



UNITED STATES
ENERGY RESEARCH AND DEVELOPMENT ADMINISTRATION

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

621
R

AREA CODE 615
TELEPHONE 483 8611

May 18, 1976

707816

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

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

We are submitting for your review and appropriate action the following information concerning the contract which will expire on 8/31/76.

1. Renewal Proposal (4)
2. Progress Report (4)
3. Financial Statement (4)
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

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

RCP&R BR
MEDLEY:ejb
5/18/76

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1034973

REPOSITORY Oak Ridge Operations
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4155 Univ. of Florida
 FOLDER CA 12-28-76

PUBLICATION BY ERDA AUTHORIZED

NOTICE OF RESEARCH PROJECT SCIENCE INFORMATION EXCHANGE SMITHSONIAN INSTITUTION

SIE NO.

ERDA CONTRACT N AT-(40-1)-4155

ENERGY RESEARCH AND DEVELOPMENT ADMINISTRATION

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.)

Department of Biochemistry J. Hillis Miller Health Center University of Florida Gainesville, Florida 32610

TITLE OF PROJECT:

Formation and Repair of Physically and Chemically Induced DNA Damage in Human 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, M.D., Ph.D., Professor and Chairman, Department of Biochemistry, 20% Kunio Shinohara, Ph.D., Postdoctoral Associate, Department of Biochemistry, 100%

NO. OF GRADUATE STUDENTS ON PROJECT: 1 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 documents. Make summaries substantive, giving initially and for each annual revision the following: OBJECTIVE; SCIENTIFIC BACKGROUND FOR STUDY; PROPOSED PROCEDURE; TEST OBJECTS AND AGENTS.

Damage introduced into DNA by various physical (e.g., ultraviolet light and ionizing radiation) or chemical agents, the spontaneous loss of a small number of DNA bases and mismatched base-pairs introduced by errors in repair or replication may impair the biological functions of DNA by similar mechanisms. Most cells have evolved elaborate mechanisms for the repair of DNA damage. Prereplication excision repair is particularly efficient in human cells. If repair does not occur or remains incomplete, the cytopathological effects may range from cell death or induction of mutations to malignant transformation and possibly cell aging. The studies of the repair of DNA damage induced by energy related physical and chemical damaging agents in human cells proposed in this program are, therefore of fundamental importance to basic molecular biology as well as the health sciences

In this program we plan to study excision repair of DNA lesions of the 5,6-dihydroxy-dihydrothymine-type induced by ionizing radiation and ultraviolet light in human cells. The mode and capacity of damage processing will be studied in normal human skin fibroblasts and in fibroblasts from patients with the hereditary diseases Xeroderma pigmentosum and Ataxia telangiectasia, which are characterized by increased cancer incidence. Since DNA repair deficiencies have been (Continued)

RESULTS TO DATE:

Preliminary results need to be confirmed.

1034974

Table with 2 columns: BUDGET (PRIMARY, SECONDARY) and PROGRAM CATEGORY NO.

Signature of Principal Investigator: Peter A. Cerutti per J. F. Rema

DATE: March 12, 1976

SUMMARY OF PROPOSED WORK (Continued)

observed in cells from patients with both diseases, the proposed study may shed light on the relationship between DNA damage, DNA repair and carcinogenesis.

The mode of processing of DNA-adducts formed in metabolizing human cells upon exposure to the energy related, ubiquitous pollutant benz(a)-pyrene will be investigated in intact normal and diseased human skin fibroblasts and in Epstein-Barr virus positive and negative human lymphoblastoid cells. The DNA-benz(a)pyrene adducts formed have been characterized chemically and the excision of individual lesions can be studied. Individual steps in the repair of benz(a)pyrene DNA-adducts will be studied in in vitro experiments using damaged DNA and isolated chromatin as exogenous substrates.

The proposed studies are expected to supply information about the mechanisms of processing of DNA lesions in human cells and the relationship between different types of DNA damage and their biological effects.

UNIVERSITY OF FLORIDA
OFFICE OF ADMINISTRATIVE AFFAIRS
FINANCE AND ACCOUNTING DIVISION
GAINESVILLE, 32611

REPLY TO: CONTRACTS AND GRANTS
106 JOHNSON HALL

TELEPHONE
AREA CODE 904 392-1235

U. S. ATOMIC ENERGY COMMISSION
STATEMENT OF ESTIMATED COSTS

A. H. Frost
U. S. Energy Research and Development Administration
Research Contracts, Procedures and Reports Branch-Contract Division
Post Office Box E
Oak Ridge, Tennessee 37830

Contract Number: E(40-1)-4155 modification #6
UFF# 229*C85

	<u>AT Share</u>
1. a. Actual Project Costs to date for the current period.	<u>\$29,227.33</u>
b. Estimated total costs for remainder of period.	<u>\$30,772.67</u>
c. Total actual and estimated cost chargeable to AEC per article A-III	<u>\$60,000.00</u>
2. Accumulated costs chargeable to AEC	<u>\$267,002.00</u>
3. Accumulated support ceiling as stated in article III of contract.	<u>\$267,002.00</u>
4. Total estimated AEC funds remaining under contract (Item 2 from Item 3).	<u>-0-</u>

1034976

ORO-4155-1

DEADLINE DATE

June 1, 1976

UNIVERSITY OF FLORIDA
Gainesville, Florida 32611

(Date Proposal Must Be Received By Granting Agency)

APPROVAL OF PROPOSAL

RESEARCH
TRAINING
OTHER
(Check One)

SEND NOTICE OF AWARD TO
DIVISION OF SPONSORED RESEARCH
219 Grinter Hall

May 12, 1976
DATE

Title of Proposal Formation & Repair of Physically and Chemically Induced DNA Damage in Human Cell

Submitted To: (Agency) U.S. Energy Research and Development Administration (E.R.D.A.)

