin: Dose response curves for the formation of products of the 6-(hydroxy or hydroperoxy)-5,6-dihydrothymine type and the release of $[^3\text{H}]\text{H}_2\text{O}$ from thymine-methyl $[^3\text{H}]$ will be determined for native HeLa chromatin prepared according to Stein and Farber (1972). A special effort will be made to obtain data in the low dose range (i.e., below 10 Krads). The results will be compared to those obtained from HeLa DNA prepared under mild conditions from chromatin. Characteristic dose response curves were obtained for native chromatin in preliminary experiments and it is hoped that the radiochemical reactions of thymine which are being determined can be used as a probe for chromatin structure.

2. Efficiency of formation of thymine damage in partially and fully reconstituted HeLa chromatin: Chromatin will be partially reconstituted by incubating histones or acidic chromosomal proteins with HeLa DNA in the proportions present in native chromatin. Complete reconstitution will be carried using DNA and all chromosomal proteins. The conditions for reconstitution and the procedures for the preparation of chromosomal proteins will be according to Stein and Farber (1972). Special attention will again be focused on "initial" efficiencies at low doses where the difference in the radiochemical reactivity of the preparations are expected to be at a maximum. The results will be compared to those obtained by Ansevin (1973) who compared radiation induced helix denaturation, single strand breakage and double strand breakage for free DNA and reconstituted chromatin from rat thymus nuclei. Our results may also shed some light on the validity of some recent chromatin models (see e.g., Clark and Felsenfeld, 1972; Paul, 1972).

III. Excision of γ -ray Damaged Thymine from the DNA in Mammalian Cells.

Recently we have demonstrated the removal from the DNA of damaged thymine following γ -irradiation in bacterial and mammalian cells (see Progress Report, Section II). The reductive assay for products of the 6-(hydroxy or hydroperoxy)-5,6-dihydrothymine type was used in these studies (Hariharan and Cerutti, 1971; 1972). The sensitivity of this assay, however, is limited. Micrococcus radiodurans was chosen as bacterial system since rather high doses can be used with this organism without loss of viability. Most of our experiments on mammalian cells were done with Chinese hamster ovary cells. Large amounts of cells and high levels of radioactivity had to be used in these experiments (e.g., at a dose of 25 Krads approximately 5×10^7 cpm in DNA-

thymine-methyl^[3H] and 10⁸ cells in monolayers were required per experimental point to be able to measure the released radiation products in the cytoplasm and medium. For a systematic study of the molecular steps of excision repair of γ -ray damaged residues in mammalian cells (and E. coli!) a more sensitive assay for radiation products will have to be developed.

A. Attempt to develop a more sensitive assay for the determination of thymine damage.

Ideally assays should be available allowing the determination of specific radiation products in the DNA before their removal by a repair process and in the cytoplasm and culture medium after excision has occurred. The reduction assay for products of the 6-(hydroxy or hydroperoxy)-5,6-dihydrothymine type (t^+) can be used for both purposes but its sensitivity is insufficient to detect t^+ in DNA extracted from cells which were irradiated with moderate doses. These are two alternatives for chemical methods for the determination of radiation products of the DNA bases. (1) The radiation products can be isolated and quantitated in their original form by chromatographic methods. Mild digestion is necessary for the determination of products in irradiated DNA. (2) A characteristic derivative of a radiation product can be isolated and used as a quantitative measure. This alternative is especially attractive if digestion of the irradiated polymer can be avoided. The reductive assay for products of the 6-(hydroxy or hydroperoxy)-5,6-dihydrothymine type is an example of this second approach.

During the coming year we will attempt to develop a more sensitive assay for γ -ray induced thymine damage. Promising preliminary results have been obtained with a procedure using a cycle of base and acid treatments of the irradiated thymine-methyl^[3H] labeled DNA or the acid soluble material contained in the cytoplasm and the culture medium. Labeled acetol is isolated as a specific degradation products of 6-(hydroxy or hydroperoxy)-5,6-dihydrothymine. A systematic study on a molecular level of the steps involved in the excision of γ -ray damaged residues in mammalian cells (and E. coli) may become feasible with this procedure.

