

APPENDIX "A"

REPOSITORY Oak Ridge Operations
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FVO 4155 Florida
 FOLDER Expenditure Statement

UNIVERSITY OF FLORIDA
 CONTRACT NO. AT-(40-1)-4155

For the contract period September 1, 1973 through August 31, 1974

ARTICLE A-I. RESEARCH TO BE PERFORMED BY CONTRACTOR

The Contractor will conduct studies on the photohydration of DNA.

The Principal Investigator, Dr. P. A. Cerutti, expects to devote approximately 20% of his time or effort to the project.

ARTICLE A-II. WAYS AND MEANS OF PERFORMANCE

(a) Items included in total estimated cost:

(1) Salaries and wages and fringe benefits: \$22,183

(2) Equipment to be purchased or fabricated by the Contractor: \$ 1,340

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

None

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

Density gradient fractionator

(3) Travel: \$ 1,000

Domestic-----\$1,000

Foreign-----\$ 0

(4) Other direct costs: \$10,514

(5) Indirect costs (based on a predetermined rate of 49.42% of salaries and wages and fringe benefits): \$10,963

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

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

(1) Items to be contributed by the Contractor:

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

(2) Items to be contributed by the Government:

None

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

None under this paragraph.

ARTICLE A-III. The total estimated cost of items under A-II (a) above for the contract period stated in this Appendix "A" is \$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 period(s):	\$ 207
(b) New funds for the current period:	\$45,793

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

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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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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.

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-dihydrothymine-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: Comparision 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

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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^{\dagger}) can be used for both purposes but its sensitivity is insufficient to detect t^{\dagger} 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

(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).

Town, C. D., Smith, K. C. and Kaplan, H. S., Rad. Res. 52, 99 (1972).

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.

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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] Zurich, Switzerland
 PRESENT NATIONALITY: [REDACTED]
 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).

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).

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.

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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.

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2. 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 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)

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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.

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], Ponce of Puerto Rico
 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

1039208

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.

1039209

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.

1039210

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



UNITED STATES
ATOMIC ENERGY COMMISSION

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

August 16, 1974

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AREA CODE 615
TELEPHONE 483-8611

University of Florida
ATTN: Dr. George K. Davis
Division of Sponsored Research
219 Graduate & International Studies Building
Gainesville, Florida 32601

Gentlemen:

PROPOSED MODIFICATION NO. 5 TO CONTRACT NO. AT-(40-1)-4155

Enclosed, in triplicate, is the subject contract document.

If this document, as submitted, is satisfactory, will you have two of the enclosed copies signed by the proper official and return them to this office. After signature and dating on behalf of the Commission, one fully signed copy of the document will be returned for your files.

Sincerely,

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

ACR:AHF

Enclosure: *1/27*
Modification 5 (in trip.)

o/c bcc: Lamar Medley

REPOSITORY *Oak Ridge Operations*
Records Holding Area
COLLECTION *Documents 1944-1994*
BOX No. *H-75-17 Bldg. 2714-H*
EUO 4155 Florida
FOLDER *Expenditure Statement*

F 5458
RCP&R Branch
Contract Div.
Brown
/ARBrown=
8-16-74

yellow

1039212A

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, 1974 - August 31, 1975

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

F 3939

JUL 12 1974

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SUMMARY

During the third year period of this project, we plan to continue our studies on the radiation chemistry of DNA. In particular, the stability of products of the 5,6-dihydroxy-dihydrothymine type in DNA and the formation of apyrimidinic sites will be investigated. The presence of significant amounts of apyrimidinic- and possibly apurinic sites in γ -irradiated DNA could explain the well known similarity between the biological effects exerted by ionizing radiation and certain alkylating agents which are known to produce apurinic sites.

Major emphasis in our program will, however, be placed on studies of the excision repair of products of the 5,6-dihydroxy-dihydrothymine type in bacteria and in normal and diseased mammalian cells. "Open systems" will be used, i.e. crude E. coli extracts on one hand, and isolated nuclei from cultured mammalian cells on the other, and the excision of damaged residues from an exogenous, damaged DNA substrate will be investigated.

