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UNIVERSITY OF FLORIDA
Gainesville, Florida 32611

APPROVAL OF RESEARCH / TRAINING PROPOSAL

707817

June 11, 1975
DATE

Title of Proposal: Formation and Repair of γ-ray Induced Nucleic Acid Base Damage in Bacteria and Mammalian Cells (deleted version)

Submitted to: (sponsor) U. S. Atomic Energy Commission

University Unit Responsible for Research or Training and Indirect Cost:

Department of Biochemistry

College of Medicine

Approval by Dean or Director:

NAME: J. McLean
TITLE: Assistant Dean for Administration

Principal Investigator:

NAME: Peter A. Cerutti, M.D., Ph.D.
TITLE: Professor and Chairman of Biochemistry
TELEPHONE: 904-392-3361

Approval by Dean or Director: (if more than one)

NAME: C. A. Stetson, M.D.
TITLE: Dean, College of Medicine

Co-Principal Investigator:

NAME:
TITLE:
TELEPHONE:

Approval by Vice Pres. Health Affairs (if applicable)

NAME:
TITLE:

Department Head:

NAME: Peter A. Cerutti, M.D., Ph.D.
TITLE: Professor and Chairman of Biochemistry

Approval by Asso. V.P. Academic Affairs (if applicable)

NAME:
TITLE:

Department Head: (if more than one involved)

NAME:
TITLE:

Official Authorized to Sign for the University:

Thomas D. Fontaine, Acting Director
DIVISION OF SPONSORED RESEARCH
UNIVERSITY OF FLORIDA
904 · 392 · 1291

Instructions to the Sponsor for addressing correspondence:

Dr. Peter A. Cerutti, Department of Biochemistry
Box 724 J. Hillis Miller Health Center, University of Florida
Gainesville, Florida 32610

P. I. Check one:
- NEW ☐
- RENEWAL ☒
- CONTINUATION ☐

JUN 16 1

4295

PAGE 1 AGENCY
PAGE 2 DSR
PAGE 3 PRINCIPAL INVESTIGATOR
PAGE 4 DEAN OR DIRECTOR

SEE REVERSE SIDE

1035008

SEP 17 1975

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

Dear Dr. Cerutti:

I wish to advise you that the Research Committee has approved renewal of your Research Contract No. E(40-1)4155, "Formation and Repair of Gamma-Ray Induced Nucleic Acid Base Damage in Bacteria and Mammalian Cells," for an additional year. You will be contacted by someone from the Oak Ridge Operations Office in the near future regarding negotiation of the renewal contract.

Your next renewal period comes after the completion of six years of operation of this contract. For every multiple of three years of operation the Division of Biomedical and Environmental Research requires a comprehensive report of the overall activities of the contract during the past three years. In particular, the following items should be included in this report:

1. The main research accomplishments, with special reference to the originally stated objectives.

2. Plans for the continuation of present objectives and possible new objectives in consideration of past results.

3. Graduate students trained, degrees granted, and post-doctoral tenures completed.

4. Bibliography, with titles of publications associated with this contract.

5. Your opinion as to the present state of knowledge in this area of research, its significance in the fields of biology and medicine and needed future investigations.

1035009

APPENDIX "A"

CONTRACTOR: UNIVERSITY OF FLORIDA
CONTRACT NO.: E-(40-1)-4155

For the contract period: September 1, 1975 through August 31, 1976

ARTICLE A-I. RESEARCH TO BE PERFORMED BY CONTRACTOR

The Contractor will conduct studies on the photobiology and 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: $21,680

(2) Equipment to be purchased or fabricated by the Contractor: $ 2,904

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

Liquid Nitrogen Refrigerator with accessories

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

Double-door CO_2 -incubator

1035010

UNITED STATES
ENERGY RESEARCH AND DEVELOPMENT ADMINISTRATION

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

AREA CODE 615
TELEPHONE 483 86

June 19, 1975

J. L. Liverman, Director, Division of Biomedical and Environmental Research, HQ

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

We are submitting for your review and appropriate action the following information concerning the contract which will expire on 8/31/75.

1. Renewal Proposal (4)
2. Progress Report (1)
3. Financial Statement (4) included in Renewal Propc
4. Form SI-SIE-78a (200-Word Summary) (3)

We shall appreciate your advising us of your decision so that we may proceed with the necessary contract action at the earliest possible date.

