II. SPECIFIC AIMS

A. FORMATION AND REPAIR OF γ-RAY AND NEAR-ULTRAVIOLET INDUCED DNA DAMAGE IN HUMAN CELLS

(1) The involvement of superoxide radicals in radiation induced DNA damage in mammalian cells

707849

(a) Ionizing radiation:

There is much evidence that OH-radicals are important intermediates in the induction of DNA damage by ionizing radiation (Johansen and Howard-Flanders, 1965; Roots and Okada, 1972; RotiRoti and Cerutti, 1974; Remsen and RotiRoti, 1977). OH-radicals are primary products of the radiolysis of water but the possiblity has to be considered that they are also formed in secondary reactions via the intermediacy of superoxide radicals ($O_2^{\cdot-}$). It is postulated that $O_2^{\cdot-}$ is formed by the reaction of solvated electrons and hydrogen atoms with molecular oxygen. The reaction of $O_2^{\cdot-}$ with hydrogen peroxide then leads to the formation of OH-radicals ($O_2^{\cdot-} + H_2O_2 \rightarrow OH + OH^- + O_2$) (Gregory et al, 1974). The involvement of $O_2^{\cdot-}$ in the oxygen enhancement of killing of E. coli by X-rays (Misra and Fridovich, 1976). and the cytotoxic effects of γ-rays in various mammalian systems is indicated by the fact that superoxide dismutase exerts a protective effect. Superoxide dismutase dismutates $O_2^{\cdot-}$ to hydrogen-peroxide and molecular oxygen ($2O_2^{\cdot-} + 2H^+ \rightarrow H_2O_2 + O_2$) Fridovich, 1975). The following central questions arise (1) Does nuclear DNA represent an important target for radiation induced $O_2^{\cdot-}$? (2) Is intra- and/or extracellularly formed $O_2^{\cdot-}$ involved in DNA damage formation?

We are attempting to answer the first question by measuring the efficiency of the formation of thymine damage by aerobic γ-rays in human carcinoma HeLa S-3 cells in the presence and absence of exogenous superoxide dismutase and/or catalase. The following experimental design will be used. HeLa cells will be prelabeled in their DNA with thymine-methyl [^{3}H] and the effect of the addition of these enzymes on the formation of tritiated water (by abstraction of tritium from the thymine-methyl substituent by OH-radicals) and of products of the 5,6-dihydroxy-dihydrothymine type will be investigated. The methodology developed in our laboratory for the determination of radiation induced thymine damage will be used. (see e.g. Swinehart et al, 1974)

The question whether intracellular levels of $O_2^{\cdot-}$ affect the efficiency of DNA damage formation by aerobic ionizing radiation will be investigated in human skin fibroblasts. As mentioned above, it is postulated that DNA damage is produced by OH-radicals which are formed by the reaction of $O_2^{\cdot-}$ with H_2O_2. Therefore, removal of H_2O_2 by catalase is expected to decrease the amount of OH-radicals which is produced by this pathway. It should be mentioned that biological results with bacteria argue against the importance of intracellular dismutase and catalase for the formation of lethal radiation damage induced by ionizing radiation (Misra and Fridovich, 1976). No biological or biochemical data of this type is available for mammalian cells. We are

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BOX No. H-75-17 Bldg 2714-H
EVO 4155 Florida
FOLDER Expenditure Statement

REPOSITORY Oak Ridge Operations
Records Holding area
COLLECTION Documents 1944-1994

5. Voucher for Concluding Payment. In the event the Contract is renewed, the remaining ten percent payment for the current period will be added to funds to be provided under the Letter of Credit for the renewal period. Therefore, submission of a voucher covering this payment is not necessary.

The renewal proposal should be signed by the principal investigator and by an official authorized to sign for the institution, and the expenditure statement should be endorsed by the proper administrative officials and six copies of each, together with six copies of the progress report and four copies of the 200-word summary should be submitted at the same time to:

U. S. Energy Research and Development Administration
Contract Division
Post Office Box E
Oak Ridge, Tennessee 37830

If you do not contemplate requesting renewal, we shall appreciate your advising us as soon as possible. In addition, we should like to invite your attention to Paragraph (b) of Article B-XX, which provides for submission of a final technical report upon expiration date of the Contract.

The certified expenditure statement required by Article B-XXIX, following the format set forth in Appendix "C" to the Contract, is to be submitted, in triplicate, within three months after the expiration of the current contract period. It is important that we receive the certified expenditure statement on time. If it is not received on time, payment may be withheld.

All references to the U. S. Atomic Energy Commission ("AEC" or "Commission") in the Contract have been changed to Energy Research and Development Administration (ERDA).

Your cooperation and assistance in this matter are appreciated.