Some of the questions which will be asked in our experiments with E. coli extracts are: (1) What are the functions of the rec-genes in the repair of γ -ray induced thymine damage in E. coli? (2) What is the role of the exr-gene in repair of γ -ray induced thymine damage in E. coli? (3) Is polynucleotide ligase responsible for the joining of the ends following the removal from the DNA of ring damaged thymine? Is it possible to learn more about the incision step with the help of ligase mutants or ligase inhibitors?

In our studies of prereplication incision repair of γ -ray damaged thymine in mammalian cells, we plan to concentrate on two major questions: (1) Are there differences in the γ -ray excision repair capability of normal and transformed human and rodent cells? (2) What is the γ -ray excision repair capability of nuclei from normal human skin-fibroblasts and nuclei from Xeroderma pigmentosum skin fibroblasts?

OUTLINE

- I. Introduction
- II. Apyrimidinic-Sites in γ -Irradiated DNA
- III. Excision Repair of γ -Ray Damaged Thymine by Crude E. Coli Extracts
 - A. The role of the rec-genes in repair of γ -ray induced thymine damage
 - B. The role of the exr-genes in repair of γ -ray induced thymine damage
 - C. The role of polynucleotide ligase in γ -ray excision repair
- IV. Excision Repair of γ -Ray Damaged Thymine by Isolated Nuclei from Normal and Diseased Mammalian Cells
 - A. The γ -ray repair capability of nuclei from normal and virally transformed mammalian cells.
 - B. The γ -ray repair capability of nuclei from normal human skin-fibroblasts and from Xeroderma pigmentosum skin-fibroblasts.
- V. References
- VI. Supporting Data
 - A. Personnel.
 - B. Publications from work supported by A.E.C.
 - C. Support received from other federal agencies.
- VII. Budget
- VIII. Financial Statement for the Present Contract Period

I. Introduction

During the third year period of this project, we plan to continue our studies on the radiation chemistry of DNA. In particular, the stability of products of the 5,6-dihydroxy-dihydrothymine type in DNA and the formation of apyrimidinic sites will be investigated. The presence of significant amounts of apyrimidinic- and possibly apurinic sites in γ -irradiated DNA could explain the well known similarity between the biological effects exerted by ionizing radiation and certain alkylating agents which are known to produce apurinic sites (cf. radiomimetic effects of methylmethanesulfonate or ethylmethanesulfonate).

Major emphasis in our program will, however, be placed on studies of the excision repair of products of the 5,6-dihydroxy-dihydrothymine type in bacteria and in normal and diseased mammalian cells. "Open systems" will be used, i.e. crude E. coli extracts prepared according to Wickner et al. (1972) on one hand, and isolated nuclei from cultured mammalian cells (Remsen and Cerutti, unpublished results) on the other, and the excision of damaged residues from an exogenous, damaged DNA substrate will be investigated. These open systems have a number of attractive features: unirradiated extracts or nuclei are used with damaged, well-defined DNA specimens or in certain experiments with selectively modified synthetic polydeoxynucleotides; complementation and reconstitution experiments can be carried out in case repair deficiencies are discovered, etc. Both the bacterial and the mammalian system are presently successfully being used in our laboratory. The selective excision of products of the 5,6-dihydroxy-dihydrothymine type from irradiated viral DNA and from OsO₄-oxidized polyd(A-T) by crude E. coli extracts and by isolated mammalian nuclei has been demonstrated (Hariharan and Cerutti, 1974 a & b; Remsen and Cerutti, 1974).

Some of the questions which will be asked in our experiments with E. coli extracts are: (1) What are the functions of the rec-genes in the repair of γ -ray induced thymine damage in E. coli? (2) What is the role of the exr-gene in repair of γ -ray induced thymine damage in E. coli? (3) Is polynucleotide ligase responsible for the joining of the ends following the removal from the DNA of ring damaged thymine? Is it possible to learn more about the incision step with the help of ligase mutants or ligase inhibitors?

In our studies of prereplication incision repair of γ -ray damaged thymine in mammalian cells, we plan to concentrate on two major questions: (1) Are there differences in the γ -ray excision repair capability of normal and transformed human and rodent cells? (2) What is the γ -ray excision repair capability of nuclei for normal human skin-fibroblasts and nuclei from Xeroderma pigmentosum skin fibroblasts?