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

ACR: LM

Enclosures:
As stated above

bcc: D. S. Zachry, w/Prog. Rpt. (2) & Form 427

RCP&R BR
MEDLEY:ejb
6/19/75

4478

Fdi

1035011

THE J. HILLIS MILLER HEALTH CENTER
UNIVERSITY OF FLORIDA

Department of Biochemistry

Phone: 904-392-3361
Gainesville, 32610

June 12, 1975

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

Dear Dr. Medley,

Enclosed is the renewal for the period of September 1, 1975 to August 31, 1976 of contract AT-(40-1)-4155 entitled "Formation and Repair of γ-ray Induced Nucleic Acid Base Damage in Bacteria and Mammalian Cells". Your consideration in granting a time extension to me was appreciated. Thank you very much.

Sincerely yours,

Peter A. Cerutti
Principal Investigator

PAC:mb

JUN 1 6 1975

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1035012

"EQUAL EMPLOYMENT OPPORTUNITY AFFIRMATIVE ACTION EMPLOYER"

SI-SIE-78a

PUBLICATION BY AEC AUTHORIZED ☐

NOTICE OF RESEARCH PROJECT
SCIENCE INFORMATION EXCHANGE
SMITHSONIAN INSTITUTION

U.S. ATOMIC ENERGY COMMISSION

SIE NO.

AEC CONTRACT NO.

SUPPORTING DIV. OR OFFICE:

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

Peter A. Cerutti, M.D., Ph.D.
Department of Biochemistry
University of Florida
Gainesville, Florida 32610

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; Professor and Chairman; Department of Biochemistry; 20%.

Kunio Shinohara; Postdoctoral Associate; Department of Biochemistry; 100%.

Maria Bravo; Research Assistant; Department of Biochemistry; 100%.

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

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.

Excision repair of DNA base damage induced by ultraviolet light, gamma rays, and chemical carcinogens will be investigated in rodent and human cells.

The repair capacity for the excision of gamma-ray damaged thymine will be compared for virally transformed mouse fibroblasts which are temperature sensitive for the maintenance of the transformed state. Attempts will be made to characterize, on a molecular level, the ultraviolet repair deficiency in the human hereditary disease, Xeroderma pigmentosum. Excision repair of DNA base damage induced by the chemical carcinogens, N-ethyl-N-nitroso-urea and N-acetoxy-2-acetylaminofluorene, will be investigated in normal and diseased human skin fibroblasts.

RESULTS TO DATE:

Formation and repair of γ-ray induced DNA base damage has been demonstrated in bacterial and mammalian cells.

	PROGRAM CATEGORY NO.
BUDGET	
PRIMARY	
SECONDARY	

Signature of Principal Investigator

DATE: June 10, 1975

INVESTIGATOR – DO NOT USE THIS SPACE

1035013

SUBMIT ORIGINAL & ONE COPY TO DSR WITH PROPOSAL

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UNIVERSITY OF FLORIDA
Gainesville, Florida 32611

APPROVAL OF RESEARCH / TRAINING PROPOSAL

June 11, 1975
DATE

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

Submitted to: (sponsor) U. S. Atomic Energy Commission

University Unit Responsible for Research or Training and Indirect Cost:

Department of Biochemistry

College of Medicine

Principal Investigator:

NAME: Peter A. Cerutti, M.D., Ph.D.
TITLE: Professor and Chairman of Biochemistry
TELEPHONE: 904-392-3361

Co-Principal Investigator:

NAME:
TITLE:
TELEPHONE:

Department Head:

NAME: Peter A. Cerutti, M.D., Ph.D.
TITLE: Professor and Chairman of Biochemistry

Department Head: (if more than one involved)

NAME:
TITLE:

Approval by Dean or Director:

NAME: J. McLean
TITLE: Assistant Dean for Administration

Approval by Dean or Director: (if more than one)

NAME: C. A. Stetson, M.D.
TITLE: Dean, College of Medicine

Approval by Vice Pres. Health Affairs (if applicable)

NAME:
TITLE:

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Gainesville, Florida 32610

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CONTINUATION	☐

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1035014

Renewal for the Period of September 1, 1975

to August 31, 1976 of Contract AT-(40-1)-4155 of the

United States Energy Research and Development Administration

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

Peter A. Cerutti
Department of Biochemistry
University of Florida
Gainesville, Florida 32611

1035015

OUTLINE

I. Introduction.

II. Comparison of Repair Capacity of Normal and Virally Transformed Mammalian Cells.

III. The Molecular Basis for the Ultraviolet Repair Deficiency in *Xeroderma pigmentosum*.

A. Development of an "Open System" for Studies of Photodimer Repair in *Xeroderma pigmentosum*.

B. Attempts to Reconstitute Excision Repair Capacity of XP Preparations and Preparations from Other Repair-Deficient Cells.