Sincerely,

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

ACR: WLB

Enclosures:
1. Guide
2. Notice of Research Project Form (5)
3. Form ERDA-427 (2)

CC: Dr. Harry H. Sisler, U of Florida

1039088

RETURN ALL COPIES TO DSR WITH THE GOAL

DEADLINE DATE October 1, 1977

(Date Proposal Must Be Received By Granting Agency)

UNIVERSITY OF FLORIDA
Gainesville, Florida 32611

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

APPROVAL OF PROPOSAL

RESEARCH ☒
TRAINING ☐
OTHER ☐
(Check One)

September 26, 1977
DATE

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

Submitted To: (Agency) U. S. Energy Research and Development Administration

(Division)

(Program)

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

Department of Biochemistry and Molecular Biology
College of Medicine

Principal Investigator: (Project Director)

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

Co-Principal Investigator: (If Applicable)

NAME: J. F. Remsen, Ph.D.
TITLE: Research Asst. Prof.
TELEPHONE: 904-392-2669

Department Head: P. A. CERUTTI

NAME: P. A. CERUTTI, M.D., Ph.D.
TITLE: Professor and Chairman

Department Head: (If more than one involved)

NAME:
TITLE:

Approval by Dean or Director:

NAME: W. B. DEAL, M.D.
TITLE: Acting Dean

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)

NAME: K. F. Finger, Ph.D.
TITLE: Acting Vice President

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

NAME:
TITLE:

Official Authorized to Sign for the University (Leave Blank).

Dr. Anthony F. Randazzo, Assistant Director
DIVISION OF SPONSORED RESEARCH
UNIVERSITY OF FLORIDA
904-392-4800

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P.I. Check one:

NEW ☐
RENEWAL ☐
CONTINUATION ☒

SEND ALL CORRESPONDENCE RELATING TO:

Grant Administration: Division of Sponsored Research

Fiscal Matters: E. Stivender, Contracts & Grants, Finance and Accounting

Scientific Matters: Dr. P. A. Cerutti, Box J-245, JHMHC

DIVISION OF BIOMEDICAL RESEARCH
UNITED STATES
ENERGY RESEARCH AND DEVELOPMENT ADMINISTRATION
OAK RIDGE OPERATIONS
P. O. BOX E
OAK RIDGE, TENNESSEE 37830
AREA CODE 615
TELEPHONE 483-8611

July 28, 1977

Dr. P. A. Cerutti
Department of Biochemistry
University of Florida
Gainesville, Florida 32611

RENEWAL OF CONTRACT NO. EY-76-S-05-4155 - UNIVERSITY OF FLORIDA

Dear Dr. Cerutti:

Your attention is invited to Article B-XX of Appendix "B" of the Contract pertaining to the submission of a renewal proposal and progress report which will be due September 1, 1977.

The renewal proposal and progress report may be submitted at any time after six months of performance under the current contract period. In any event, they should reach us no later than the due date. Later submission will, in all probability, result in our receipt of the decision on renewal after the current contract period has expired. Therefore, we urge you to submit any renewal proposal no later than the above date. The information to be submitted is as follows:

1. Progress Report. Include information requested in Paragraph (a) of Article B-XX of Appendix "B". As indicated therein, the progress report should briefly describe the scope of the investigations undertaken and the significant results obtained. Technical reports, preprints, and articles prepared for publication during the current period should be listed with bibliographic references. Reprints of all such material not previously submitted should be appended and material contained therein need not be duplicated in the report. Progress reports and preprints should be numbered consecutively using a system based on the format "ORO - last four digits of the contract number - report number".

   The submission of a completed Form ERDA-427. "Contractor's Recommendation for Disposition of Research Document," is required with respect to Annual Progress Reports, Special Topical Reports, and the Final Report.

1039090

2. Renewal Proposal. Include information outlined in the enclosed "Guide for the Submission of Research Proposals from Educational Institutions," revised May, 1975, 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 ofthe project including support by both the institution and 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 $_____

(2) Estimated total cost for remainder of period $_____

(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 $_____

(4) Accumulated costs chargeable to ERDA (include costs reported in certified statement for preceding period(s) and the costs stated in Item (3) above) $_____

(5) Accumulated ERDA Support Ceiling as stated in Article III of Contract $_____

(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 $_____

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.

BY ERDA AUTHORIZED ☐

SCIENCE INFORMATION EXCHANGE
SMITHSONIAN INSTITUTION

ENERGY RESEARCH AND DEVELOPMENT ADMINISTRATION

ERDA CONTRACT

SUPPORTING DIV. OR OFFICE: COLLEGE OF MEDICINE

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

Department of Biochemistry and Molecular Biology
J. Hillis Miller Health Center, Box J-245
University of Florida
Gainesville, Florida 32610

TITLE OF PROJECT:

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

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.

P. A. Cerutti, Professor and Chairman, Principal Investigator	20%
J. F. Remsen, Research Assistant Professor, Co-Principal Investigator	30%
M. S. Netrawali, Postdoctoral Research Fellow	60%

NO. OF GRADUATE STUDENTS ON PROJECT: 1 NO. OF GRADUATE STUDENT MAN-YEARS: .60

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.

Our program concentrates on studies of the formation and repair of DNA damage induced in human cells by energy-related physical and chemical carcinogens. An important goal is the development of a functional classification of DNA damage which should allow predictions about the biological effects of a given cla of lesions on the basis of their structural characteristics.

We are attempting to answer the question whether superoxide radicals are intermediates in the formation of DNA damage in mammalian cells by these forms of radiation. The molecular mechanism of repair of near-ultraviolet induced DNA damage will be investigated in normal human skin fibroblasts and skin fibroblasts from patients with the genetic diseases Xeroderma pigmentosum and Ataxia telangiectasia.