II. Apyrimidinic-Sites in γ -Irradiated DNA

As pointed out in the introductory paragraph and in section I(5) of the "Progress Report", the discovery that ring damaged thymine residues are in part "spontaneously" released from γ -irradiated DNA has important implications for our understanding of the relationship between γ -ray repair and repair of DNA lesions induced by alkylating agents such as MMS and EMS. The further chemical characterization of the damage introduced into DNA by ionizing radiation may allow a structural definition of the DNA lesions which are recognized by the various "repair-endonucleases". Such studies are a prerequisite for the characterization of the damage recognized by γ -endonuclease from M. luteus ("endonuclease sensitive sites,"), γ -endonuclease from E. coli (Wallace, 1974), endonuclease II from E. coli (Friedberg and Gold, 1969), apurinic site enzymes from various bacterial, plant and mammalian sources (Verley et al., 1973).

We propose to continue our studies on the formation of apyrimidinic- and possibly apurinic sites in γ -irradiated DNA. Gamma-irradiated pseudomonas phage PM-2 DNA labeled by thymine-methyl[³H] and OsO₄-oxidized synthetic polyd(A-T)-thymine-methyl[³H] are being used in our studies. (Note: OsO₄ selectively oxidizes thymine in polyd(A-T) to 5,6-dihydroxy-dihydrothymine. Products of the 5,6-dihydroxy-dihydrothymine type (t') represent a major class of lesions produced by ionizing-radiation in DNA. OsO₄-oxidized polyd(A-T), in contrast to γ -irradiated DNA, does not contain adenine damage and, most importantly, does not contain radiation-induced strand breakage. OsO₄-oxidized polyd(A-T) in contrast to γ -irradiated PM-2 DNA, therefore, represents a chemically well defined DNA substrate). We plan to analyze the low molecular weight material released at neutrality from γ -irradiated PM-2 DNA and OsO₄-oxidized polyd(A-T) upon incubation at 37° by ion-exchange chromatography on Dowex 50(H⁺) and DEAE-Sephadex columns. Authentic thymine, thymidine and thymidylic acid will be cochromatographed as markers. A second series of samples will be pretreated with bacterial alkaline phosphatase before application to the columns. Appropriate portions of the eluates will be pooled and analyzed for their content in t' by the alkali-acid degradation assay (Hariharan and Cerutti, 1974, see Section I(1) of "Progress Report"). These experiments will show whether t' is indeed released from the DNA as free base under formation of internal apyrimidinic sites. At the same time, it will be interesting to see how much intact thymine, thymidine and thymidylic acid is released from irradiated DNA under mild, physiological conditions (a measure of "latent" damage of the DNA backbone). For the case of OsO₄-oxidized polyd(A-T), the chromatographic analysis of the low molecular weight fraction will be complemented by sedimentation analysis of the polymeric material on alkaline sucrose gradients or on neutral sucrose gradients under denaturing conditions.

Depending on the progress in these experiments the generality of our findings concerning "athyminic sites" in γ -irradiated PM-2 DNA will be tested. The question whether apurinic sites are formed in γ -irradiated DNA will be studied in an analogous series of experiments using DNA specifically labeled in adenine instead of thymine. The preparation of DNA specifically labeled in adenine is straightforward. However, the development of an assay for γ -ray induced adenine damage along the line of our assay for t' may require a considerable amount of work.

III. Repair of γ -Ray Damaged Thymine by Crude E. Coli Extracts

A. The role of the rec-genes in repair of γ -ray induced thymine damage.

The involvement of the recombination enzyme systems in the repair of UV- and γ -ray induced DNA damage in E. coli was implicated when it was found that recA-mutants which can perform all the steps of zygote formation but are unable to link DNA molecules to form recombinants are much more sensitive to UV-light and ionizing radiation (2.5 to 5-fold more sensitive) than the wild type strains (Clark and Margulies, 1965; Howard-Flanders and Boyce, 1966; Howard-Flanders and Theriot, 1966). For the case of UV-induced damage which escaped excision repair, it was shown that the recombination enzymes are involved in reconstituting the integrity of the genome following DNA replication by sister strand exchange (postreplication repair; Rupp and Howard-Flanders, 1968; Ganesan and Smith, 1972; Rupp, et al., 1971).