(Division) _____

(Program) _____

University Unit Responsible for Research or Training and the Unit to Receive the Appropriate Indirect Cost Return

Dept. of Biochemistry
College of Medicine

Approval by Dean or Director:

W. B. Deel
NAME: J.P. McLean,
TITLE: Assoc. Dean, College of Medicine

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

NAME:
TITLE:

Approval by Vice President Health Affairs
(For All Projects Involving J.H.M.H.C. Personnel)

C. Stetson
NAME: Chandler A. Stetson,
TITLE: Vice President for Health Affairs

Approval by Vice-President Academic Affairs
(For All Projects Emanating From Research Centers)

NAME:
TITLE:

Official Authorized to Sign for the University (Leave Blank)

Patricia B. Rambo
NAME: Patricia B. Rambo, Assistant Director
DIVISION OF SPONSORED RESEARCH
UNIVERSITY OF FLORIDA
904-392-4800

Principal Investigator: (Project Director)

P. A. Cerutti
NAME: P. A. Cerutti, M.D., Ph.D.
TITLE: Professor & Chairman
TELEPHONE: (904) 392-3361

Co-Principal Investigator: (If Applicable)

NAME:
TITLE:
TELEPHONE:

Department Head:

P. A. Cerutti
NAME: P. A. Cerutti, M.D., Ph.D.
TITLE: Professor & Chairman

Department Head: (If more than one involved)

NAME:
TITLE:

SEND ALL CORRESPONDENCE RELATING TO:

Grant Administration: Division of Sponsored Research, 211 Grinter Hall

Fiscal Matters: Gene Stivender, Contracts & Grants, 106 Johnson Hall

Scientific Matters: P. A. Cerutti, M.D., Ph.D., Principal Investigator

P.I. Check one:

NEW

RENEWAL

CONTINUATION

1034977

FORMATION AND REPAIR OF PHYSICALLY AND
CHEMICALLY INDUCED DNA DAMAGE IN HUMAN CELLS

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

September 1, 1976 - August 31, 1979

RENEWAL PROPOSAL FOR CONTRACT No. AT-(40-1)-4155 OF
THE U. S. ENERGY RESEARCH AND DEVELOPMENT ADMINISTRATION

1034978

SUMMARY

Damage introduced into DNA by various physical (e.g., ultraviolet light and ionizing radiation) or chemical agents, the spontaneous loss of a small number of DNA bases and mismatched base-pairs introduced by errors in repair or replication may impair the biological functions of DNA by similar mechanisms. Most cells have evolved elaborate mechanisms for the repair of DNA damage or more generally of defects in the DNA structure. Prereplication excision repair is particularly efficient in human cells. If repair does not occur or remains incomplete, the cytopathological effects may range from cell death, induction of mutations to malignant transformation and possibly cell aging. The studies of the repair of DNA damage induced by energy related physical and chemical damaging agents in human cells proposed in this program are, therefore, of fundamental importance to basic molecular biology as well as the health sciences.

In this program we plan to study excision repair of DNA lesions of the 5,6-dihydroxy-dihydrothymine-type induced by ionizing radiation and ultraviolet light in human cells. The mode and capacity of damage processing will be studied in normal human skin fibroblasts and in fibroblasts from patients with the hereditary diseases Xeroderma pigmentosum and Ataxia telangiectasia which are characterized by increased cancer incidence. Since DNA repair deficiencies have been observed in cells from patients with both diseases, the proposed study may shed light on the relationship between DNA damage, DNA-repair and carcinogenesis.

The mode of processing of DNA-adducts formed in metabolizing human cells upon exposure to the energy related, ubiquitous pollutant benz(a)pyrene will be investigated in intact normal and diseased human skin fibroblasts and in Epstein-Barr virus positive and negative human lymphoblastoid cells. The DNA-benz(a)pyrene adducts formed have been chemically characterized and the excision of individual lesions can be studied. Individual steps in the repair of benz(a)pyrene DNA-adducts will be studied in in vitro experiments using damaged DNA and isolated chromatin as exogenous substrates.

The proposed studies are expected to supply information about the mechanisms of processing of DNA lesions in human cells and the relationship between different types of DNA damage and their biological effects.

OUTLINE

- I. Introduction
- II. Specific Aims
 - A. Excision repair of gamma-ray induced products of the 5,6-dihydroxy-dihydrothymine type (t^{γ}) by normal and Ataxia telangiectasia (AT) skin fibroblasts.
 - B. Formation and repair of products of the 5,6-dihydroxy-dihydrothymine type induced by ultraviolet light in human cells.
 - 1. Formation of products of the 5,6-dihydroxy-dihydrothymine type (t^{UV}) by ultraviolet light.
 - 2. Excision repair of ultraviolet-induced products of the 5,6-dihydroxy-dihydrothymine type (t^{UV}) by normal, Xeroderma pigmentosum (XP) and Ataxia telangiectasia (AT) skin fibroblasts.
 - C. Repair of DNA-benz(a)pyrene adducts by human cells
 - 1. Formation of DNA-benz(a)pyrene adducts in cultured human cells.
 - 2. Repair of DNA-benz(a)pyrene adducts in intact rodent and human cells.
 - 3. In vitro excision of DNA-benz(a)pyrene adducts from free DNA and isolated chromatin by cellular extracts from human cells.
- III. References
- IV. Supporting Data
 - A. Personnel. Biographical sketches and selected personal publications related to present proposal; Personnel contributing to the program which is not supported by E.R.D.A.
 - B. Support received from other federal agencies.
 - C. Publications of work supported by A.E.C.-E.R.D.A., see "Final Report".
 - D. Facilities.
 - E. Budget for three-year period and budget justification.

I. INTRODUCTION

A short introductory discussion is given in order to relate the specific aims of our program to the fundamental questions concerning DNA damage formation, DNA repair and their biological consequences. An account of the state of the field and our contributions to it is given in the "Final Report" for the last three years of our A.E.C.-E.R.D.A. Contract and in some recent review articles by the principal investigator.

Epidemiological evidence indicates that many forms of human disease are induced by toxic agents in our environment, e.g. the most common forms of cancer, such as cancer of the lung and large intestines appear to be due to pollutants in the air or to dietary factors. There are physical and chemical toxic agents. Physical agents include sunlight, ionizing radiation and heat. Chemical damaging agents are found in our natural environment or normal diet or are added to the environment in the form of insecticides, herbicides, drugs, food additives, industrial pollutants, etc. The chromosomal DNA represents a sensitive cellular target to many of these agents. The biological consequences of DNA damage can be cell death, mutation, malignant transformation and possibly cellular aging. The structural identification of the DNA damage produced by the long list of physical and chemical damaging agents, the characterization of the mechanisms of damage processing employed by living cells and the elucidation of the relationship between different types of DNA damage and specific biological effects are of obvious importance to basic molecular biology and the Health Sciences. For several years my laboratory has been addressing itself to these questions.

The heterocyclic bases of DNA in situ in the living cell undergo photochemical reactions upon exposure to ultraviolet light and radical addition reactions leading to ring-saturation or homolytic fragmentation upon exposure to ionizing radiation. They react readily with a large variety of electrophilic chemicals in nucleophilic substitution reactions at the ring- or exocyclic heteroatoms. The pyrimidine bases are far more reactive than the purines to ultraviolet light and slightly more reactive to ionizing radiation. In contrast, the purines react more readily with most electrophilic chemicals. A great variety of structural changes is induced, therefore, by the large number of DNA damaging agents, in particular since most agents produce more than one type of lesion. The following principal alternatives exist for the processing of all DNA base damage in dividing cells (1) Pre-Replication: the damage is removed by excision repair or remains in the DNA (2) Replication: the remaining damage does not interfere with replication, the damage acts as temporary or permanent replication block, the damage is circumvented or removed during replication (3) Post-Replication: incomplete daughter strands are completed, remaining damage may be removed. The relative importance of these alternatives for damage processing is expected to vary for different cell types and different classes of DNA lesions. The biological effect(s) of a given class of lesions will be co-determined by the structural characteristics of the lesions, the quality and extent of the local distortion of the DNA helix and the mode(s) of damage processing used by the cell. It may be speculated that certain lesions have particularly high killing-, mutagenic-, or transformation-efficiency.