Our studies with chemical DNA damaging agents concentrate on the energy-related ubiquitous pollutant benzo(a)pyrene. We are planning to continue our studies of the repair of covalent benzo(a)pyrene-DNA adducts in the human alveolar tumor line A549 and skin fibroblasts from normal individuals and patients with Xeroderma pigmentosum and Ataxia telangiectasia.

RESULTS TO DATE:

Excision repair of thymine products of the 5,6-dihydroxy-dihydrothymine type which are induced by ionizing radiation and ultraviolet light has been demonstrated in normal human skin fibroblasts and skin fibroblasts from Xeroderma pigmentosum and Ataxia telangiectasia patients. The removal from DNA of covalent benzo(a)pyrene-purine adducts was studied in baby hamster kidney cells and mouse embryo fibroblasts.

	PROGRAM CATEGORY NO.
BUDGET	
PRIMARY	
SECONDARY	

Signature of Principal Investigator P. A. CERUTTI

DATE: 9/26/77

INVESTIGATOR -- DO NOT USE THIS SPACE

1039092

ERDA-326

UNIVERSITY-TYPE CONTRACTOR'S RECOMMENDATION FOR DISPOSITION OF SCIENTIFIC AND TECHNICAL DOCUMENT

(See Instructions on Reverse Side)

1. ERDA REPORT NO.

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

3. TYPE OF DOCUMENT (Check one):

- [x] a. Scientific and technical report
- [ ] b. Conference paper:
  - Title of conference
  - Date of conference
  - Exact location of conference
  - Sponsoring organization
- [ ] c. Other (Specify)

4. RECOMMENDED ANNOUNCEMENT AND DISTRIBUTION (Check one):

- [ ] a. ERDA's normal announcement and distribution procedures may be followed.
- [x] b. Make available only within ERDA and to ERDA contractors and other U.S. Government agencies and their contractors.

5. REASON FOR RECOMMENDED RESTRICTIONS:

Privileged scientific information

6. SUBMITTED BY: NAME AND POSITION (Please print or type)

P. A. CERUTTI, M.D., Ph.D., Professor and Chairman
Dept. of Biochemistry and Molecular Biology
University of Florida
Gainesville, Florida 32610

Organization

University of Florida

Signature P. A. CERUTTI

Date September 26, 1977

FOR ERDA USE ONLY

7. ERDA CONTRACT ADMINISTRATOR'S COMMENTS, IF ANY, ON ABOVE ANNOUNCEMENT AND DISTRIBUTION RECOMMENDATION:

8. PATENT CLEARANCE:

- [ ] a. ERDA patent clearance has been granted by responsible ERDA patent group.
- [ ] b. Report has been sent to responsible ERDA patent group for clearance
- [ ] c. Patent clearance not required.

1039093

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

P. A. Cerutti, Principal Investigator

J. F. Remsen, Co-Principal Investigator

Department of Biochemistry and Molecular Biology
University of Florida
Gainesville, Florida

December 1, 1977 - November 30, 1978

RENEWAL PROPOSAL FOR CONTRACT NO. EY-76-S-05-4155

1039094

SUMMARY

During the period of Dec. 1, 1977 to Nov. 30, 1978 of our E.R.D.A. contract, we are planning to continue our studies of the formation and repair of DNA damage induced in human cells by energy-related physical and chemical carcinogens. An important goal of our program is the development of a functional classification of DNA damage which should allow predictions about the biological effects of a given class of lesions on the basis of their structural characteristics.

In our studies with physical carcinogens we will focus on the mechanism of action of aerobic γ-rays and near-ultraviolet light. We will attempt to answer the question whether superoxide radicals are intermediates in the formation of DNA damage in mammalian cells by these forms of radiation. The molecular mechanism of repair of near-ultraviolet induced DNA damage will be investigated in normal human skin fibroblasts and skin fibroblasts from patients with the genetic diseases Xeroderma pigmentosum and Ataxia telangiectasia. These diseases are characterized clinically by increased susceptibility for the formation of malignancies and on a cellular-molecular level by deficiencies in DNA repair.

Our studies with chemical DNA damaging agents concentrate on the energy-related ubiquitous pollutant benzo(a)pyrene. In mechanistic studies we are attempting to demonstrate that benzo(a)pyrene induces radical-type damage in metabolizing cells by indirect action in addition to covalent adducts between the polycylic hydrocarbon moiety and the purine bases.

We are planning to continue our studies of the repair of covalent benzo(a)pyrene-DNA adducts in the human alveolar tumor line A549 and skin fibroblasts from normal individuals and patients with Xeroderma pigmentosum and Ataxia telangiectasia. Different facets of the repair of these lesions will be studied. (1) removal of covalent benzo(a)pyrene-DNA adducts by direct chromatographic analysis (2) breakage and resealing of benzo(a)pyrene damaged parent DNA (3) effect of benzo(a)pyrene damage on the synthesis of daughter strands (4) effect of the chromosomal distribution of benzo(a)pyrene lesions on their repairability.

1039095

OUTLINE

I. Introduction

II. Specific Aims

A. Formation and repair of γ-ray and near-ultraviolet induced DNA damage in human cells.

(1) The involvement of superoxide radicals in radiation-induced DNA damage in mammalian cells

a. Ionizing radiation
b. Near ultraviolet light

(2) Repair of DNA damage induced by near-ultraviolet light in Xeroderma pigmentosum and Ataxia telangiectasia

a. Study of the repair of near-ultraviolet damage in XP- and AT- skin fibroblasts by the alkaline-elution method.
b. Study of the repair of near-ultraviolet damage in XP- and AT- skin fibroblasts: disappearance of endonuclease sensitive sites.