IV. Excision Repair of DNA Damage Induced by Chemical Carcinogens in Human Cells.

A. Development of an "Open System" for the Study of the Excision of DNA Damage Induced by Chemical Carcinogens.

B. Repair of N-ethyl-N-nitroso-urea and N-acetoxy-2-acetylamino-fluorene Induced DNA Damage by Preparations from *Xeroderma pigmentosum* (XP) and Fanconi's Anemia (FA) Skin Fibroblasts.

C. Excision - Competition Studies.

V. References.

VI. Supporting Data.

A. Personnel.

B. Publications of Work Supported by E.R.D.A. (see "Progress Report").

C. Support Received from Other Federal Agencies.

VII. Budget and Budget Justification.

VIII. Financial Statement for the Present Contract Period.

1035016

I. Introduction.

Exposure of cells to certain chemical and physical agents can lead to impairment of their biological functions and ultimately to cell death. While detrimental effects can result from damage to various cellular components, chromosomal DNA represents a particularly sensitive target due to its high molecular weight, its chemical make-up, and due to the fact that each cell contains only one or at the most a few copies of each chromosome. DNA damage can be lethal, mutagenic, or carcinogenic and may lead to cellular aging. Important physical agents causing DNA damage are ultraviolet light, ionizing radiation, and heat; important chemicals are alkylating and arylalkylating agents, polycyclic aromatic systems with a tendency to intercalate between the DNA bases and bifunctional reagents leading to intra- or interstrand crosslinking.

In our program we are addressing ourselves to the following fundamental questions concerning the chemical and biological effects of DNA damaging agents in mammalian and particularly in human cells: (1) What are the factors which determine why certain DNA damaging agents are potent mutagens but weak carcinogens and vice versa? (2) What are the reasons for the high or low killing efficiencies of different agents? (3) What determines whether certain lesions are removed efficiently from the DNA by repair processes while others remain in the DNA, some apparently without adverse biological effects? (4) How many different excision repair "pathways" does a human cell possess? We are approaching these questions by studies of the formation and excision repair of DNA damage induced by ionizing radiation, ultraviolet light and selected chemical carcinogens in normal human cells and cells from patients with the hereditary diseases Xeroderma pigmentosum and Franconi's Anemia.

1035017

II. Comparison of Repair Capacity of Normal and Virally Transformed Mammalian Cells.

We are planning to continue our comparative studies of the repair capacity of "normal and transformed" mammalian cells (for an account of the present state of this work, see section III B of the "Progress Report"). In particular, virally transformed cell lines are being used which are temperature sensitive for the phenotypic expression of the transformed growth state. This allows the comparison of the two growth states of cells which possess identical chromosomal configurations. A mutant simian virus 40 transformed mouse fibroblast 3T3 line (SV 3T3 ts H6-15) will be studied which expresses the transformed phenotype at 32°, but not at 39° (see Renger and Basilico, 1972). These phenotypic changes are fully reversible. In contrast to the temperature-sensitive polyoma transformed 3T3 (PY 3T3 ts-3) which we have studied during the last six months, the SV 3T3 ts H6-15 line appears to owe its behavior to a cellular rather than a viral alteration. The virus rescued from these transformed cells seems to be completely normal with respect to both growth and transforming ability.

We will study the capacity of whole cell sonicates and nuclear sonicates prepared from SV 3T3 ts H6-15 cells grown either at 32° or at 39° to excise gamma-ray products of the 5,6-dihydroxy-dihydrothymine type (t′) from an exogenous DNA substrate. The sonicates in these experiments will be prepared as described by Mattern and Cerutti (1975) and Cerutti and Remsen (1975). As damaged substrates γ-irradiated bacteriophage λ DNA and thymidine-methyl[^{3}H] labeled sonicated HeLa nuclei will be used. The following comments justify the use of the latter substrate. Dr. Hariharan, in our laboratory, has recently discovered that heavily γ-irradiated HeLa nuclei are unable to remove t′-type products from their DNA. Excision of t′, however, was observed upon addition of unirradiated whole cell sonicates or nuclear sonicates. This observation indicates that t′ lesions are removed not only from naked DNA, but also from chromatin.

The capacity for excision of photodimers is much lower in rodent cells relative to human cells (see e.g. in Cleaver, 1974). Similarly, we have found that the *in vitro* capacity of whole cell sonicates and nuclear sonicates from 3T3 is only approximately half that of analogous preparations from human cells. It should be interesting to extend our comparative studies of the repair capacities of the transformed and normal growth state to a human system. Virally transformed human cells which are temperature sensitive for the expression of the transformed state are available from Dr. J. Butel (Baylor University), and extension of the program is planned depending on the availability of time and resources.

It is clear that the open system used in our experiments is uniquely suited for biochemical analysis in case differences in repair capacity are discovered between the normal and transformed growth states.