B. The molecular mechanism of the excision from the DNA of γ -ray damaged thymine in mammalian cells.

If it is possible to substantially increase the sensitivity of our methods for the determination of thymine damage experiments on the cell cycle dependence of product excision may become possible. This would be of particular interest since it has so far not been possible to relate the changes in the radiosensitivity of mammalian cells with the cell cycle (see e.g., Terasima and Tolmach, 1961; Sinclair and Morton, 1963) to the efficiency of the production or repair of a particular type of DNA damage. Pulse labeling of the following type may be most promising: Chinese hamster ovary cells

1035057

(CHO) or human embryonic lung fibroblasts WI-38 will be pulse labeled for a short time period (e.g., one hour) with [³H]thymidine and chased with cold thymidine (e.g., for one-half to one hour). The kinetics of product excision will be studied for the case where irradiation immediately followed termination of the labeling procedures (mostly S-phase cells will be irradiated under these conditions with approximately 20% G₂ contamination for a 10-hour S-period). Alternatively, irradiation will be carried out after a lag period comparable to the length of S (a mixture of G₂, M, G₁ cells will be irradiated under these conditions (for the assay for damaged thymine, see introductory paragraph to Section III).

Studies on the effect of metabolic inhibitors on excision repair may become possible with the help of a more sensitive assay for base damage. Especially interesting would be inhibitors of protein synthesis (e.g., cycloheximide; e.g., Terasima and Yasukama, 1967; Doida and Okada, 1972), RNA-synthesis (actinomycin D (see e.g., Tobey *et al*, 1966; Doida and Okada, 1972) and DNA-synthesis (e.g., hydroxyurea and fluorodeoxyuridine).

IV. References

Roti Roti, J. and Cerutti, P. A., Rad. Res. Soc. Annual Meeting, St. Louis, 1973; Abstr. EC-1.

Dean, C. J., Ormerod, M. G., Serianni, R. W. and Alexander, P., Nature 222, 1042 (1969).

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Swinehart, J., Lin, W-S. and Cerutti, P. A., Biophysical Soc. 7th Annual Meeting, Columbus, Ohio, 1973; Abstr. WPM-F3.

Ward, J. and Kuo, I., Int. J. Radiat. Biol. 18, 381 (1970).

Brent, T., Biophysical Soc. 7th Annual Meeting, Columbus, Ohio, 1973; Abstr. WMP-F8.

Carrier, W. L. and Setlow, R. B., Biophysical Soc. 7th Annual Meeting, Columbus, Ohio, 1973; Abstr. WMP-F9.

Van der Schans, G. P., Bleichordt, J. F. and Blok, J., Inter. J. Radiat. Biol. 23, 133 (1973).

Stein, G. and Farber, E., Proc. Natl. Acad. Sci. USA, 69, 2918 (1972).

Ansevin, A., Biophysical Soc. 7th Annual Meeting, Columbus, Ohio, 1973; Abstr. WMP-F5.

Clark, R. and Felsenfeld, G., Nature New Biol. 240, 226 (1972).

Paul, J., Nature 238, 444 (1972).

Hariharan, P. V. and Cerutti, P. A., Nature New Biol. 229, 247 (1971).

Hariharan, P. V. and Cerutti, P. A., J. Mol. Biol. 66, 65 (1972).

Terasima, T. and Tolmach, L. J., Nature 190, 1219 (1961).

Sinclair, W. K. and Morton, R. A., Nature 199, 1158 (1963).

Terasima, T. and Yasukawa, M., Expt. Cell Res. 44, 669 (1967).

Doida, Y. and Okada, S., Cell Tissue Kinet. 5, 15 (1972).

Tobey, R. A., Peterson, D. F., Anderson, E. D. and Puck, T. T., Biophys. J. 6, 567 (1966).

V. 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]
 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, University of Florida, Gainesville, Florida.
 1966 - 1970 Assistant Professor, Department of Biochemistry Sciences, Princeton University, Princeton, New Jersey.
 1964 - 1966 Research Associate NIAMD and NHI (with Drs. Nirenberg, Witkop and Udenfried), National Institutes of Health, Bethesda, Maryland.

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

Principal Investigator; 20% 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).

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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).

M. G. Pleiss and P. A. Cerutti - Phototransformation of 4-Thiouridine in Escherichia coli Valine Transfer Ribonucleic Acid to Uridine, Cytidine and N⁴-Methylcytidine. Biochemistry 10, 3093 (1971).

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. V. Hariharan and P. A. Cerutti - Repair of Strand Breaks in Gamma-irradiated Micrococcus radiodurans. Int. J. Radiat. Biol. 22, 301 (1972).