B. Formation and repair of benzo(a)pyrene induced DNA damage in mammalian cells.

(1) Induction of DNA damage by oxygen radical species as a consequence of benzo(a)pyrene metabolism.

(2) Excision repair of covalent benzo(a)pyrene-purine adducts in human alveolar tumor cells.

(3) Chromosomal distribution of covalent benzo(a)pyrene-purine adducts in human alveolar tumor cells A549 and in secondary mouse embryo fibroblasts.

(4) Formation of covalent benzo(a)pyrene-purine adducts in cultured explants of peripheral human lung.

(5) Repair of covalent benzo(a)pyrene-purine adducts in XP- and AT- skin fibroblasts.

a. Study of the repair of benzo(a)pyrene-purine adducts in XP- and AT- skin fibroblasts by alkaline-elution method.
b. Study of the repair of benzo(a)pyrene-purine adducts in XP- and AT- skin fibroblasts: disappearance of endonuclease sensitive sites.

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III. References

IV. Supporting Data

A. Personnel

B. Support received from other federal agencies

C. Facilities

D. Budget for period of Dec. 1, 1977 to Nov. 30, 1978.

1039097

I. INTRODUCTION

Over the last decade it has become evident that human cancer is to a large part caused by exogenous environmental factors. While mankind has always been exposed to ultraviolet light, ionizing radiation and certain natural toxins, the amounts and types of agents in our environment have been steadily increasing with industrialization, urbanization and "modernization" of our life style which are accompanied by extraordinary needs for energy. Not in the least, the development of organic, nutritional, pharmaceutical and agricultural chemistry, etc., with all their recognized blessings has often led to exposure to chemicals which had not been sufficiently tested for detrimental side effects. Great efforts are being made to identify and eliminate such health hazards and to evaluate the risk and benefit of important chemicals such as drugs, food additives, herbicides, pesticides, etc. Our program concentrates on the elucidation of the cellular and molecular mechanisms of malignant transformation of human cells by energy-related chemical and physical agents. Emphasis is placed on two major aspects of this general topic.

(1) Identification and classification of carcinogen-induced chromosomal damage in human cells:

Many physical and chemical agents in the environment that are hazardous to human health have been demonstrated to produce damage in the chromosomal DNA. The characterization of DNA damage induced by important energy-related human carcinogens represents a major goal in our studies. A first class of lesions under investigation are produced by active oxygen radical species. Such radicals, most importantly hydroxyl radicals, are formed in the cell by ionizing radiation and ultraviolet light and indirectly as a consequence of polycyclic hydrocarbon metabolism.

A second class of DNA lesions which are under investigation in cultured human tissues are induced by ubiquitous environmental polycyclic hydrocarbon carcinogens such as benzo(a)pyrene. Several research teams including our own have identified benzo(a)pyrene metabolites which act as potent proximate mutagens and carcinogens in rodent cells and are intermediates in the covalent binding of benzo(a)pyrene to DNA. A major aim in our work with these and other carcinogens is to develop a classification of DNA damage which allows predictions about the biological effects of specific lesions on the basis of their structural properties.

(2) Cellular mechanisms of DNA repair and their relationship to mutagenesis and carcinogenesis:

In mammalian cells damaged residues can be removed and the structure of the DNA totally reconstituted by error-free repair pathways which operate with high fidelity. The capacity of a cell to remove a particular lesion by an error-free pathway to a large degree determines the magnitude of the biological effects. Lesions which are not excised in time before DNA replication reaches the damaged segment - persistent lesions - may lead to cell death, mutation, initiation of malignant transformation or possibly cellular aging.

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In our program we are attempting to elucidate the mechanisms of DNA repair and the relationship of DNA repair to mutagenesis and carcinogenesis in human cells. Attention is focused on the repair of DNA lesions induced by active oxygen radical intermediates (produced mostly by physical carcinogens) and by polycyclic hydrocarbon carcinogens. The molecular biology of the repair of DNA lesions produced by these agents is being investigated in epitheloid cells and fibroblasts from normal individuals and patients with the hereditary diseases Xeroderma pigmentosum and Ataxia telangiectasia. These diseases are characterized by predisposition for cancer and evidence has been obtained on the cellular and molecular level for deficiencies in DNA repair. They promise to be extremely interesting for the elucidation of the relationship between chromosomal damage induced by environmental factors, damage repair and carcinogenesis in man.

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planning to study this question by comparing the efficiency of the formation of thymine damage by aerobic γ-rays in normal skin fibroblasts and fibroblasts from patients with Acatalasemia (Aebi and Suter, 1972). The general experimental design described in the previous paragraph will be used.