III. The Molecular Basis for the Ultraviolet Repair Deficiency in *Xeroderma pigmentosum*.

The present status of *Xeroderma pigmentosum* (XP) research has recently been summarized in excellent reviews and the important implications of this

disease to human carcinogenesis have been discussed (Cleaver, 1974; Robbins et al., 1974). It suffices to say that cells from most XP patients are unable to perform excision repair of DNA containing UV-induced pyrimidine dimers at a normal rate. The repair deficiency manifests itself by defects in dimer removal, host cell reactivation, and repair replication (as reflected in equilibrium density gradient centrifugation, bromodeoxyuridine photolysis, and unscheduled synthesis). Resealing of X-ray induced single strand breaks, X-ray induced unscheduled synthesis, and the removal of products of the 5,6-dihydroxy-dihydrothymine type from exogenous DNA (Hariharan and Cerutti, 1975) are normal in XP. Complementation experiments, using cell fusion, indicate that the disease cannot be due simply to a single gene mutation. Five complementation groups have been defined. Cleaver (1969) had originally speculated that a deficient UV-endonuclease was responsible for XP, but considerable doubt has recently arisen concerning this conclusion. The lesions responsible for the DNA repair deficiency in XP have not been identified in molecular terms. Excision of photodimers from bacteriophage T_7 DNA by crude extracts of human lymphocytes WIL-II (Duncan et al., 1975), normal human skin fibroblasts, and XP skin fibroblasts of complementation group A has been demonstrated (E. Friedberg, personal communication). Surprisingly, no difference could be detected between the excision capacity of the preparations derived from XP (group A) and normal cells. Similar results were obtained by Duker and Teebor (1975) who used crude extracts of XP cells of four different complementation groups. These authors measured the capacity of the extracts to incise UV-irradiated superhelical pseudomonas phage PM2 DNA (cf. Brent, 1972). Their results are in qualitative agreement with those of Bacchetti et al. (1972).

In our program, we plan to compare the photodimer excision capacity of nuclear sonicates and whole cell sonicates of normal skin fibroblasts and XP skin fibroblasts of complementation groups A, B, and C. The possibility has to be considered that an excision deficiency can be demonstrated in nuclear preparations, but not in whole cell preparations. It should be mentioned in this context that the deficiency for the excision of γ-ray damaged thymine from exogenous DNA, in Fanconi's Anemia, is more pronounced in nuclear sonicates than in whole cell sonicates. In order to avoid duplication of work, we will remain in communication with Dr. E. Friedberg about progress made in this part of our program.

A. Development of an "Open System" for Studies of Photodimer Repair in *Xeroderma pigmentosum*.

We are planning to study the excision of cyclobutane-type photodimers from ultraviolet-irradiated bacteriophage λ-DNA by whole cell sonicates and nuclear sonicates prepared from normal and XP skin fibroblasts. The experimental design will be similar to that presently being used for the study of the repair of γ-ray induced thymine damage in XP cells, Fanconi's Anemia cells, normal skin fibroblasts, human diploid lung fibroblasts, and HeLa cells (Mattern and Cerutti, 1975; Cerutti and Remsen, 1975; "Progress Report"). Bacteriophage λ DNA, labeled with thymine-methyl[^{3}H], will be irradiated with monochromatic light of 280 nm and the dimer content measured according to Goldman and Friedberg (1973). The irradiated DNA will be incubated with 5×10^6 nuclear or cellular equivalents in the presence of ATP, an ATP-generating system, bovine serum albumin, dithiothreitol,

and the four deoxynucleosidetriphosphates. The amount of photodimers remaining in the acid-precipitable fraction of the bacteriophage DNA and the total amount of thymine radioactivity rendered acid soluble will be measured as a function of the length of incubation. The photodimer excision capacity of normal skin fibroblasts and WI-38 cells, and later of XP cells, will be investigated. We are planning to first concentrate on the XP lines CRL 1223 (<2% excision), CRL 1199 (3-7% excision), and CRL 1166 (15-25% excision) from the American Type Culture Collection corresponding to the complementation groups A, B, and C. These cell lines are presently cultured in our laboratory. (Note: excision of γ-ray products of the 5,6-dihydroxy-dihydrothymine type, but not of photodimers, by whole cell and nuclear sonicates from human tissue has been demonstrated in our laboratory. Duncan et al. (1975) have shown excision of photodimers from UV-irradiated T_7 DNA which had been pretreated with T_4 UV-endonuclease by crude extracts of human lymphocyte cells WIL-II. UV-irradiated whole cell extracts of these cells also removed part of the photodimers from their own DNA.)

B. Attempts to Reconstitute Excision Repair Capacity of XP Preparations and Preparations from Other Repair-Deficient Cells.