J. Y. Vanderhoek and P. A. Cerutti - The Stability of Deoxycytidine Photohydrates in the Mononucleotide, Oligonucleotides and DNA. Biochem. Biophys. Res. Commun., in press.

P. Cerutti - Photochemie der Nukleinsäuren, Houben-Weyl Band IV/3 Kapitel IX, Organische Photochemie (E. Müller, ed.), Georg Thieme Verlag, Stuttgart; in press.

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 Breach Science Publishers, New York, in press.

1035062

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

1035063

2. Joseph L. Roti Roti

NAME: Joseph Lee Roti Roti, Ph.D.
 TITLE: Postdoctoral Associate
 BIRTHDATE/BIRTHPLACE: [REDACTED]
 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 C of the proposal, i.e., the radiochemical reactivity of thymine in native and reconstituted HeLa chromatin. He will devote 100% of his time to the project.

SELECTED PERSONAL PUBLICATIONS IN AREAS RELATED TO PRESENT PROPOSAL.

- J. L. Roti Roti, S. Okada and H. Eberle, Protein Synthesis During the Cell Cycle of L5178Y. Exptl. Cell Res. 76, 200 (1973).
- J. L. Roti Roti and S. Okada, A Mathematical Model of the Cell Cycle of L5178Y. Cell and Tissue Kinetics 6, 111 (1973)

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. 77, 346 (1973).

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. Exptl. Cell Res. 77, 351 (1973).

J. L. Roti Roti, Matrix Stimulation of Cell Cycle Regulation, submitted for publication.

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

J. L. Roti Roti and P. A. Cerutti, Radiochemical Reactivity of Thymine in the DNA in Chinese Hamster Ovary Cells "in situ", manuscript in preparation.

3. Reba K. Wright

NAME Reba Kay Wright, Ph.D.
 TITLE: Postdoctoral Associate
 BIRTHDATE/BIRTHPLACE: [REDACTED]
 PRESENT NATIONALITY: USA
 SEX: Female
 SOCIAL SECURITY NUMBER: [REDACTED]

EDUCATION:

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

HONORS:

NASA Traineeship, 1965-1968
 University of Miami Research Fellowship, 1968-1969
 NSF Traineeship, 1969-1972
 Chi Beta Phi, 1964
 Sigma Xi, 1972
 American Association for Advancement of Science, 1965

RESEARCH AND/OR PROFESSIONAL EXPERIENCE:

1973 - Postdoctoral Associate with Dr. P. A. Cerutti, Department of Biochemistry, University of Florida, Gainesville, Florida

7/1968 - 8/1968 Research Technician, Emory University, Atlanta, Georgia.

6/1965 - 9/1965 Research Technician, Oak Ridge Associated University, Oak Ridge, Tennessee.

6/1964 - 8/1964 Student Research, AEC Summer Student Research, Oak Ridge Institute of Nuclear Studies, Oak Ridge, Tennessee

6/1963 - 8/1963 Student Research, NSF Summer Student Research at Southwestern at Memphis, Memphis, Tennessee

6/1962 - 9/1962 Research Technician, Department of Clinical Physiology, University of Tennessee Medical School, Memphis, Tennessee

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

Will carry out the major portion of our study of thymine damage (Section III). She will devote 100% of her time to this project.

SELECTED PERSONAL PUBLICATIONS IN AREAS RELATED TO PRESENT PROPOSAL.

F. Snyder and R. Wright, Effect of Localized Irradiation on the Metabolism of Bone-Marrow Lipids. *Radiation. Res.* 25, 417 (1965).

F. Binkley, N. King, E. Milikin, R. K. Wright, C. H. O'Neal and I. J. Wundrum, Brush Border Particulates of Renal Tissue, *Science* 162, 1009 (1968).

V. Ziboh, R. Wright and S. L. Hsia, Effects of Insulin on the Uptake and Metabolism of Glucose by Rat Skin in vitro. *Arch. Biochem. Biophys.* 146, 93 (1971).

R. K. Wright and S. L. Hsia, Effects of Insulin, Prostaglandin E₂ and Epinephrine on the Formation of Cyclic AMP by Human Skin. *Fed. Proc.* 30, 1205Abs (1971).

S. L. Hsia, R. Wright, S. H. Mandy and K. M. Halprin. Abnormalities in Adenyl Cyclase of Psoriatic Skin. Joint Meeting of the Society for Investigative Dermatology and European Society for Dermatological Research, Amsterdam, Netherland, pp. 10-11Abs, May 17-19, 1972.