The molecular steps in which the rec-enzymes participate in γ -ray repair have not been elucidated. There is considerable evidence that the recA, recB and recC functions participate in the slow, growth dependent resealing of radiation induced DNA strand breakage in E. coli first described by McGrath and Williams (1966) ("type III" repair of single strand breaks according to the nomenclature of Smith and his collaborators, see in Town, et al., 1973). While recA-mutants are completely deficient in this slow resealing process (Kapp and Smith, 1970) some residual resealing appears to occur in recBrecC-mutants (Town et al., 1973). It may be speculated that the recBrecC-enzyme (exonuclease V) may be needed for end-preparation, i.e removal of damaged or undamaged residues at the breaks. From studies of the effects of certain drugs on slow break repair in wild type E. coli and rec⁻-mutants it was suggested by Town et al. (1973) that the rec-functions may additionally participate in other repair processes, e.g. the repair of damage at nucleic acid bases (cf. in analogy to the recA-controlled repair of UV-base damage; Rupp and Howard-Flanders, 1968; Smith and Meun, 1970).

During the coming year we plan to investigate the role of the rec-gene products in the excision repair of γ -ray induced thymine damage. Our "open E. coli system" will be used. Crude extracts will be prepared essentially according to Wickner, et al. (1972) from E. coli endo I⁻ recA⁻, E. coli recA⁻ recB⁻. The capacity of these extracts to excise products of the 5,6-dihydroxy-dihydrothymine type (t') from γ -irradiated PM-2 DNA and from OsO₄-oxidized polyd(A-T) will be studied. (As mentioned in the introductory paragraph γ -irradiated PM-2 DNA contains a variety of lesions in addition to t' while OsO₄-oxidized polyd(A-T) only contains 5,6-dihydroxy-dihydrothymine and some apyrimidinic sites). Special attention will be given to the selectivity of the excision process by the extracts from the different mutants (i.e. the number of non-damaged residues removed from the DNA per ring damaged residue t').

B. The role of the exr-gene in repair of γ -ray induced thymine damage.

E. coli mutants deficient in the exrA (lexA)-gene are 2 to 4 times more sensitive to X-rays than the corresponding wild type strain. The exrA-gene product is required for the slow resealing of radiation-induced DNA strand breaks ("type III" repair, Youngs and Smith, 1973) and may be responsible for error-prone repair leading to radiation induced mutagenesis in E. coli. As for the rec-genes it has been speculated that the exr-function may participate in repair processes other than strand resealing (Town, et al., 1973).

We plan to carry out a study of the excision repair capability of E. coli exrA⁻ using the "open E. coli system" and the exogenous DNA substrates described in the preceding section.

C. The role of polynucleotide ligase in γ -ray excision repair; single strand breaks associated with the excision of damaged thymine residues from OsO₄-oxidized polyd(A-T).

The role of polynucleotide ligase in the final resealing step in excision repair of UV-photodimers in bacteria has been clearly demonstrated (see e.g. Grossman, 1974). Corresponding experiments have not been carried out for γ -ray excision repair. We are planning to study the involvement of polynucleotide ligase of E. coli in the excision repair of 5,6-dihydroxy-dihydrothymine (t') in OsO₄-oxidized poly(A-T). The removal from the polymer of t' by crude extracts of E. coli endo I⁻ lig₄ at the permissive and non-permissive temperature will be measured by our standard techniques and the completion of the repair process, i.e. gap-filling and resealing, will be followed by alkaline sucrose gradient sedimentation (Note: no degradation of OsO₄-oxidized polyd(A-T) occurs on the alkaline sucrose gradients carried out in a SW 50.1

rotor at 20° for 12 hrs at 45,000 rpm). An analogous series of experiments will be carried out in the presence and absence of the ligase-inhibitor nicotinamidemononucleotide (NMN). These experiments will show whether polynucleotide ligase is responsible for the last resealing step in γ -ray excision repair. If strand rejoining is indeed deficient at the non-permissive temperature of the lig₄-mutant or in the wild type in the presence of NMN as suggested by some preliminary experiments the correlation of the product excision data with those obtained by sedimentation analysis should allow an estimate of the endonucleolytic breaks introduced into the polymer per ring damage thymine residue. Some experiments along these lines are presently being carried out and look rather promising.