This part of our program will be carried out in collaboration with Dr. L. Grossman, Department of Biochemical and Biophysical Sciences, Johns Hopkins University, Baltimore, Maryland.

A prerequisite for the proposed experiments is the detection of deficiencies for the excision of photodimers from exogenous, UV-irradiated DNA by nuclear preparation (or whole cell sonicates) from XP cells. At a later state in this program, reconstitution experiments will also be attempted with preparations from other repair-deficient cells. The experimental design outlined in the preceding paragraph will be used.

1. Mixing experiments: Attempts will be made to reestablish the XP complementation groups which were defined by somatic cell hybridization. Nuclear preparations (or whole cell sonicates) prepared from XP cells of the different complementation groups will be mixed and assayed for their photodimer excision capacity.

2. Reconstitution of excision capacity of XP preparations by addition of repair enzymes: The major advantages of the "open system" for the study of DNA repair of mammalian cells developed in our laboratory are: (a) chemically and physico-chemically well-defined exogenous, damaged DNA substrates can be used; and (b) cellular fractions or highly purified bacterial or mammalian repair enzymes with well-defined biochemical characteristics can be added in attempts to resupply a deficient excision repair function. If successful, this approach will allow it to pinpoint and characterize the deficient steps in cells obtained from patients with repair diseases, such as XP, Fanconi's Anemia, and, possibly also, Hutchinson-Gilford Progeria and senescent diploid human skin fibroblasts WI-38 (Mattern and Cerutti, 1975).

The purified repair enzymes (correndo- and correxonucleases) will be prepared by Dr. Grossman's laboratory, who is a leader in this field. There is little doubt that much can be gained by this collaboration. It

would be difficult, time consuming, and expensive for our laboratory to attempt the purification of relatively large amounts of these enzymes and to independently reestablish optimal conditions for their use. Dr. Grossman has agreed to supply the following enzymes for this purpose: Micrococcus luteus correndonuclease II (peak I and II), correndonuclease III (specific for alkylated and γ-irradiated DNA), the apurinic site enzyme, the correctional exonucleases from M. luteus, E. coli and placenta, and the M. luteus DNA polymerases.

IV. Excision Repair of DNA Damage Induced by Chemical Carcinogens.

Up to now, all attempts to transform cultured human cells by chemicals have failed (cells from a patient with the hereditary disease, Fanconi's Anemia, may represent a first exception; C. Heidelberger, personal communication. It has been speculated, on the other hand, that between 60-80% of human cancer may be directly or indirectly induced by environmental chemical and physical carcinogens.). In contrast, chemical and physical transformation of rodent cells has been demonstrated in many laboratories and is very actively being investigated. The possibility has to be considered that differences in the mode of repair of DNA damage between rodent and human cells may, in part, be responsible for their different transformability by chemical carcinogens.

Impressive evidence for a direct relationship between repair of DNA damage and carcinogenesis has recently been obtained. Good examples are the studies of the hereditary disease, Xeroderma pigmentosum (see previous sections, and reviews by Cleaver, 1974, and Robbins et al., 1974), the studies of ultraviolet-induced tumor formation in the fish poecelia formosa by Hart and Setlow (1973), the work of Roberts et al. (1974), and Ikenaga et al. (1975). The first part of our working hypothesis, within the framework of the somatic mutation theory of cancer, is: cells with efficient excision repair pathways which operate with high accuracy (e.g., human cells) are less susceptible to transformation by chemical and physical DNA damaging agents than cells with inefficient excision repair which process DNA damage mostly by the less accurate postreplication-recombination pathways. It is interesting to note, in support of this hypothesis, that Fanconi's Anemia cells which can be chemically transformed, according to Heidelberger's preliminary results, are deficient in excision repair of γ-ray induced thymine damage of the 5,6-dihydroxy-dihydrothymine type (Cerutti and Remsen, 1975). The second part of our working hypothesis is: DNA lesions which are excised inefficiently in human cells and exhibit low killing efficiency may be particularly active in transformation either by direct miscoding or by induction of error-prone repair.

The list of chemical and physical carcinogens is very long, and it appears more likely that classes of lesions with common structural features are repaired by common repair pathways than that specific repair enzymes exist for each individual lesion or for a specific DNA damaging agent. Structurally related lesions are also expected to have similar biological effects regardless of the agent responsible for their formation. Rather than the structural features of the damaged bases, the effect of the lesions on the local conformation of the DNA helix may largely determine their biological effects. The extent and type of the helix distortion are

expected to differ for different types of lesions. Comparable conformational changes will result from structurally related base modifications, regardless of the agent responsible for their formation. We have recently proposed (Cerutti, 1975) to distinguish three classes of DNA base damage on this basis:

CLASS I: Monofunctional lesions causing negligible helix distortion (products with essentially unaltered base pairing and base stacking capacity).

