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

Peter A. Cerutti

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September 1, 1974 - August 31, 1975

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

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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 OsO_4 -oxidized synthetic polyd(A-T)-thymine-methyl[^3H] are being used in our studies. (Note: OsO_4 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. OsO_4 -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. OsO_4 -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 OsO_4 -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 OsO_4 -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 "athymic 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 lig_4 -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 OsO_4 -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 OsO_4 -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 OsO_4 -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.

The notion that unrepaired DNA damage may ultimately lead to malignant transformation has recently obtained strong support by the work of Hart and Setlow (1973). These authors were able to demonstrate a direct correlation between neoplastic transformation and the level of pyrimidine photodimers in DNA for the fish *Poecelia formosa*. Advantage was taken of the presence of photoreactivating enzyme in fish cells which allowed the selective reversion of photodimers. Another result relating DNA damage to malignant transformation has been obtained by Ikenaga, et al., (1974). A decrease in the transformation frequency of 4-nitroquinoline-1-oxide treated mouse cells with increasing liquid holding time following treatment with the carcinogen was observed. Since 4-nitroquinoline-1-oxide damages the purine bases in DNA in situ, the decrease in transformation frequency by liquid holding was attributed to DNA repair. There are, of course, many examples for chemically or radiation induced carcinogenesis which have been related to the induction of an oncogenic virus. In the cases mentioned above, such an interpretation appears less obvious since reversion of DNA damage was accompanied by a decrease in transformation frequency.

Only for the case of Xeroderma pigmentosum (XP) has a DNA repair deficiency actually been demonstrated. A depression or complete absence of unscheduled DNA synthesis was found in XP-cells following exposure to UV-light but not to ionizing radiation. Mostly on the basis of this observation, it was concluded that the endonucleolytic incision step in prereplication repair of UV-photodimers was deficient in XP-cells (Cleaver, 1969). While it is still generally accepted that some early step in UV-excision repair is defective in XP, considerable doubt has recently arisen whether a defective or missing UV-endonuclease indeed represents the molecular basis for this disease. It has become questionable whether UV-photodimers are the crucial DNA lesions in XP (Bacchetti, et al., 1972). Complementation studies indicate that the disease cannot be due simply to a single gene mutation (see e.g. Keijzer and Bootsma, 1973); for an up-to-date appraisal of the present status of Xeroderma research, it is referred to the review by Cleaver (1974).

A systematic study of γ -ray repair in XP-cells will help to define the molecular basis for this interesting disease and shed some light on the relationship between unrepaired DNA damage and malignant transformation. While the repair of radiation-induced single strand breakage and unscheduled synthesis were normal in XP-skin fibroblasts, no information is available concerning their capability to excise γ -ray damaged nucleic acid residues. We are planning to study the excision of products of the 5,6-dihydroxy-dihydrothymine type from γ -irradiated PM-2 DNA and OsO₄-oxidized polyd(A-T) by isolated nuclei from XP-skin fibroblasts. (We have obtained six XP-lines from ATCC and Dr. G. Todaro (SV-40 transformed XP) and we have not encountered any serious problems in culturing these cells.)

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VI. Supporting Data

A. Personnel

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

1. Peter A. Cerutti

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

EDUCATION:

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

HONORS:

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

RESEARCH AND/OR PROFESSIONAL EXPERIENCE:

1971 - Professor and Chairman, Department of Biochemistry, 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

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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).

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

M. R. Mattern, P. V. Hariharan, D. E. Dunlap and P. A. Cerutti. DNA Degradation and Excision Repair in Gamma-Irradiated Chinese Hamster Ovary Cells. Nature New Biol. 245, 230 (1973).

P. A. Cerutti. Effects of Ionizing Radiation on Mammalian Cells. Naturwissenschaften 61, 51 (1974).

J. L. Roti Roti, G. S. Stein and P. A. Cerutti. Reactivity of Thymine to Gamma-ray in HeLa Chromatin and Nucleoprotein Preparation. Biochemistry 13, 1900 (1974).

J. L. Swinehart, W. S. Lin and P. A. Cerutti. Gamma-ray Induced Damage in Thymine in Mononucleotide Mixtures and in Single- and Double-Stranded DNA. Rad. Res. 58, 166 (1974).

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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.

J. L. Roti Roti and P. A. Cerutti. Gamma-ray Induced Thymine Damage in Mammalian Cells. Int. J. Radiat. Biol., in press.

P. V. Hariharan and P. A. Cerutti, Excision of Damaged Thymine Residues from Gamm-Irradiated Polyd(A-T) by Crude E. Coli Extracts. Proc. Natl. Acad. Sci., in press.