A major long range goal in this area of research and in our own work is to develop experimental data which will allow the definition of classes of DNA base damage on the basis of the chemical structure of the lesions and their effect on local DNA (and chromatin) conformation. A useful classification should allow predictions about the mode(s) of damage processing and the predominant biological effect(s) elicited by a specific lesion in a given type of cell. This represents obviously a long range goal which can only be achieved by the cooperative efforts of many laboratories working on different aspects of this problem. Relevant studies extend from the chemical structure elucidation of the lesions induced in DNA (in situ in the cell) by important physical and chemical damaging agents, to studies of the effect of specific lesions on DNA (and chromatin) conformation, to the characterization of the molecular steps of DNA (and chromatin) repair and the enzymes which are involved, to the definition of the cellular and molecular processes leading to specific biological endpoints.

In this framework we are concentrating on studies of the formation and prereplication excision repair of two different types of DNA lesions in mammalian and, in particular, in human cells. The first type of lesions are monomeric, ring-saturated thymine derivatives of the 5,6-dihydroxy-dihydrothymine type which are formed in DNA as major lesions by ionizing radiation and as minor lesion by ultraviolet light. Products of this type are expected to introduce "significant but minor distortion of the DNA helix" according to a preliminary classification of DNA base damage proposed by us as a working hypothesis (Cerutti, 1975). The second type of lesions are the guanine- and adenine- benz(a)pyrene adducts formed in the DNA of metabolizing mammalian cells. The benz(a)pyrene substituent is bulky and its introduction into DNA is expected to cause "major helix distortion". Prereplication excision repair of these two categories of lesions is being investigated in normal human skin fibroblasts and skin fibroblasts from patients with the autosomal recessive diseases Xeroderma pigmentosum and Ataxia telangiectasia. It is hoped that the comparison of the repair capabilities of the normal and diseased cells which have established or suggested deficiencies in DNA repair will yield information about the molecular steps in human DNA repair and allow the construction of experimental systems for the identification and characterization of human repair enzymes. Since the incidence of the formation of malignancies is increased in patients with these diseases, the proposed studies may also shed light on the relationship between DNA-damage, DNA-repair and carcinogenesis.

II. SPECIFIC AIMS

A. Excision repair of gamma-ray induced products of the 5,6-dihydroxy-dihydrothymine type (t^Y) by normal and Ataxia telangiectasia (AT) skin fibroblasts.

Since a number of years we have focused our efforts on studies of the excision repair of ionizing radiation induced DNA base damage in mammalian cells (see "Final Report"). This part of the proposed program represents a continuation of these interests.

Recent results of Taylor et al, (1975; 1976) and Paterson et al (1976) suggest that the human autosomal recessive disease Ataxia telangiectasia (AT) may represent an "ionizing radiation counterpart" to Xeroderma pigmentosum (XP). A short summary of the present state of AT research is given in the "Final Report" (Section B(3)). In particular, there is an indication that the repair of anoxic X-ray damage other than strand breakage may be deficient in some AT-skin fibroblast lines. We are planning to study excision repair of damage of the 5,6-dihydroxy-dihydrothymine type (t^Y) introduced under aerobic and anoxic conditions using the assays and experimental design developed in our laboratory.

Excision of t^Y (aerobic and anoxic) will be studied in intact normal and AT-skin fibroblasts at high gamma-ray doses. Normal and AT-cells will be grown and labeled with thymidine-methyl(3H) in special small roller bottles. Following irradiation, dead cells will be removed by gentle shaking and the cultures will be incubated at 37° in fresh growth medium. The disappearance of t^Y from acid precipitable DNA will be determined by the alkali-acid degradation assay. This protocol is similar to that used previously by Mattern et al (1975). Normal human skin fibroblasts and several AT-lines are presently being cultured in our laboratory.

A series of in vitro t^Y -excision experiments are planned with isolated chromatin as exogenous substrate. These experiments will be analogous to those described for in vitro t^{UV} -excision by AT and XP cell extracts (see below in Section B(2b)).

B. Formation and Repair of products of the 5,6-dihydroxy-dihydrothymine type induced by ultraviolet light in human cells.

Bachetti et al (1972) described an endonuclease activity in homogenates of HeLa S-3 cells and several strains of XP skin fibroblasts which recognizes damage other than pyrimidine photodimers in ultraviolet irradiated DNA. They later purified an endonuclease from calf thymus which is capable of incising photoreactivated, ultraviolet-irradiated DNA and gamma-irradiated DNA. It was estimated that this enzyme recognizes lesions which are formed at an approximately 35 times lower level than pyrimidine photodimers in DNA irradiated at 254nm. An enzyme with similar properties has been isolated by VanLancker and Tomura (1974) from rat liver. Evidence for the presence of

non-photoreactivable lesions in the DNA of uv-irradiated chicken fibroblasts was obtained by Paterson and Lohman (1975). Feldberg and Grossman (Second International Workshop on DNA repair mechanisms, The Netherlands, May 1976) have recently described a DNA binding protein from human cells which recognizes non-dimer damage in uv-irradiated DNA. Thymine-photoproducts other than cyclobutane-type dimers were detected in uv-irradiated DNA by Wacker (1963) and evidence suggesting the formation of 5,6-dihydrothymine in DNA was later presented by Yamane *et al* (1967). A considerable amount of data is available on the photo-oxidation of free thymine to ring-saturated hydroxy-hydroperoxides (Daniels and Grimison, 1967; Hahn and Wang, 1974). The formation of thymine-photohydrate (6-hydroxy-dihydrothymine) was suggested by Miller and Cerutti (1969) and clearly demonstrated by Fisher and Johns (1973). We have recently started a search for ultraviolet products of the 5,6-dihydroxy-dihydrothymine type in free DNA and DNA *in situ* in HeLa S-3 cells using the alkali-acid degradation assay (Hariharan and Cerutti, 1974). Our results indicate that such products are formed and we propose to carry out a systematic study of the photochemistry and photobiology of these lesions.