R. K. Wright, S. H. Mandy, K. M. Halprin and S. L. Hsia, Defects and Deficiency of Adenyl Cyclase in Psoriatic Skin. *Arch. Dermatol.* 107, 47 (1973).

S. L. Hsia, R. Wright, S. H. Mandy and K. M. Halprin, Adenyl Cyclase in Normal and Psoriatic Skin. *J. Invest. Derm.* 59, 109 (1972).

4. Maria V. McMacken

NAME: Maria Vigo McMacken
 TITLE: Research Assistant
 BIRTHDATE/BIRTHPLACE: [REDACTED]
 PRESENT NATIONALITY: USA
 SEX: Female
 SOCIAL SECURITY NUMBER: [REDACTED]

EDUCATION:

Institution	Degree	Year	Speciality
[REDACTED]	[REDACTED]	[REDACTED]	[REDACTED]

RESEARCH AND/OR PROFESSIONAL EXPERIENCE:

- 1973 - Research Assistant with Dr. P. A. Cerutti, Department of Biochemistry, University of Florida, Gainesville, Florida.
- 1970 - 1971 Research Associate, Yale University, New Haven, Connecticut.
- 1968 - 1969 Research Assistant, University of Wisconsin, Madison, Wisconsin.
- 1965 - 1966 Research Assistant, University of Wisconsin, Madison, Wisconsin.

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

Ms. McMacken's major responsibility will be the routine culturing of mammalian cells and will assist in our experiments on the repair of thymine damage in Chinese hamster ovary cells and WI-38 cells. 50% of her time will be devoted to this project.

SELECTED PERSONAL PUBLICATIONS IN AREAS RELATED TO PRESENT PROPOSAL.

D. R. Wilken, M. McMacken and A. Rodriguez - Choline and Betaine Aldehyde Oxidation by Rat Liver Mitochondria. Biochim. Biophys. Acta 216, 305 (1970).

5. Personnel contributing to the program but for whom no support is requested from AEC.

P. V. Hariharan, Ph.D.	Associate Scientist and Instructor in Biochemistry
Miss J. Swinehart	Graduate Student (terminates in Fall, 1973)
Mr. M. Mattern	Graduate Student

1035069

B. Publications resulting from work supported by A.E.C.

1. Papers published or in press:

P. V. Hariharan and P. A. Cerutti, Repair of Strand Breaks in Gamma-irradiated Micrococcus radiodurans. Int. J. Radiat. Biol. 22, 301 (1972).

J. Y. Vanderhoek and P. A. Cerutti, The Stability of Deoxycytidine Photohydrates in the Mononucleotide, Oligonucleotides and DNA. Biochem. Biophys. Res. Commun., in press.

P. A. Cerutti, "Base Damage in DNA Induced by Ionizing Radiation", Chapter VI in Photochemistry and Photobiology of Nucleic Acids, (S. Wang and M. Patrick, eds.), Gordon Breach Science Publishers, New York, in press.

P. A. Cerutti, "Deoxycytidine Photohydration in DNA", Chapter IC in Photochemistry and Photobiology of Nucleic Acids, (S. Wang and M. Patrick, eds.), Gordon Breach Science Publishers, New York, in press.

2. Papers submitted for publication:

J. Roti Roti and P. Cerutti, Gamma-ray Induced Thymine Damage in Mammalian Cells.

M. Mattern, P. V. Hariharan, B. E. Dunlap and P. A. Cerutti, DNA-Degradation and Excision Repair in γ -irradiated Chinese Hamster Ovary Cells.

3. Papers communicated at national meetings:

P. A. Cerutti, Repair of Gamma-ray Induced Thymine Damage in Chinese Hamster Ovary Cells, IV International Congress of Biophysics, Moscow, 1972.

J. Roti Roti and P. A. Cerutti, Radiochemical Reactivity of Thymine in DNA of Chinese Hamster Ovary Cells in situ, Rad. Res. Soc. Annual Meeting, St. Louis, 1973; Abstr. EC-1.

J. Swinehart, W-S. Lin and P. A. Cerutti, Gamma-ray Induced Thymine Damage in the Mononucleotide and Single and Double Stranded DNA, Biophysical Society 7th Annual Meeting, Columbus, Ohio, 1973, Abstr. WMP-F3.

P. A. Cerutti, Ultraviolet and Gamma-ray Induced Pyrimidine Damage in Polynucleotides in vitro and in vivo, Gordon Conference on Nucleic Acids, New Hampshire, 1973.