CLASS II: Monofunctional lesions causing minor helix distortion (products with only slightly altered base pairing and base stacking properties, such as ring-saturation, ring-contraction, ring-fragmentation, and ring-elimination products).

CLASS III: Lesions causing major helix distortion (monofunctional lesions with bulky substituents or substantially altered base pairing properties; intercalation damage; intra- and interstrand crosslinks; crosslinks between DNA and other cellular components).

An important goal in this part of our program will be to distinguish the repair pathways which accomplish the removal of different types of DNA lesions in human cells. Initially, we are planning to limit our studies to two carcinogens, N-ethyl-N-nitroso-urea (ENUA) and N-acetoxy-2-acetyl-aminofluorene (N-acetoxy-AAF), which produce DNA damage of all three major classes distinguished above. Both carcinogens are widely used in chemical carcinogenesis. They represent ultimate carcinogens which do not need to be metabolically activated.

ENUA ethylates the purine bases by the SN_1 and SN_2 substitution mechanisms, the major products in DNA being N_7-ethyl-guanine, N_3-ethyl-adenine, O_6-ethyl-guanine, and apurinic sites. For ENUA, the ratio of O_6-ethyl-guanine over N_7-ethyl-guanine is 0.7 which is higher than for any of the other simple alkylating agents (Lawley, 1974; Lawley, 1975; Lawley and Warren, 1975). There is considerable evidence that the mutagenic and carcinogenic potential of alkylating agents in rodent cells is related to the extent of O_6-alkyl-guanine formation (Loveless, 1969; Lawley, 1974; Frei and Joshi, 1974; Maitra and Frei, 1975). O_6-methyl-guanine has also been shown to miscode in an *in vitro* RNA polymerase system (Gerchman and Ludlum, 1973). It will, therefore, be particularly interesting to study the capacity of human cells to excise O_6-ethyl-guanine. The ethylation lesions introduced by ENUA may partially produce radiomimetic and partially ultraviolet-mimetic biological effects.

The pro-carcinogen 2-acetylaminofluorene (AAF) and its metabolic activation has been studied extensively, in particular by E. and J. Miller (see e.g. Miller and Miller, 1974). N-acetoxy-AAF represents a synthetic derivative which produces the same type of lesions in DNA without activation as AAF *in vivo* (Miller *et al.*, 1966; Krick *et al.*, 1967); that is, predominantly the "C_8-arylalkylation" product of guanine. Introduction of the bulky AAF residue into DNA is expected to cause major distortion of the DNA helix (Class III A according to our classification scheme; Cerutti, 1975; see e.g. Fujimura *et al.*, 1972; Weinstein and Grunberger, 1974).

C_8-AAF-guanine is expected to produce ultraviolet-mimetic biological effects. It has been shown that N-acetoxy-AAF induces ultraviolet-type, "long" type repair in normal diploid human skin fibroblasts (Regan and Setlow, 1974). A deficiency in the induction of "long" type repair and unscheduled DNA synthesis by N-acetoxy-AAF was observed in Xeroderma pigmentosum cells (Regan and Setlow, 1974; Stich et al., 1973).

The following general research plan will be followed:

A. Development of an "Open System" for the Study of the Excision of DNA Damage Induced by Chemical Carcinogens.

Whole cell sonicates and nuclear preparations from cultured human cells will be used (human carcinoma HeLa S-3; diploid human lung fibroblasts WI-38; normal human skin fibroblasts). Chemically- and physicochemically-defined exogenous DNA substrates which were treated with radioactively labeled ENUA or N-acetoxy-AAF will be prepared. (Chromatographic assays for the analysis of the damaged DNA are available from the literature, see e.g. Kritikar and Goldthwait, 1974; the cellular preparations and the incubation conditions will be developed using our studies on the excision repair of γ-ray induced thymine as a starting point; see previous sections, and Cerutti and Remsen, 1975). Excision kinetics and excision capacity are the major parameters to be determined.

B. Repair of ENUA and N-acetoxy-AAF Induced DNA Damage by Preparations from Xeroderma pigmentosum (XP) and Fanconi's Anemia (FA) Skin Fibroblasts.

The study of the excision capacity of XP and FA cells for the various lesions produced by ENUA and N-acetoxy-AAF which fall into all three classes of base damage according to our classification (see section IV; Cerutti, 1975) may help to distinguish different excision repair pathways in human cells. While XP cells are deficient in photodimer excision (Class III type damage), a deficiency for the excision of γ-ray induced thymine damage (Class II type damage) was detected in FA preparations (Cerutti and Remsen, 1975).