The following should be recognized: The alkali-acid degradation assay measures a group of structurally similar thymine ring-saturation products rather than a single product in irradiated DNA. It is expected that the product distribution and the relative yields of the different components are different for uv and ionizing radiation. Furthermore, the spectrum of other DNA lesions (i.e. lesions not determined by the alkali-acid degradation assay) is very different for ultraviolet- and ionizing radiation. For instance, pyrimidine-photodimers are the major lesions produced by uv-light in DNA while strand breakage occurs only in very low yield - single strand breakage is a major type of lesion formed by ionizing radiation but pyrimidine-dimerization does not occur. In summary, DNA exposed *in vivo* or *in vitro* to ultraviolet light or ionizing radiation both contain monomeric, ring-saturated thymine products but differ in many other respects from each other. These differences may be important factors in determining the mode of repair and the biological effects of these lesions.

(1) Formation of products of the 5,6-dihydroxy-dihydrothymine type (t^{uv}) by ultraviolet light

- (a) Properties of the ultraviolet-induced thymine lesions determined by the alkali-acid degradation assay.

Experiments are presently being carried out and will be continued to characterize the lesions measured in uv-irradiated DNA by the alkali-acid degradation assay (t^{uv}) and to clearly distinguish them from thymine-photodimers. (1) The formation of t^{uv} from TMP-methyl(3H) is being determined. TMP in dilute aqueous solution does not photodimerize. The assay yield for the formation of t^{uv} relative to total ring-saturation will be measured by comparing the fraction of TMP which has reacted to t^{uv} as a function of dose to total thymine ring-saturation (measured by the loss of the absorption at 260nm). (2) According to Yamane *et al* (1967) 5,6-dihydrothymine is formed in DNA by uv-light. Based on chemical considerations, it is unlikely that dihydrothymine interferes with the alkali-acid degradation assay but this question should be checked experimentally. Dihydrothymidine-methyl(3H) will be prepared by catalytic reduction of thymidine-methyl(3H)

according to Green and Cohen (1957) and treated under our standard assay conditions (3) The action spectrum of the formation of t^{uv} and thymine-photodimers will be compared for free DNA and DNA in-situ in HeLa S-3 cells (see sections b & c below) (4) Two step irradiation experiments with 280 and 240nm light are planned in order to compare the photostability of t^{uv} relative to thymine-photodimers (5) The chemical stability of t^{uv} in DNA to changes in pH and temperature will be measured and will allow a clear distinction of t^{uv} from thymine-photohydrates. (6) It is likely that radical addition-photooxidation reactions are responsible for the formation of t^{uv} . A study of the effect of radical scavengers (such as histidine) and mild reducing agents (such as cystein) is planned and should yield information concerning the chemical mechanism of t^{uv} formation (cf. Roti-Roti and Cerutti, 1974).

(b) Formation of t^{uv} in DNA in vitro

The efficiency of formation of t^{uv} and thymine-photodimers in highly purified thymine-methyl(3H) labeled coliphage T7 DNA will be determined. Light in the 240, 260, 280 and 313nm region of the uv-spectrum from our Schoffel-High-Intensity UV-Monochromator will be used. This data will yield action spectra and ratios of t^{uv} over photodimers in this range of the uv-spectrum and is of obvious importance to photobiology.

(c) Formation of t^{uv} in situ in HeLa S-3 cells

An analogous series of experiments to those described in the previous section will be carried out with HeLa S-3 cells which were labeled in their DNA with thymidine-methyl(3H) and chased with cold thymidine to deplete the radioactive pool. Action-spectra for t^{uv} and photodimer formation will be obtained and ratios of t^{uv} over photodimers calculated.

(2) Excision repair of ultraviolet-induced products of the 5,6-dihydroxy-dihydrothymine type (t^{uv}) by normal, Xeroderma pigmentosum (XP) and Ataxia telangiectasia (AT) skin fibroblasts

For a description of the state of XP and AT research in relation to our program, it is referred to Section C(3) (p. 11) of the "Final Report". In short, with the exception of XP-variants all XP-skin fibroblasts lines possess decreased levels of uv-induced unscheduled DNA synthesis and as far as such data is available decreased capacities for photodimer removal. Nuclear fractions from normal and XP cells (complementation groups A, B, C, and D) have comparable capacities for the in vitro excision of products of the 5,6-dihydroxy-dihydrothymine type from gamma-irradiated exogenous DNA and according to preliminary results from uv-irradiated DNA (Cerutti and Remsen, in press, and Hariharan and Cerutti, unpublished results). Several strains of AT skin fibroblasts exhibit increased sensitivities to X-rays (aerobic). Some AT-strains appear partially deficient in the removal of X-ray induced (anoxic) M. luteus gamma-endonuclease sensitive sites as well as in their capability to carry

out repair replication (Paterson *et al*, 1976). We are planning to study the excision repair of t^{uv} (and t^Y , see below) in intact XP- and AT-skin-fibroblasts and in in vitro component systems..

(a) High dose experiments with intact cells

Monolayer cultures of normal, XP- and AT-skin fibroblasts (from American Type Culture Collection; all lines are presently being cultured in our laboratory) will be grown in large Petri dishes and prelabeled in their DNA with thymidine-methyl(3H). The cultures will be irradiated with a high dose of light at 254nm (Hg low pressure lamp) at $0^{\circ}C$. Dead cells will be removed by gentle shaking and the cultures will be incubated at 37° in fresh growth medium for different lengths of time. The disappearance of t^{uv} from acid precipitable DNA will be determined by the alkali-acid degradation assay. This protocol is similar to that used previously by Mattern *et al* (1975).

(b) Excision of t^{uv} from isolated normal, XP- and AT-Chromatin by cellular extracts

The construction of "open systems" allowing simple mixing and reconstitution experiments is crucial for the elucidation of the molecular deficiencies in these diseases. This may be rather more difficult than it appeared initially. It was disappointing when Friedberg, Cleaver, Duker and Teebor and their collaborators discovered that crude preparations of XP-cells had normal capacities to incise uv-irradiated exogenous phage DNA and to excise photodimers from free DNA. More encouraging results were obtained in our own laboratory in similar experiments with Fanconi's Anemia preparations (Remsen and Cerutti, in press).

We are planning experiments using isolated chromatin as exogenous substrate. Normal-, XP- and AT- cells will be labeled in their DNA with thymidine-methyl(3H) and the chromatin isolated by a modification of the procedure used by RotiRoti *et al* (1974). The chromatin will be irradiated with a high dose of monochromatic light and unirradiated, unlabeled cell extracts will be added in large excess. Removal of t^{uv} from the exogenous chromatin will be determined (incubation conditions are similar to those described by Mattern and Cerutti, 1975). Preliminary experiments indicate that the "spontaneous" release of t^{uv} from chromatin is small for skin-fibroblast preparations but substantial ($\approx 15\%$) for HeLa S-3 chromatin. This experimental design allows potentially interesting permutations relative to the origine of the chromatin-substrate and the cell extracts: XP-cell extracts with chromatin of normal cells; XP-cell extracts with XP-chromatin (from the same and different complementation groups) etc.