(b) Near-ultraviolet light

We have demonstrated (see Section I(2) of Progress Report) that monomeric ring-saturated thymine products of the 5,6-dihydroxydihydrothymine type (t^{uv}) are formed by near-ultraviolet light in HeLa cells with an efficiency which is comparable to cyclobutane-type pyrimidine dimerization (Hariharan and Cerutti, 1977). The mechanism of t^{uv} formation has not been investigated. The possibility that $O_2^{\cdot -}$ and OH radicals are formed as intermediates in these reactions has to be considered (Rosenthal, 1975). Therefore, we are proposing to carry out a series of experiments of the type described above for γ-rays with near-ultraviolet light. These experiments are of practical relevance for health and energy research since t^{uv}-production relative to pyrimidine dimerization reaches a maximum in that part of the solar spectrum which is suspected to be most effective for the induction of skin cancer in man (Hariharan and Cerutti, 1977).

(2) Repair of DNA damage induced by near-ultraviolet light in Xeroderma pigmentsoum and Ataxia telangiectasia

Numerous reviews have been written about Xeroderma pigmentosum (XP) and Ataxia telangiectasia (AT) (Cleaver, 1974; Robbins et al, 1974; Kraemer, in press) and the relevance of these diseases to cancer research. Aspects of special importance to our research program have been discussed in last year's renewal proposal and in our publications and are not repeated here.(Remsen and Cerutti, 1976; Remsen and Cerutti, 1977; Cerutti and Remsen, in press).

Most of the photobiology of XP has been carried out with light of wavelengths below 300nm (far uv). This is mostly due to the ready availability of monochromatic light sources at 254nm and to the fact that most of the bacterial photobiology and photochemistry has been carried out in this wavelength range. However, the solar irradiation reaching the surface of the earth does not contain significant amounts of radiation below 300nm. In contrast, near ultraviolet of wavelengths 300-380nm represents a portion of the sun spectrum which is of particular importance to energy and health research, e.g. a tentative action spectrum for the induction of actinic cancer in man has its maximum between 305 and 315 nm (Hariharan and Cerutti, 1977; Setlow, 1974). Far- and near ultraviolet photochemistry and photobiology may be quite different, e.g. we have shown that photolesions other than cyclobutane-type photodimers are formed in significant yields by 313nm light. Therefore, it is of obvious importance to study the near ultraviolet photobiology of normal skin fibroblasts and of XP cells. We are planning to study the molecular mechanisms of the repair of DNA lesions introduced by monochromatic light at 313nm in normal and XP-skin fibroblasts.

In contrast to XP, AT skin fibroblasts are not sensitive to (far) ultraviolet light in culture and according to the limited amount of data available, they repair UV-endonuclease sensitive sites normally (Kraemer, in press). A number of AT-lines have been shown to be deficient in the repair of anoxic γ-ray damage measured by the determination of repair replication and the removal of M. luteus γ-endonuclease sensitive sites, on the other hand (Paterson et al, 1976). In vitro excision of products of the 5,6-dihydroxy-dihydrothymine type from γ-ray irradiated DNA or chromatin by AT-preparations was normal (Remsen and Cerutti, 1977). These findings do not allow the construction of a molecular model for the (repair) deficiency in AT, indeed the situation is confusing. We hope to clarify the situation by a comparative study of the repair of γ-ray and near-ultraviolet induced DNA lesions in intact AT-skin fibroblasts. Note that both γ-rays and near-ultraviolet induce products of the 5,6-dihydroxy-dihydrothymine type. The following experimental design will be used in our experiments with XP and AT fibroblasts.

(a) Study of the repair of near-ultraviolet damage in XP- and AT- skin fibroblasts by the alkaline-elution method:

Skin fibroblasts will be grown in petri dishes and labeled in their DNA with ^{14}C-thymidine. They will then be irradiated with a small dose of 313nm light from our Schoffel High Intensity Monochromator and incubated for different time periods at 37°. Before the cells are harvested, they will be pulsed for 30 min. with ^{3}H-thymidine and chased with fresh non-radioactive media for 60 min. The cells will then be harvested by trypsinization and collected on a filter disc. The size distribution of the parental and newly synthesized DNA will then be analysed by the very sensitive alkaline elution procedure developed by Kohn and collaborators (1976). A similar protocol is successfully being used in our laboratory for the study of the repair of benzo(a)pyrene damage on daughter strand synthesis (^{3}H-label) and the overall DNA synthetic capacity of the cultures (^{3}H/^{14}C ratios) as a function of the duration of postirradiation incubation (human alveolar tumor line A549).

(b) Study of the repair of near-ultraviolet damage in XP- and AT- skin fibroblasts: disappearance of endonuclease sensitive sites.

The following approach will be taken to investigate the removal of specific classes of near-ultraviolet lesions from the DNA in XP and AT cells. The cells will be grown, labeled, irradiated and applied on filter discs as described in the preceding paragraph with the exception that they will not receive a ^{3}H-thymidine pulse before the termination of posttreatment incubation. After the cells have been lysed on the filter, they will be treated with repair-endonuclease before their DNA is analyzed by the alkaline elution procedure, The retention time and the quantity of the DNA fragments which are eluted from the filter will depend on the number of lesions in the DNA which are recognized by a specific endonuclease. This combination of the endonuclease-sensitive site assay with the Kohn-alkaline elution procedure promises to yield an

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extremely sensitive tool for the study of excision repair in intact cells. The following endonucleases will be used in our studies: (1) UV-endonuclease from M. luteus which recognizes cyclobutane-type pyrimidine dimers (2) peak III endonuclease from M. luteus which apparently recognizes products of the 5,6-dihydroxy-dihydrothymine type introduced into DNA by osmium tetroxide oxidation or γ-irradiation (3) apurinic site enzyme from E. coli. All these enzymes have been biochemically characterized and will be supplied to us in highly purified form by Dr. L. Crossman's laboratory, Johns Hopkins, (1974), (Gates and S. Linn, 1977).