IV. Excision Repair of γ -Ray Damaged Thymine by Isolated Nuclei From Normal and Diseased Mammalian Cells.

As mentioned in the "Introduction", we have developed an "open system" using isolated nuclei preparations for the study of excision repair of γ -ray or OsO₄-induced thymine damage of the 5,6-dihydroxy-dihydrothymine type (t') and some of the advantages and disadvantages of our experimental design have been mentioned. This system is presently successfully being used in preliminary studies. Selective excision of t' from γ -irradiated bacteriophage PM-2 DNA or OsO₄-oxidized polyd(A-T) has been demonstrated by nuclei from human carcinoma HeLa S-3 cells and human embryonic lung fibroblasts WI-38 (Remsen, Mattern and Cerutti, unpublished results). Approximately 25% of t' was removed from acid precipitable DNA within 30 min. of incubation with HeLa or WI-38 nuclei preparations complemented by an ATP generating system and the four deoxynucleosidetriphosphates. Only 6% undamaged thymine was rendered acid soluble during the same time period. We are presently optimizing the conditions of our system and we are establishing some of its basic properties: e.g. determination of optimal pH, ionic milieu, nuclei concentration; what is the effect of freezing of the nuclei preparations (Mammalian endonucleases appear to be extremely sensitive to freezing!)? What is the effect of SH-inhibitors? In what form are the damaged residues removed from the DNA by the nuclei?, etc.

During the coming year we are planning to concentrate on (a) a comparison of the γ -ray repair capability of nuclei from normal and virally transformed mammalian cells; (b) studies of the γ -ray repair capability of nuclei from normal human skin fibroblasts and from Xeroderma pigmentosum skin fibroblasts. Most of the experiments proposed below will be carried out in parallel with γ -irradiated PM-2 DNA and OsO₄-oxidized polyd(A-T). The comparison of results obtained with the two substrates allows an assessment of the effect of DNA damage other than t', in particular, of radiation induced strand breaks, on the excision repair process.

A. The γ -ray excision repair capability of nuclei from normal and virally transformed mammalian cells.

Ionizing radiation remains to be the most successful tool for the therapy of cancer. The discovery of significant differences in the repair capabilities of normal and malignant cells could suggest new avenues for the improvement of radiotherapeutic procedures. No deficiencies in the repair of radiation-induced single strand breaks, unscheduled synthesis or repair replication was observed in malignant cells (see e.g. in Painter, 1970, 1972). An increased rate of rejoining of single strand breaks was observed in chronic lymphocytic leukemic cells (Huang et al., 1972). Except for our preliminary results with HeLa S-3 cells, no data is available on the γ -ray excision repair capacity of transformed relative to normal cells.

We plan to compare the capability for excision repair of γ -ray products of the 5,6-dihydroxy-dihydrothymine type (t') of isolated nuclei from normal and virally transformed cells. Our standard isolated nuclei system will be used with γ -irradiated PM-2 DNA and OsO₄-oxidized polyd(A-T) as exogenous substrates. We also plan to carry out experiments concerning the gap filling step of the excision repair process. The following cells will be used as sources for the preparation of nuclei: human embryonic lungfibroblasts WI-38 >< SV-40 transformed WI-38; mouse embryo 3T3 cells >< SV-40 and polyoma transformed 3T3; ts-3 3T3 (tsPy) at the permissive and non-permissive temperature (ts-3 3T3 is a polyoma transformed cell line which exhibits all the characteristics of a transformed line at the permissive temperature but has normal growth properties at the non-permissive temperature; the temperature sensitivity in ts-3 3T3 has been shown to reside in the virus (Eckhart et al., 1971); ts sptr 3T3 at the permissive and the non-permissive temperature (ts sptr 3T3 is a spontaneously transformed 3T3 line which is temperature sensitive for the expression of the transformation characteristics) obtained from Dr. K. Noonan of our Department).

B. The γ -ray repair capability of nuclei from normal human skin-fibroblasts and from Xeroderma pigmentosum skin-fibroblasts.

There are a number of hereditary diseases in humans which are characterized by an increased frequency for the development of leukemia and systemic cancer (e.g. Fanconi's Anemia, Bloom's Syndrome, Louis-Bar Syndrome, Xeroderma pigmentosum). The possibility has to be considered that a deficiency in DNA repair may form the molecular basis for some of these diseases.