C. Repair of DNA-benz(a)pyrene adducts by human cells

There are numerous DNA damaging agents which introduce sterically bulky substituents. Aromatic amines such as 2-aminonaphthalene, benzidine and 4-amino-biphenyl and polycyclic hydrocarbons contained in tars, oils, soots and cigarette smoke such as benz(a)pyrene, benz(a)anthracene, dibenz-(a,h)anthracene and their methylated derivatives are potent carcinogens. All these chemicals have to be metabolically activated to the reactive, ultimate carcinogen. There are many examples where the formation of DNA as well as RNA and protein adducts can be demonstrated in animals and cultured cells exposed to these agents. Reactive derivatives of a number of these compounds have been synthesized which do not need to be metabolized in order to react with the cellular macromolecules in vitro and in vivo. Examples for such agents are 7-bromomethylbenz(a)anthracene, N-acetoxy-2-acetyl-amino-fluorene, benz(a)pyrene-4,5-oxide and most recently 7,8-dihydroxy-9,10-epoxy-tetrahydrobenz(a)pyrene. Reaction of these chemicals with DNA leads to lesions involving the purine bases which are expected to cause major distortion of the helix (Class III type damage "Causing major helix distortion" according to our classification; Cerutti, 1975). It is expected that the mode by which cells process such lesions together with the chemical properties of the damaged residues co-determine the biological effects of these damaging agents. Since chemicals of the type listed above are produced industrially or represent ubiquitous pollutants of our environment and since their detrimental influence on human health are well established, the study of their effects on the cellular and molecular level is of practical importance. (For recent reviews, see e.g. Heidelberger (1973; 1975; Kriek (1974); Sims and Grover (1974); Trosko and Chu (1975).

Important studies on the structure of the DNA lesions and their repair in mammalian cells have been carried out with 7-bromomethylbenz(a)-anthracene (7-BrMeBA). Lieberman and Dipple (1972) demonstrated the excision of N⁶-(benz(a)anthryl-7-methyl)deoxyadenosine and of N²-(benz(a)-anthryl-7-methyl)deoxyguanosine from the DNA of non-dividing human lymphocytes. The excision of the adenosine lesion was considerably faster than of the guanosine lesion. Similar studies have more recently been carried out by the same authors with rapidly dividing hamster V79 cells and human HeLa S-3 cells (A. Dipple and J. Roberts, personal communication). In lymphocytes from Xeroderma pigmentosum patients 7-BrMeBA induced only approximately one-tenth the level of unscheduled DNA synthesis relative to lymphocytes from normal controls suggesting the participation of ultraviolet-type repair in the processing of at least some of the damage produced by this carcinogen (Slor, 1973). An endonuclease was isolated from rat liver which recognizes lesions in ultraviolet and 7-BrMeBA treated DNA (Maher et al, 1974). This enzyme may participate in the excision of 7-BrMeBA lesions in vivo. Kriek (1974) and Kriek and Westra (personal comm.) have identified a repairable major lesion, N-(guanine-8-yl)-2-acetylaminofluorene and a persistent minor lesion, 3-(guanine-N-yl)-2-acetylaminofluorene in the liver of rats which had received 2-acetylaminofluorene or its activated

derivatives. Evidence for the formation of persistent damage in rat liver DNA was obtained earlier by Warwick and Roberts (1967) following the administration of the carcinogen butter yellow (dimethylaminoazobenzene). Excision of purine adducts formed by 4-nitroquinoline-N-oxide occurred in normal human amnion cells (60% excised in 24 hrs) but not XP cells. Mouse A31-714 cells (a 3T3 clone) also had the capability to remove 4NQO lesions. (Ikenaga et al, 1975; three stable 4-NQO adducts: 2 on G, 1 on A, some unstable products releasing 4-NQO; exact structure of adducts is unknown(?)). An interesting relationship between the proliferative state of human peripheral lymphocytes and their capacity to excise DNA damage induced by N-acetoxy-2-acetylaminofluorene was recently observed by Strauss (TNO 2nd Int. Workshop on DNA Repair, Holland, 1976). The possibility that some chemical carcinogens may, besides altering the DNA, act as inhibitors of repair has to be considered (such a situation may exist for benz(a)pyrene, R. Rasmussen, personal comm., and 7-BrMeBA damage, J. Roberts and A. Dipple, TNO Meeting on DNA repair, Holland, 1976).

Indirect conclusions concerning the repair of DNA lesions induced by the groups of chemicals under discussion comes from the determination of unscheduled DNA synthesis and related phenomena in cultured cells (for human cells, see e.g., Regan and Setlow, 1974; Stich et al, 1973; Strauss et al, 1975). Further valuable information is being derived from comparative studies of the toxicity and mutagenicity of these chemicals in rodent cells (e.g. Duncan and Brookes, 1973), in normal and Xeroderma pigmentosum skin fibroblasts (Maher et al, 1975a,b; Maher and Wessel, 1975; Maher and McCormick, personal comm.) and in cells from other human diseases with repair deficiencies.

We propose to study the mode(s) of damage processing used by human cells for DNA lesions induced by benz(a)pyrene. As mentioned above polycyclic hydrocarbons are ubiquitous contaminants of our environment (*). They are carcinogenic in animals and probably in man. Most of these chemicals have to be metabolically activated in order to exert their biological effects. Exciting progress has been made during the last few years in the identification of the biologically most active and important metabolites of benz(a)pyrene (see e.g. Borgen et al, 1973; Rasmussen and Wang, 1974). As first proposed by Sims et al (1974) and Sims and Grover (1974) and now clearly established by the work of Brookes, Gelboin, Jerina and Sachs and their collaborators, the most strongly mutagenic metabolites of benz(a)pyrene are the two isomeric 7,8-diol-9,10-epoxide-derivatives ((±)7 α ,8 β -dihydroxy-9 α ,10 α -epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene: diol-epoxide II \equiv fast isomer \equiv syn-isomer; (±) 7 α ,8 β -dihydroxy-9 β ,10 β -epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene: diol-epoxide I \equiv slow isomer \equiv anti-isomer; Huberman et al, 1976; Wislocki et al, 1976; syn-anti nomenclature is used by P. Brookes and collaborators).

- (*) Committee on biological effects of atmospheric pollutants (1972) "Particular Polycyclic Organic Matter" (National Academy of Sciences, Washington, D. C.)