B. FORMATION AND REPAIR OF BENZO(A)PYRENE INDUCED DNA DAMAGE IN MAMMALIAN CELLS

(1) Induction of DNA damage by oxygen radical species as a consequence of benzo(a)pyrene metabolism

Most of the stable, and therefore readily identifiable metabolites of polycyclic hydrocarbons have been shown to be products of two-electron oxidation reactions. However, there is considerable evidence which indicates that one-electron oxidations also take place in these metabolic processes. The one-electron oxidations are known to produce organic free radicals. If the oxidant is molecular oxygen, reactive oxygen species such as the super-oxide radical anion, the hydroxyl radical, and/or hydrogen peroxide can be produced as side products in these reactions. These reactive reduced oxygen species can cause cell injury and are known to be involved in radiation damage (RotiRoti and Cerutti, 1974; Remsen and RotiRoti, 1977).

One of the major metabolites of B(a)P metaolism is 6-hydroxy-benzo(a)pyrene and its degradation product, the 6-oxo-benzo(a)pyrene radical. The 6-oxo-benzo(a)pyrene radical is then rapidly oxidized to three major diones, 6,12- B(a)P dione (15%, 1,6-B(a)P dione (41%), and 3,6- B(a)P dione (44%) (Lesko et al, 1975). In this oxidation process, it is most likely that hydrogen peroxide (H_2O_2), superoxide radicals, and hydroxyl radicals are transiently formed.

Incubation of B(a)P-diones with T_7 DNA produces DNA strand breakage as measured by alkaline sedimentation. This ability to damage DNA is inhibited by removing oxygen and by the presence of either super-oxide dismutase or EDTA. Alternately, it is increased by the presence of a cellular reducing agent, NADPH, or a transition metal ion, Cu^{++} (Ts'o, in press). Thus it has been suggested that DNA strand scission is caused by reactive reduced oxygen species produced in a one-electron redox reaction involving B(a)P diones. In addition to strand breaks, hydrogen peroxide or peroxide and hydroxyl radical generators are known to cause DNA strand breaks, base modifications, crosslinking, and inactivation of transforming activity of DNA (Lorentzen, et al. 1975; RotiRoti and Cerutti, 1974; Remsen and RotiRoti, 1977). We are attempting to establish that the active oxygen species formed as a consequence of this B(a)P metabolism leads to DNA-Thymine damage in intact mammalian cells.

1039102

The basic experimental design will involve double labeling of BHK cells with both tritium and carbon-14 (the ^3H will be on the thymine methyl group and the ^{14}C will be on the 2-position of the thymine ring) at about a 16 to 1 ratio respectively. The double-labeled cells will then be incubated with concentrations of B(a)P in the range of 2μgs/ml to 10 μgs/ml. After an incubation period sufficient for metabolism of the B(a)P to take place (approx. 20-60 hrs). The cells will be harvested by trypsinization (0.05% Trypsin), treated with TCA, neutralized in NaOH, and an aliquot counted directly in aquasol. Hydrogen abstraction from the thymine-methyl [^3H] and concomitant decrease in the $^3H/^{14}C$ ratio can be taken as indirect evidence of B(a)P radical attack at that point on the DNA. In addition, the [^3H]H_2O produced by the attack of OH-radicals at the thymine-methyl group will be followed by analyzing a portion (approx. 1/3 of the total amount of the medium) of the TCA soluble fraction according to the column chromatography procedure detailed by Swinehart et al (1974).

(2) Excision repair of covalent benzo(a)pyrene-purine adducts in human alveolar tumor cells

This project represents a continuation of our work with rodent cells which is described in last year's Renewal Proposal and the Progress Report for 1976/77 which is enclosed. The human alveolar tumor line A549 (Lieber et al, 1976) has retained some differentiated functions in culture, e.g. it produces surfactant and metabolizes polycyclic aromatic hydrocarbons. Alveolar lung cells may represent an important target in human lung carcinogenesis. While bronchogenic carcinomas are the major form of lung cancer in humans, a minor but significant fraction of cancer originates from alveolar cells. Therefore, it seemed particularly interesting to us to study the molecular steps of excisions repair of benzo(a)pyrene lesions in A549 cells. The experimental design is the same as in our studies with rodent cells (Shinohara and Cerutti, 1977) and it is not further discussed here.