C. Support received from other federal agencies.

Support is obtained from the National Institutes of Health (Grant No. 5 R01 GM 18617-03) for a project entitled "Structure and Function of Ribonucleic Acid" in the amount of \$36,431 (direct costs) for the current year.

1035071

VI. BudgetBudget for the period September 1, 1973 to August 31, 19741. Salaries

Name	Title	% of Time		
J. L. Roti Roti, Ph.D.	Postdoctoral Associate	100	\$ 9,515	(no fringe benefit)
R. K. Wright, Ph.D.	Postdoctoral Associate	100	8,300	(no fringe benefit)
M. V. McMacken	Research Assistant	50	4,855	
	Fringe Benefits (10.5%)		<u>510</u>	
	(includes unemployment compensation)			
	SUBTOTAL		\$23,180	

2. Permanent Equipment

Isco density gradient fractionator			
Model 640			\$ 1,340
universal flow cell (5mm) and top holder			170
reel for 42 scintillation vials, 30 mm diameter			60
Sero-utility bath, large (Precision Scientific)			250
Automatic pipettes with tips; 10-200 μ l			150
Two Dewan condenser assemblies (for flash evaporator)			<u>180</u>
	SUBTOTAL		\$ 2,150

3. Supplies

Chemicals		\$ 1,000
Radioisotopes		2,500
Biochemicals		1,500
Tissue-culture media		2,500
Glass and plastic ware		<u>2,500</u>
	SUBTOTAL	\$10,000

4. Other Expenses

Instrument service contracts	\$ 1,500
Publication costs	<u>1,000</u>
	SUBTOTAL
	\$ 2,500

5. Travel (Domestic)

	<u>\$ 1,000</u>
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TOTAL DIRECT COSTS	\$38,830
INDIRECT COSTS (49.42% of salaries, wages and fringe benefits)	11,456
TOTAL	<u>\$50,286</u>

1035072

VII. Financial Statement for the Present Contract Period

1. Total actual project cost to date for the current period.	\$ 22,213.29
2. Estimated total cost for remainder of period.	\$ 23,786.71
3. Total actual 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.	\$ 46,000.00
4. Accumulated costs chargeable to AEC	\$115,001.64
5. Accumulated AEC Support Ceiling as stated in Article III of Contract.	\$115,209.00
6. Total estimated AEC funds remaining under Contract.	\$ 207.36

PUBLICATION
BY AEC
AUTHORIZED

NOTICE OF RESEARCH PROJECT
SCIENCE INFORMATION EXCHANGE
SMITHSONIAN INSTITUTION

U.S. ATOMIC ENERGY COMMISSION

SIE NO.

AEC CONTRACT NO.

AT(40-1)-4155

SUPPORTING DIV. OR OFFICE: DIVISION OF BIOLOGY AND MEDICINE

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

Department of Biochemistry
College of Arts and Sciences
University of Florida
Gainesville, Florida 32601

TITLE OF PROJECT:

Formation and Repair of γ -ray Induced Nucleic Acid Base Damage in Bacteria and Mammalian Cells.

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.

Peter A. Cerutti	Department of Biochemistry	Principal Investigator	20%
Reba K. Wright	Department of Biochemistry	Postdoctoral Associate	100%
Joseph L. Roti Roti	Department of Biochemistry	Postdoctoral Associate	100%
Maria V. McMacken	Department of Biochemistry	Research Assistant	50%

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

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

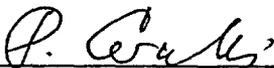
During the coming year we plan to continue our studies on the formation and repair of thymine damage induced by γ -rays in the DNA of bacterial and mammalian cells.

The following problems concerning the radiochemical reactivity of thymine in DNA in vitro and in vivo will be investigated. (1) Is there an oxygen effect for production of thymine damage in free E. coli DNA? (2) Is the formation of thymine damage in native DNA preceded by local denaturation of the polymer? (3) What are the molecular reasons for the pronounced protection of the thymine residues to γ -ray induced damage in the DNA in situ in mammalian cells? The radiochemical reactivity of thymine in isolated native and reconstituted HeLa chromatin will be investigated.

A major effort will be made to improve our present methods for the determination of radiation induced thymine damage. If methods of sufficient sensitivity can be developed the molecular mechanism of the excision from the DNA of γ -ray damaged thymine in mammalian cells will be investigated.