While the fast isomer of the diol-epoxide is more mutagenic and more cytotoxic in *Salmonella typhimurium* than the slow isomer (Wislocki et al, 1976 and D. Jerina, personal comm.) the opposite result is obtained with Chinese hamster V79 cells (mutation to ouabain or 8-azaguanine resistance; Huberman et al, 1976; Wislocki et al, 1976; P. Brookes, personal communication). According to these results, the slow isomer of the diol-epoxide is an extremely potent mutagen in V79 at essentially non-toxic doses and has been referred to as a "supermutagen". The work of P. Brookes, P. Sims and B. Weinstein and their collaborators strongly suggests that the major "in vivo" DNA adduct formed by benz(a)pyrene by metabolizing cells (such as mouse embryo cells, baby hamster kidney cells, primary human lung cells) involves guanine (see e.g. Osborne et al, 1975; Brookes et al, 1975; King et al, 1975; Thompson et al, 1976; Alexandrov et al, 1976; Pietropaolo and Weinstein, 1975; Sims et al, 1975; P. Brookes, personal communication). Substitution at the O⁶-position of guanine has been excluded and all available evidence indicates that the benz(a)pyrene moiety is linked to the exocyclic N²-aminofunction of guanine. A minor product involving adenine may be formed at approximately 1/12 the rate of the guanine adduct (P. Brookes, personal comm.). In our program we plan to elucidate the mode(s) of damage processing used by human cells for these "in vivo" purine benz(a)pyrene adducts.

The principal investigator recently spent six weeks at the Chemical Carcinogenesis Division, Chester Beatty Cancer Hospital in England and had the opportunity to learn techniques and to participate in many discussions related to the topic of this part of the proposal with Drs. Peter Brookes, Phil Lawley and John Roberts. His stay at the Chester Beatty was in part supported by E.R.D.A.. The specific program outlined below is not meant to be comprehensive but should give the reviewers sufficient information about the type of experiments that are planned. In this fast moving field, it will undoubtedly become necessary to modify some of our protocols and to add and to delete certain experiments.

(1) Formation of DNA-benz(a)pyrene adducts in cultured human cells.

Metabolism of polycyclic hydrocarbons has been observed in human tissues (see e.g. Kellerman et al, 1973; Grover et al, 1973; Bast et al, 1974; Booth et al, 1974). The formation of a specific "in vivo" benz(a)pyrene adduct in intact, metabolizing cells has been demonstrated by LH Sephadex chromatography of DNA digests from mouse embryo cells and baby hamster kidney (BHK) cells and distinguished from in vitro products in DNA reacted with benz(a)pyrene-4,5-oxide (Baird et al, 1975) or with benz(a)pyrene activated by rat liver microsomes (King et al 1975).

In a first part of our program we will attempt to demonstrate benz(a)pyrene metabolism and the formation of benz(a)pyrene-DNA adducts in cultured human skin-fibroblasts and lymphoblastoid cells (an informal poll of investigators in this field concerning the occurrence or absence of benz(a)pyrene metabolism in cultured human skin fibroblasts yielded inconclusive results! Preliminary experiments look promising). In case no or insufficient metabolism is observed in human cells, cocultivation with BHK will be attempted or feeder layers may be used. (A similar approach has recently been taken by L. Sachs and his collaborators for the demonstration of polycyclic hydrocarbon mutagenicity in V79 hamster cells.)

The methodology will be similar to that of King *et al*, 1975 and has successfully been used in experiments with BHK-cells in our laboratory. The skin fibroblast lines are from ATCC and have been cultured by us for a number of years, while Epstein Barr Virus (EBV) positive and negative lymphoma lines were recently obtained from Dr. G. Klein (Stockholm; see Steinitz and Klein, 1975). It will be interesting to see whether the presence of EBV in part of the lymphoma lines (EHRA-Ramos, EHRB-Ramos) affects benz(a)pyrene metabolism and DNA-adduct formation.

(2) Repair of DNA-benz(a)pyrene adducts in intact rodent and human cells.

(a) Excision of DNA-benz(a)pyrene adducts.

The fate of DNA benz(a)pyrene adducts during post-treatment growth will be investigated in baby hamster kidney cells (BHK-clone 21), normal and Xeroderma pigmentosum (XP) human skin fibroblasts and EBV positive and negative human lymphoblastoid cells. The following experimental design will be used. Cells will be labeled with 2-¹⁴C-thymidine before and during incubation with (³H)-benz(a)pyrene (low, non-toxic benz(a)pyrene doses will be used). The cells will be washed and growth continued up to two additional generations (growth will be monitored using appropriate cell counting procedures). Following post-treatment incubation high molecular weight DNA will be extracted by a modified Kirby procedure (see e.g. Baird *et al*, 1975). A change in the ³H/¹⁴C ratio of the DNA as a function of incubation time will give a first indication of the fate of DNA-benz(a)pyrene adducts. The DNA samples will then be digested enzymatically and the nucleoside mixtures chromatographed on LH-Sephadex (see e.g. Baird *et al*, 1975). The ratio of the (³H)-content in the benz(a)pyrene-adduct peak over total C¹⁴ as a function of the length of post-treatment incubation will yield unambiguous information concerning the removal or persistence of these products (P.S. recovery of radioactivity from the LH-Sephadex columns is very good according to our experience with BHK). Differences between normal and XP-skin fibroblasts, if observed, may allow conclusions about repair mechanisms which are operative in the excision of benz(a)pyrene adducts and differences between EBV positive and negative lymphoblastoid lines about the effect of the presence of the EBV-genome on repair.

The following should be kept in mind in interpreting the results of these experiments. Prolonged treatment of cultures with (³H)benz(a)pyrene is necessary to obtain measurable amounts of DNA-adduct. It is conceivable that a steady state is reached between damage formation and removal, that transitory damage is formed which is rapidly removed and escapes detection and that only irreparable damage accumulates in the DNA. In order to distinguish some of these possibilities, experiments are planned at a later stage in which active benz(a)pyrene metabolites, i.e. the (³H)-labeled 7,8-dihydroxy-9,10-epoxides, will be directly reacted with the cultured cells. (Thanks to the courtesy of Dr. Don Jerina small quantities of the radioactive metabolites are available in our laboratory; see below.)

- (b) Incision of DNA in human cells treated with the 7,8-diol-9,10-epoxy-metabolites of benz(a)pyrene.

A very sensitive filter elution procedure which allows the detection of DNA strand breakage and avoids the problems of alkaline sucrose gradient sedimentation of high molecular weight DNA has been developed by Kohn *et al* (1974) and successfully used in XP-research (Fornace *et al*, 1976). We are proposing to study repair of benz(a)pyrene damage in normal and diseased human cells (Xeroderma pigmentosum, Fanconi's Anemia, Ataxia telangiectasia) using this procedure. Prelabeled ((³H)-thymidine) cells will be treated with the non-radioactive slow and fast 7,8-diol-9,10-epoxy-metabolites of benz(a)pyrene (from Dr. D. Jerina). Since metabolism is unnecessary DNA damage is expected to be formed rapidly and it should be possible to follow the kinetics of DNA fragmentation and resealing during post-treatment growth of the cultures.