(3) Chromosomal distribution of covalent benzo(a)pyrene-purine adducts in human alveolar tumor cells A549 and in secondary mouse embryo fibroblasts

The effect of local chromatin structure on the reactivity of DNA towards the attack by damaging agents and the repairability of lesions in different parts of the chromosomes may play an important role in physical and chemical carcinogenesis. With the recent progress in the understanding of chromatin structure (Kornberg, 1977) and the development of simple methods for the distinction of nucleosomal and spacer DNA, it has become possible to approach this problem experimentally. Several studies on the distribution of alkylation and arylalkylation damage in the chromosomal DNA have been carried out (Cooper et al, 1975; Metzger et al, 1977; Metzger et al, 1977; Jahn and Litman, 1977). Higher damage levels have usually been detected in the spacer DNA relative to nucleosomal DNA. In most studies in vitro component systems and directly acting proximate carcinogens were used. In order to better simulate the conditions of polycyclic aromatic hydrocarbon carcinogenesis in the animal

1039103

and in man we are analyzing the chromosomal distribution of covalent benzo(a)pyrene-purine adducts in the actively metabolizing alveolar tumor line A549 and in secondary mouse embryo fibroblasts which had been exposed to benzo(a)pyrene. Additionally we are comparing the relative rates of the removal of benzo(a)pyrene adducts in nucleosomal and spacer DNA during posttreatment incubation.

Growth, labeling and carcinogen treatment of the cultures have been described by Shinohara and Cerutti (1977). Following different lengths of incubation, nuclei are prepared and treated with micrococcal nuclease under the conditions of Sollner-Webb and Felsenfeld (1975). The nuclease resistant, nucleosomal DNA is precipitated with alcohol and further analyzed for its content in benzo(a)pyrene-purine adducts by Sephadex LH 20 chromatography as described by Shinohara and Cerutti (1977a). The initial damage level and the kinetics of product removal are compared between total DNA and nucleosomal DNA. (Co-sponosred by N.I.H.)

(4) Formation of covalent benzo(a)pyrene-purine adducts in cultured explants of peripheral human lung

We are investigating the formation of covalent benzo(a)-pyrene-DNA adducts in organ-cultured specimens of peripheral human lung obtained from surgery. The culture media and the growth conditions developed by Harris and collaborators for bronchial explants are being used (Barret et al, 1976) and the protocol the ^{14}C-thymidine labeling, the treatment with ^{3}H-benzo(a)pyrene, DNA-extraction, DNA-digestion and chromatographic analysis are similar to our published methods (Shinohara and Cerutti, 1977a). Evidence for the formation of diasteromeric deoxyguanosine adducts (^{2}N-(10-{7β,8β,9α- and 9β-trihydroxy-7,8,9,10-tetrahydrobenzo(a)pyrene}yl)deoxyguanosine) was obtained in all six lung specimens which have so far been investigated. The 7β,8α,9α-diastereomer represents the major adduct. This work is being continued. In particular, we are attempting to further corroborate the structural assignments by High Pressure Liquid Chromatography in collaboration with Dr. Don Jerina at the N.I.A.M.D. A preliminary report of this work is in press (Shinohara and Cerutti, 1977b).

(5) Repair of covalent benzo(a)pyrene-purine adducts in Xeroderma pigmentosum and Ataxia telangiectasia skin fibroblasts.

It can be safely concluded that XP-fibroblasts are deficient in excision repair of cyclobutane type pyrimidine dimers (Cleaver, 1974; Robbins et al, 1974; Kraemer, in press) and data from Paterson and collaborators suggest that some AT-fibroblasts are deficient in the excision of anoxic radiation lesions (Paterson et al., 1976). One of the major goals in our laboratory reminds to develop a classification of DNA damage which allows predictions about the biological effects of a structurally related group of lesions (Cerutti, 1974; Cerutti, 1975). One possibility is to organize lesions according to their repairability by cells with genetic DNA repair defects. This approach has been taken by a number of investigators (Cleaver, 1974; Regan and Setlow, 1973). It should also be kept in mind that the clinical symptoms in patients with genetic DNA repair defects may be due to their inability to repair an entire class of related lesions rather than a single prototype. Therefore, the study of the repair of a variety of structurally related and unrelated DNA lesions in cells with genetic repair defects should yield valuable information.

1039104

We are planning to investigate the repair of benzo(a)-pyrene adducts in skin fibroblasts from XP and AT patients. Since cultured human skin fibroblasts have essentially lost their ability to metabolize polycyclic aromatic hydrocarbons the highly reactive diol-epoxide metabolites of benzo(a)pyrene, i.e. 7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo(a)pyrene and the corresponding-9β,10β-isomer, will be used (Remsen *et al*, 1977).

(a) Study of the repair of benzo(a)pyrene-purine adducts in XP- and AT- skin fibroblasts by the alkaline-elution method:

The design of these experiments will be as described above for our studies of the repair of near-ultraviolet damage (see Section II-A(2a)).

(b) Study of the repair of benzo(a)pyrene-purine adducts in XP- and AT- skin fibroblasts: disappearance of endonuclease sensitive sites:

The design of these experiments will be as described above for our studies of the repair of near-ultraviolet damage (see Section II-A(2b)).

1039105

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1039107

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] Zurich, Switzerland
PRESENT NATIONALITY: USA
SEX: Male
SOCIAL SECURITY NUMBER: [redacted]
EDUCATION:

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

1039108

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).

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1039113

2, Joyce F. Remsen

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PRESENT NATIONALITY: USA
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SOCIAL SECURITY NUMBER: [redacted]
EDUCATION:

Institution	Degree	Year	Speciality

PROFESSIONAL ACTIVITIES: American Association for the Advancement of Science
American Institute of Biological Scientists
American Society for Photobiology
Radiation Research Society

RESEARCH AND/OR PROFESSIONAL EXPERIENCE:

1975-Present	Research Assistant Professor University of Florida, Gainesville, Fla.
1971-1975	Instructor, University of Florida Gainesville, Florida
1960-1964	Information Scientist, E.R. Squibb & Sons, New Brunswick, New Jersey
1957-1958	Technician, Boyce Thompson Institute for Plant Research, Yonkers, New York

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

Co-principal investigator: 30% of time devoted to project.