A first indication that a deficiency in the repair of chromosomal damage may exist in Fanconi's Anemia comes from the detection of an increased efficiency of formation of chromosomal aberrations by ionizing radiation (Higurashi and Conen, 1971) and DNA cross-linking agents (Sasaki and Tonomura, 1973) in FA lymphocytes and skin fibroblasts. Enhanced sensitivity to cell killing was observed for mitomycin C, but not for γ-rays and ethylmethane-sulfonate (Finkelberg et al., 1974). A deficiency in the excision of photodimers was recently described for the FA skin fibroblast line CCl 122 from ATCC (Poon et al., 1974); an independent laboratory, however, has not been able to reproduce these results (J. Regan, personal communication). The preliminary results on chemical transformation of cells obtained by C. Heidelberger (see above) make it particularly interesting to study carcinogen excision repair in FA. Reconstitution experiments analogous to those outlined above for Xeroderma pigmentosum will be carried out in the case that deficiencies for the excision of specific chemical lesions are observed. Partially purified fractions from normal skin

fibroblasts and highly purified bacterial and mammalian repair enzymes will be used in collaboration with Dr. L. Grossman whose laboratory will supply the purified repair enzymes. It is clear that this extensive program cannot be completed within the next budget period, but it is hoped that a good start can be made.

C. Excision - Competition Studies.

Characterization and distinction of excision repair pathways in human cells will be attempted by excision-competition studies. The effect of one type of DNA damage on the excision of a second type of damage, by human cell preparations, will be investigated. For example, the effect of the addition of unlabeled UV-irradiated bacteriophage λ-DNA on the excision of products of the 5,6-dihydroxy-dihydrothymine type (t′) from labeled, γ-irradiated λ-DNA and vice versa will be investigated. Suppression of the removal of t′ is expected if the same pathways are used for the excision of ultraviolet products and t′. The same approach will be used for the comparison of different chemical carcinogens and chemical carcinogens with radiation (ionizing radiation, ultraviolet). It is a prerequisite for the interpretation of results obtained in such studies that the excision kinetics and the repair capacity for each individual type of lesion which is being compared is known (see above, IV B), and a detailed research plan cannot be given at the present time.

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V. References.

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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]
PRESENT NATIONALITY:	USA
SEX:	Male
SOCIAL SECURITY NUMBER:	[redacted]

EDUCATION:

Institution	Degree	Year	Specialty
[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 and H. Schmid - Photoreaktionen von Methanol mit N-Heterocyclen (1. Mitteilung) Helv. Chim. Acta 45, 1992 (1962).

P. Cerutti and H. Schmid - Photoreaktionen von Methanol mit N-Heterocyclem (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 and 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).

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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).

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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 radiourans. 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 N^4-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. 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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J. L. Roti Roti and P. A. Cerutti. Gamma-Ray Induced Thymine Damage in Mammalian Cells. Int. J. Radiat. Biol. 25, 413 (1974).

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J. L. Swinehart and P. A. Cerutti. Gamma-Ray Induced Thymine Damage in the DNA in Coliphage ϕX174 and in E. coli. Int. J. Radiat. Biol. 27, 83 (1975).

B. Dunlap and P. A. Cerutti. Apyrimidinic Sites in Gamma-Irradiated DNA. FEBS Letters 51, 188 (1975).

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P. A. Cerutti. Excision Repair of DNA Base Damage. Life Sciences 15, 1567 (1974).

M. R. Mattern and P. A. Cerutti. Age-Dependent Excision Repair of Damaged Thymine from γ-Irradiated DNA by Isolated Nuclei from Human Fibroblasts. Nature 254, 450 (1975).

M. R. Mattern, P. V. Hariharan, and P. A. Cerutti. Selective Excision of Gamma-Ray Damaged Thymine from the DNA of Cultured Mammalian Cells. Biochim. Biophys. Acta.; In Press.

P. A. Cerutti, "Deoxycytidine Photohydration in DNA," in Photochemistry and Photobiology of Nucleic Acids (S. Wang and M. Patrick, Eds.) Academic Press, New York; In Press.

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P. A. Cerutti, "Structural Classification of DNA Base Damage," in Molecular Mechanisms for the Repair of DNA (P. C. Hanawalt and R. B. Setlow, Eds.) Plenum Press, New York; In Press.

P. V. Hariharan, J. F. Remsen, and P. A. Cerutti, "Excision Repair of Gamma-Ray Damaged Thymine in Bacterial and Mammalian Cells," in Molecular Mechanisms for the Repair of DNA (P. C. Hanawalt and R. B. Setlow, Eds.) Plenum Press, New York; In Press.

P. A. Cerutti, "Gamma-Ray Excision Repair in Normal and Diseased Human Cells," in Proceedings of the Symposium on the Biology of Radiation Carcinogenesis; In Press.