- (3) In vitro excision of DNA-benz(a)pyrene adducts from free DNA and isolated chromatin by cellular extracts from human cells.

The capacity of preparations from normal human skin fibroblasts and lymphoblastoid cells to remove purine-benz(a)pyrene adducts from exogenous DNA and isolated chromatin will be measured. Analogous experiments have been described above in section IIA and IIB(2b) for our studies of excision repair of monomeric, ring-saturated thymine damage induced by gamma-rays and ultraviolet light. It is referred to those earlier sections of the proposal for a discussion of the experimental design and some of the advantages and the drawbacks of these "open systems."

The following exogenous substrates will be used (1) ¹⁴C-thymidine labeled coliphage T7-DNA treated with the slow or fast, (³H)-labeled isomer of the 7,8-diol-9,10-epoxy-metabolite of benz(a)pyrene (2) ¹⁴C-thymidine labeled chromatin isolated from skin-fibroblasts treated with the slow or fast, (³H)-labeled, benz(a)pyrene metabolite. The disappearance

of the adducts from high molecular weight DNA will be investigated. It is evident that a multi-step process is measured which may include endonucleolytic incision (cf. Maher et al, 1974), cleavage of the N-glycosidic bond in the adduct by a glycosidase (cf. Kritikar et al, 1975; Lindahl, 1976) followed by chain scission by an apurinic-site enzyme (cf. Verly et al, 1973) and exonucleolytic degradation (cf. Doniger and Grossman, 1975). Attempts will also be made to study the steps leading to DNA fragmentation using the filter election method developed by Fornace et al (1976: see previous section).

Preliminary observations from our studies on the repair of benz(a)pyrene adducts in BHK-cells indicate that acid precipitation of the DNA following incubation of the substrate with the cell extracts may not yield satisfactory results. Therefore, we are in the process of developing a simple column chromatography step for the isolation of high molecular weight DNA from the reaction mixtures. In the experiments with isolated chromatin the DNA will be extracted by a simplified Kirby procedure since it is expected that chromosomal proteins will become labeled by reaction with the benz(a)pyrene metabolites.

If the experiments discussed above are successful, it will be of obvious interest to attempt the purification of enzymes activities involved in the removal of the benz(a)pyrene adducts from DNA and to use cell preparations from Xeroderma pigmentosum, Fanconi's Anemia and Ataxia telangiectasia cells.

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IV. Supporting Data

A. Personnel

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

1. Peter A. Cerutti

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

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

PROFESSIONAL ACTIVITIES:

Since 1957 Member of the "Swiss Medical Association:
Since 1960 Member of the "Swiss Chemical Society"
Since 1966 Member of the "American Chemical Society:
Since 1968 Member of the "American Society of Biological Chemists"
Since 1969 Vice President "American-Swiss Foundation for
Scientific Exchange Inc."
Since 1972 Member of the American Society for Photobiology
Since 1972 Member of the New York Academy of Sciences
Since 1973 Member of the Biophysical Society
Since 1975 Consultant for the National Cancer Institute
Since 1976 Associate Editor, "Radiation Research", Official Organ
of the Radiation Research Society, Academic Press

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.

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

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Contributions to Books and Monographs

P. Cerutti - The Selective Modification of Uridine in Ribonucleic Acid; Methods in Enzymology (Sidney P. Colowick and N. O. Kaplan, eds.), Vol. XII, Nucleic Acids, Part B. (L. Grossman and K. Moldave, eds.) p. 461; Academic Press, 1968.

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M. G. Pleiss and P. Cerutti - Photochemical Transformation of 4-Thiouridine in Escherichia coli Transfer Ribonucleic Acid; Methods in Enzymology, Part C (L. Grossman and K. Moldave, eds.), p. 144; Academic Press, 1971.

P. Cerutti - Repairable Damage in DNA: Overview in "Molecular Mechanisms for the Repair of DNA" Part A, p. e (eds. P. Hanawalt and R. Setlow) Plenum Press, New York (1975).

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P. Cerutti and J. Remsen - Gamma-Ray Excision Repair in Normal and Diseased Human Cells in "Biology of Radiation Carcinogenesis", p. 93 (eds. T. Yuhas, R. Tennant and J. Regan) Raven Press, N.W. 1976.

P. Cerutti - Photochemie der Nukleinsaureren, Houben-Weyl Band IV/3 Kapitel IX, Organische Photochemie (E. Mullerd, ed), Georg Thieme Verlag, Stuttgart, 1976).

J. Vanderhoek and P. Cerutti - Photochemie der Imine, Hydrazone und Azine, Houben-Weyl Band 4/5b, p. 1104 "Photochemie, Teilband ii" (E. Muller, ed), Georg Thieme Verlag, Stuttgart, 1976.

P. Cerutti - Base Damage in Deoxyribonucleic Acid Induced by Ionizing Radiation, Chapter VI in: "Photochemistry and Photobiology of Nucleic Acids" Vol. II, p. 375 (S. Wang and M. Patrick, eds.) Academic Press, New York, 1976.

P. Cerutti and J. Vanderhoek - Deoxycytidine Photohydration in DNA, Chapter IC in: "Photochemistry and Photobiology of Nucleic Acids" Vol. II, p. 83, (S. Wang and M. Patrick, eds.) Academic Press, New York, 1976.

2. Kunio Shinohara

NAME: Kunio Shinohara, Ph.D.
TITLE: Postdoctoral Associate
BIRTHDATE: [REDACTED]
PRESENT NATIONALITY: Japan
SEX: Male
SOCIAL SECURITY NUMBER: [REDACTED]
EDUCATION: [REDACTED]

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

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-. Chem.-Biol. Int. 12, 101 (1976).

3. Personnel not supported by E.R.D.A. participating in the program.

Dr. Joyce Remsen, Research Assistant Professor, Dept. of Biochemistry.

The collaboration with Dr. Remsen has been very fruitful over many years and adds considerable strength to our program. Dr. Remsen will participate in our work on gamma-ray repair in Ataxia telangiectasia and in our studies on the repair of benz(a)pyrene damage.

Dr. Palghat Hariharan, Research Assistant Professor, Dept. of Biochemistry.

Dr. Hariharan has made many important contributions to our program. The collaboration with him will continue. In particular, he will participate in our studies on the repair of ring-saturated, monomeric thymine damage formed by ultraviolet light in normal and Xeroderma pigmentosum cells.

Mr. George Feldman, graduate student, NCI predoctoral fellow.