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2. Michael R. Mattern

NAME: Michael Ross Mattern
 TITLE: Postdoctoral Associate
 BIRTHDATE/BIRTHPLACE: [REDACTED] Palmerton, Penn.
 PRESENT NATIONALITY: U.S.A.
 SEX: Male
 SOCIAL SECURITY NUMBER: [REDACTED]

EDUCATION:

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

RESEARCH AND/OR PROFESSIONAL EXPERIENCE:

Thesis work on "Repair of Chromosomal Damage in Mammalian Cells" in Dr. P. Cerutti's laboratory, Department of Biochemistry, University of Florida (as Princeton student).

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

Dr. M. Mattern will devote 100% of his effort to the study of the repair of γ -ray induced thymine damage in mammalian cells.

SELECTED PERSONAL PUBLICATIONS IN AREAS RELATED TO PRESENT PROPOSAL:

J. F. Remsen, M. Mattern, N. Miller and P. Cerutti - Photohydration of uridine in the RNA of Coliphage R17. Lethality of uridine photohydrates and nonlethality of cyclobutane-type photodimers, *Biochemistry* 10, 524 (1971).

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

M. Mattern, P. Hariharan, D. E. Dunlap and P. A. Cerutti, DNA degradation and excision repair in gamma-irradiated Chinese hamster ovary cells. *Nature New Biol.* 245, 230 (1973).

* Expected in August, 1974

3. Brian E. Dunlap

NAME: Brian Eric Dunlap
 TITLE: Postdoctoral Associate
 BIRTHDATE/BIRTHPLACE: [REDACTED] Orange, New Jersey
 PRESENT NATIONALITY: USA
 SEX: Male
 SOCIAL SECURITY NUMBER: [REDACTED]

EDUCATION:

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

RESEARCH AND/OR PROFESSIONAL EXPERIENCE:

- 1971 - Postdoctoral Associate with Dr. P. A. Cerutti, Department of Biochemistry, University of Florida, Gainesville, Florida.
- 1955 - 1956 Research Assistant with Dr. Mary Shorb, University of Maryland, College Park, Maryland

SELECTED PERSONAL PUBLICATIONS IN AREAS RELATED TO PRESENT PROPOSAL:

- M. R. Mattern, P. V. Hariharan, B. E. Dunlap and P. A. Cerutti, 1974. DNA Degradation and Excision Repair in γ -Irradiated Chinese Hamster Ovary Cells. Nature New Biology 245, 230.
- B. E. Dunlap, and P. A. Cerutti, 1974. Spontaneous Release of Thymine, Damaged Thymine and Oligonucleotides After γ -Irradiation of PM-2 DNA. Manuscript in preparation.

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

Dr. B. Dunlap will have major responsibility for our work on the formation of apyrimidinic sites in γ -irradiated DNA and he will devote 100% of his effort to this project.

VII. Budget

Budget for the period September 1, 1974 to August 31, 1975

1.	<u>Salaries</u>		20	- 0 -
	<i>Peter A. Cerotti, M.D., P.H.D.</i>			
	Name	Title	% of Time	
	B. Dunlap, Ph.D.	Postdoctoral Associate	100	\$ 10,000 (No F.B.)
	M. Mattern, Ph.D.	Postdoctoral Associate	100	9,000 (No F.B.)
				<hr/>
			SUBTOTAL	\$ 19,000
2.	<u>Permanent Equipment</u>			
	Swinging bucket rotor Ti 60 (4.4 ml, 45 rpm)			
	(for Beckman L2-65B)			\$ 2,795
3.	<u>Supplies</u>			
	Chemicals			\$ 1,500
	Radioisotopes			\$ 2,500 1,965
	Biochemicals			\$ 2,000
	Tissue-culture media			\$ 3,000
	Glass and plastic ware			\$ 3,000 2,350
				<hr/>
			SUBTOTAL	\$ 12,000 11,465 10,865
4.	<u>Other Expenses</u>			
	Instrument Service Contracts			\$ 1,500
	Publication Costs			\$ 1,000
				<hr/>
			SUBTOTAL	\$ 2,500
5.	<u>Travel (domestic)</u>			\$ 1,500
				<hr/>
			TOTAL DIRECT COSTS	\$ 37,795 37,240 36,610
6.	<u>Indirect Costs</u> (49.42% of salaries, wages and fringe benefits)			\$ 8,740 9,390
				<hr/>
			TOTAL	\$ 46,535 46,000

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Per unit inc changes made per telcon with Dr. Cerotti.