2. Kunio Shinohara

NAME:	Kunio Shinohara, Ph.D.
TITLE	Postdoctoral Associate
BIRTHDATE:	
PRESENT NATIONALITY:	Japan
SEX:	Male
SOCIAL SECURITY NO.:	-----

EDUCATION:

Institution	Degree	Year	Specialty
Waseda University	B.S.	1966	Engineering
Waseda University	M.S.	1968	Physics
Tokyo University	Ph.D.	1969-1972	Biochemistry
Tokyo University		1972-1975	Radiation Biology

RESEARCH AND/OR PROFESSIONAL EXPERIENCE:

Research Official, Division of Biology, National Institute of Radiological Sciences; Since 1972.

RESPONSIBILITY IN THE PROPOSED PROJECT AND PERCENT TIME DEVOTED TO IT:

Studies on the excision repair of DNA base damage induced by chemical carcinogens in normal and diseased human cells; 100% of time devoted to project.

PUBLICATIONS:

Shinohara, K. and Okada, S. Radiosensitivities of Murine Lymphoma L5178Y Cells in a Multi-Cellular Colony System. J. Radiat. Res. 13, 109 (1972).

Okada, S. and Shinohara, K. An Automatic Synchronizer for Suspension Culture of L5178Y Cells. Cell Tissue Kinet. 7, 195 (1974).

Shinohara, K. and Matsudaira, H. Effects of Methylazoxymethanol Acetate on L5178Y Cells. -Caffeine Sensitization- In preparation.

3. Two graduate students, Mr. Farrel Fort and Mr. George Feldman, will participate in this program.

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B. Publications of Work Supported by E.R.D.A. (See "Progress Report").

C. Support Received from Other Federal Agencies.

Support is obtained from the National Institute of General Medical Sciences (Grant No. 2R01-GM-18617-05) for our studies on the repair of γ-ray induced DNA base damage (thymine and cytosine) in human carcinoma HeLa cells, Fanconi's Anemia, Bloom's Syndrome, and Louis-Bar Syndrome skin fibroblasts. Direct costs for the current year: $48,784.

VII. Budget and Budget Justification.

A. Budget for the Period of September 1, 1975 to August 31, 1976.

1. Salaries

Name	Title/% of Time	
K. Shinohara	Ph.D., Postdoctoral Associate/100%	$ 9,000
M. Bravo	Research Assistant/100%	10,828
a) Fringe Benefits		
M. Bravo 15.5%		1,678
Health Insurance		120
K. Shinohara .6%		54
		$21,680

2. Permanent Equipment

Double-door CO_2-incubator; National Model 3321	$ 2,150
Liquid Nitrogen Refrigerator with accessories; Union Carbide LD-40	754
	2,904

3. Supplies

Chemicals	$ 3,500	
Radioisotopes	3,000	
Biochemicals	2,000	-63
Tissue Culture Media	6,000	
Glass and Plastic Ware	5,000	
	$19,500	$18,40

4. Travel

Domestic	$ 1,000
Foreign	997
	$ 1,997

5. Other Expenses

Instrument Service Contracts	$ 2,000
Publication Costs	1,500
	$ 3,500

6. Indirect Costs

51% of Salaries, Wages, and Fringe Benefits	$11,057
TOTAL	$ 60,638 # 60,000

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2. Renewal Proposal. Include information outlined on pages 8 through 16 of the enclosed "Guide for the Submission of Research Proposals from Educational Institutions," revised September, 1972, unless the information is already contained in earlier proposals or in the accompanying progress report. Careful attention should be given to including the necessary information in the budget for the next period.

3. Financial Statement. Include in the renewal proposal a financial statement for the present contract period. The statement should be based on the estimated total costs of the project including support by both the institution and the ERDA as shown in A-II (a) of Appendix "A" to the Contract covering the current period. The statement should reflect:

(1)	Total actual project cost to date for the current period	$ 30,532.66
(2)	Estimated total cost for remainder of period	$ 15,467.34
(3)	Total actual and estimated cost chargeable to ERDA 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 ERDA (include costs reported in certified statement for preceding period(s) and the costs stated in Item (3) above)	$ 207,002.00
(5)	Accumulated ERDA Support Ceiling as stated in Article III of Contract	$ 207,002.00
(6)	Total estimated ERDA funds remaining under Contract (subtract Item (4) from (5)) which may be used to reduce amount of new funds required from ERDA for proposed renewal period	$ -0-

4. 200-Word Summary. The enclosed form "Notice of Research Project" should be completed in 200 words or less summarizing the proposed research. Four copies of the completed form should be forwarded to us together with the renewal proposal and progress report.