B. Support received from other Federal Agencies.

Support is obtained from the National Institute of General Medical Sciences (Grant No. 2R01-GM-18617-06) for our studies on:

(1) The repair of gamma-ray induced DNA base damage (thymine and cytosine) in human carcinoma HeLa, normal human skin fibroblasts and Fanconi's Anemia skin fibroblasts.

(2) Excision repair of alkylation damage induced by the carcinogen ethylnitrosourea in human skin fibroblasts and lymphoblastoid cells.

Direct costs for current year: \$43,838.55

C. Publications of work supported by A.E.C.-E.R.D.A.

See "Final Report".

D. Facilities available

A 2,000 square foot area of laboratory space on the third floor of the Medical Sciences Building (J. Hillis Miller Health Center) has been renovated for my research group. Most of the equipment necessary to carry out this project is already available to us, including three tissue-culture hoods, microscopes, Coulter counter, roller apparatus, CO₂ incubators, ¹³⁷Cs-source, a Schöffel high intensity Ultraviolet Monochromator, centrifuges, etc. A special NCB-hood recommended by the National Cancer Institute for the handling of biohazards is presently being installed in one of my laboratories. Additionally, the Department of Biochemistry possesses all the usual communal facilities such as cold rooms, constant temperature rooms, autoclaves, etc. Office space, secretarial services, the departmental library, and the Health Center Library are available.

E. BUDGET AND BUDGET JUSTIFICATION

Budget for the Period of September 1, 1976 to August 31, 1977.

1. Salaries

Name	Title / % of Time	Amount
K. Shinohara	Ph.D., Post-doctoral Associate (100%)	\$12,172.60
W. Dusek	Lab. Technologist II (100%)	8,668.54
	Fringe Benefits (Ms. Dusek) 15.5% + Health Ins.	1,505.62
	Fringe Benefits (Dr. Shinohara) .6% W.C.	<u>73.04</u>
	Total Salaries, Wages, & Fringe Benefits	\$22,419.80

2. Permanent Equipment

International, refrigerated centrifuge IEC 2294; \$3695		\$3,695.00
Rotors: IEC 269 (with accessories)		237.00
IEC 259 (with accessories)		552.00
Beckman Liquid Scintillation Counter with 300 sample changer; simultaneous counting on 3 channels; quench correction Total price, \$18,500; contribution requested from E.R.D.A., \$6,500.00.		\$6,500.00

3. Supplies

Chemicals	\$4,500.00
Radioisotopes	3,000.00
Biochemicals	2,000.00
Tissue Culture Media	6,000.00
Glass and Plastic Ware	4,000.00

4. Travel

Domestic	\$2,000.00
Foreign	0

5. Other Expenses

Instrument Service Contracts	\$2,500.00
Publication Costs	1,500.00

6. Indirect Costs (51% of Salaries, Wages and Fringe Benefits)	\$11,434.10
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TOTAL: \$70,337.90

BUDGET AND BUDGET JUSTIFICATION

Budget for the Period of September 1, 1977 to August 31, 1978

1. Salaries

Name	Title / % of Time	Amount
To be Appointed	Ph.D., Post-doctoral Associate (100%)	\$10,500.00
W. Dusek	Laboratory Technologist II (100%)	9,275.34
	Fringe Benefits (Ms. Dusek) 15.5% + Health Ins.	1,599.68
	Fringe Benefits (Post-doc. Assoc.) .6% W.C.	<u>63.00</u>
	Total Salaries, Wages, & Fringe Benefits	\$21,438.02

2. Permanent Equipment

Unspecified \$ 2,000.00

3. Supplies

Chemicals	\$ 4,500.00
Radioisotopes	3,000.00
Biochemicals	2,000.00
Tissue Culture Media	6,000.00
Glass and Plastic Ware	4,000.00

4. Travel

Domestic	\$ 2,000.00
Foreign	1,500.00

5. Other Expenses

Instrument Service Contracts	\$ 2,500.00
Publication Costs	1,500.00

6. Indirect Costs (51% of Salaries, Wages, and Fringe Benefits)

\$10,933.39

TOTAL: \$61,371.41

BUDGET AND BUDGET JUSTIFICATION

Budget for the Period of September 1, 1978 to August 31, 1979

1. Salaries

Name	Title / % of Time	Amount
To Be Appointed	Ph.D., Post-doctoral Associate (100%)	\$11,550.00
W. Dusek	Laboratory Technologist II (100%)	9,924.61
	Fringe Benefits (Ms. Dusek) 15.5% + Health Ins.	1,700.32
	Fringe Benefits (Post-doc. Assoc.) .6% W.C.	<u>69.30</u>
	Total Salaries, Wages, & Fringe Benefits	\$23,244.23

2. Permanent Equipment 0

3. Supplies

Chemicals	\$ 4,500.00
Radioisotopes	3,000.00
Biochemicals	2,000.00
Tissue Culture Media	6,000.00
Glass and Plastic Ware	4,000.00

4. Travel

Domestic	\$ 2,000.00
Foreign	0

5. Other Expenses

Instrument Service Contracts	\$ 2,500.00
Publication Costs	1,500.00

6. Indirect Costs (51% of Salaries, Wages and Fringe Benefits) \$11,854.56

TOTAL: \$60,598.79

BUDGET JUSTIFICATION

Request for a Liquid Scintillation Counter: The major bottleneck in instrumentation in our research group is liquid scintillation counting. Mostly the investigators have to wait in line until they can count their samples and this situation diminishes our productivity significantly. Approximately 10 samples per experimental point have to be counted in our standard assay for gamma-ray products of the 5,6-dihydroxy-dihydrothymine type and approximately 150 samples have to be counted for each LH-Sephadex chromatogram used for the analysis of DNA-benz(a)pyrene adducts. Our needs for counting time are therefore very substantial. At present, we have one 200 sample counter directly assigned to our group, and we are competing with other research groups for access to the three departmental counters. The purchase of an additional instrument would, therefore, enhance our productivity substantially. A contribution of \$12,000 towards the purchase of such an instrument has been made by the National Institutes of General Medical Science.

Request for a refrigerated, low speed International Type IEC 2294 centrifuge with large capacity swinging bucket rotors:

Such an instrument is needed for the isolation of subcellular fractions such as isolated nuclei and chromatin. The instrument which is presently being used is an International Centrifuge type PR2 of venerable age which obviously is on its last leg. Repair and refurbishing of this instrument would be rather costly and probably not worthwhile. From the proposal, it should be evident that this is a crucial piece of equipment without which the project cannot be completed.

Supply funds

Our tissue culture operation is at least "medium scale". Relatively large numbers of cells are needed in most of our experiments (usually 5×10^6 cells per experimental point). Expensive radioisotopes have to be used in all our experiments.