RESULTS TO DATE:

	PROGRAM CATEGORY NO.
BUDGET	
PRIMARY	
SECONDARY	


Signature of Principal Investigator

DATE: May 30, 1973

INVESTIGATOR - DO NOT USE THIS SPACE

1035074

THE J. HILLIS MILLER HEALTH CENTER
UNIVERSITY OF FLORIDA

Department of Biochemistry

September 20, 1973

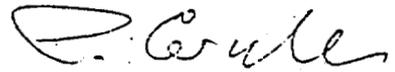
Phone: 904-392-3361
Gainesville, 32610

Dr. Lamar Medley
Contract Division
U.S. Atomic Energy Commission
Post Office Box E
Oak Ridge, Tennessee 37830

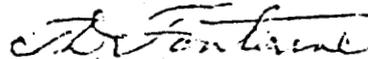
Dear Dr. Medley:

Enclosed please find a revised budget for the period September 1, 1973 to August 31, 1974 of contract AT-(40-1)-4155.

Sincerely yours,



Peter A. Cerutti
Principal Investigator



Budget Approved: Thomas D. Fontaine, Acting
Director, Sponsored Research

PAC:alf

Enclosures *ok*

E 4318

SEP 25 1973

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Revised Budget for A.E.C. Contract AT(40-1)-4155
for the Period September 1, 1973 to August 31, 1974

1. Salaries

<u>Name</u>	<u>Title</u>	<u>% of Time</u>	
S. Locke	Lab Technologist II	100	\$ 7,600
R. K. Wright, Ph.D.	Postdoctoral Associate	100	8,300 (no fringe benefits)
M. V. McMacken	Research Assistant	50	4,855
	Fringe Benefits (10.5%)		<u>1,428</u>
	(includes unemployment compensation plus \$120 health insurance)		
	SUBTOTAL		\$22,183

2. Permanent Equipment

Isco density gradient fractionator		
Model 640		1,340
universal flow cell (5mm) and top holder		170
reel for 42 scintillation vials, 30 mm diameter		<u>60</u>
	SUBTOTAL	\$ 1,570

3. Supplies

Chemicals	\$ 1,000
Radioisotopes	2,500
Biochemicals	1,000
Tissue-culture media	2,034
Glass and plastic ware	<u>2,000</u>
	SUBTOTAL
	\$ 8,534

4. Other Expenses

Instrument service contracts	\$ 1,000
Publication costs	<u>750</u>
	SUBTOTAL
	\$ 1,750

5. Travel (Domestic)

\$ 1,000

TOTAL DIRECT COSTS	\$35,037
INDIRECT COSTS (49.42% of salaries, wages and fringe benefits)	\$10,963
TOTAL	\$46,000

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SEP 26 1973

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R
USAEC, A. H. FROST, JR., CHIEF
RESEARCH CONTRACTS, PROCEDURES AND
REPORTS BRANCH, CONTRACT DIVISION
OAK RIDGE, TENNESSEE 37830

ORIGINAL SIGNED BY
Lamar Medley

A. H. FROST, JR.

SEPTEMBER 24, 1973

DR. P. A. CERUTTI, DEPT. OF BIOCHEMISTRY,
UNIVERSITY OF FLORIDA, GAINESVILLE, FLORIDA 32601

INFO: DR. GEORGE K. DAVIS, DIRECTOR, DIV. OF SPONSORED RESEARCH
UNIVERSITY OF FLORIDA, GAINESVILLE, FLORIDA 32601

UNCLASSIFIED/NOFORN

WE HAVE AUTHORIZATION TO EXTEND CONTRACT NO. AT-(40-1)-4155
THROUGH AUG. 31, 1974 WITH \$45,793 NEW AEC FUNDS PLUS \$207
ESTIMATED AEC BALANCE FROM PRIOR TERM FOR ESTIMATED PROJECT
COST OF \$46,000. PLEASE SUBMIT REVISED BUDGET ASAP BASED ON
APPROVED AEC FINANCING. PLEASE HAVE BUDGET ENDORSED BY
APPROPRIATE ADMINISTRATIVE OFFICIAL OF UNIVERSITY.

ACR:IM - 102

MEDLEY:ejb
3-4105
Room 1011

RECEIVED
ORO-AEC
TELETYPE SECTION
SEP 24 2 51 PM '73

CONTRACTS - 4155 (73) C.A

E 4274

OFFICE ▶	RCP&R BR				
SURNAME ▶	MEDLEY:ejb				
DATE ▶	9/24/73				

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