1039114

1. Cerutti, P. A., Miller, N., Pleiss, M. G., Remsen, J. F., and Ramsay, W. J., Photohydration of uridine in the RNA of coliphage R17. I. Reductive assay for uridine photohydration. Proc. Natl. Acad. Sci. 64: 731 (1969).

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4. Remsen, Joyce F., and Cerutti, Peter A., Ultraviolet inactivation and miscoding of irradiated R17-RNA in vitro. Biochem. Biophys. Res. Commun. 48:430 (1972).

5. Remsen, Joyce F., Matsushita, Tatsuo, Chirikjian, Jack G., and Davis, Frank F., Enzymatic synthesis of deoxypseudouridylic acid and a study of certain of its properties. Biochim. Biophys. Acta 281:481 (1972).

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10. Remsen, J.F. and Cerutti, P. A., Deficiency of gamma-ray excision repair in skin fibroblasts from patients with Fanconi's anemia. Proc. Natl. Acad. Sci., USA 73 2419 (1976).

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1039115

12. Remsen, J., Jerina, D., Yagi, H. and Cerutti, P., In vitro reaction of radioactive 7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydrobenzo-(a)pyrene and 7β,8α-dihydroxy-9β,10β-epoxy-7,8,9,10-tetrahydrobenzo(a)-pyrene with DNA. Biochem. Biophys. Res. Commun. 74 934 (1977).

13. Remsen, J.F. and Cerutti, P.A., Excision of gamma-ray induced thymine lesions by preparations from Ataxia telangiectasia fibroblasts. Mutation Res. 43 139 (1977).

14. Cerutti, P., Shinohara, K. and Remsen, J., Repair of DNA damage induced by ionizing radiation and benzo(a)pyrene in mammalian cells. J. Toxicol. and Environmental Health 2 1375 (1977).

15. Remsen, J. F. and RotiRoti, J. L., Formation of 5,6-dihydroxydihydrothymine type products in DNA by hydroxyl radicals. Int. J. Radiat. Biol. 32 191 (1977).

16. Cerutti, P., Shinohara, K., Ide, M. L., and Remsen, J. "Formation and repair of benzo(a)pyrene-induced DNA damage in mammalian cells", in: Polycyclic Hydrocarbons and Cancer: Chemistry, Molecular Biology and Environment (in press).

17. Cerutti, P. A. and Remsen, J. F., "Formation and repair of DNA damage induced by oxygen radical species in human cells", in: DNA Repair Processes: Cellular Senesence and Somatic Cell Genetics (eds.: W. W. Nichols and D. Murphy. Symposium Specialists, Miami) 1977 (in press).

1039116

3. M. S. Netrawali

NAME: M.S. Netrawali, Ph.D
TITLE: Postdoctoral research fellow
BIRTHDATE/BIRTHPLACE: [redacted]
PRESENT NATIONALITY: India
SEX: Male
EDUCATION:

Institution	Degree	Year	Specialty
[redacted]	[redacted]	[redacted]	[redacted]
Free University of Brussels, Prof. J. Brachee's Laboratory	Govt. of Belgium Scholar for higher research in nuclear sciences	1964-1967	
French Atomic Energy Commission Saclay, France	one of the 20 selected participants in the Advanced course in radiobiology	1963	

RESEARCH AND/OR PROFESSIONAL EXPERIENCE:

1970-Present Senior Scientist and Group Leader
Nucleic acids structure-function
Biochemistry & Food Technology Div.
Bhabha Atomic Research Centre, Trombay
Bombay, India

Worked for 6 months in the Radiobiology Laboratory, Atomic Energy Research Centr Mol, Belgium.

Research Guide for the post-graduate degree of the University of Bombay.

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; 60% of time devoted to project.

1039117

SELECTED PERSONAL PUBLICATIONS IN AREAS RELATED TO PRESENT PROPOSAL:

K. Pasupathy, M.S. Netrawali, D. S. Pradhan and A. Sreenivasan. Rejoining of single-strand scissions in nuclear and mitochondrial DNAs in Tetrahymena pyriformis. Radiat. Res. 66, 147-157, 1976.

R. K. Prakah, M.S. Netrawali, D. S. Pradhan. Rifampicin sensitivity of residual RNA synthesis in E. coli cells exposed to ultraviolet radiation and, combination of ultraviolet and gamma radiations. Photochem. Photobiol. 24, 295-297, 1976.

K. G. Chetty, M.S. Netrawali, D. S. Pradhan; Mechanisms underlying decreased protein and RNA synthesis in the rat spleen following whole-body x-irradiation. Strahlentheranie, 151, 228-235, 1976.

S. B. Kulkarni, M. S. Netrawali, D. S. Pradhan, and A. Sreenivasan. Action of hydrocortisone at translational level in rat liver. Mole. Cell Endocrinol. 4, 195-202, 1976.

M.N.S. Rao, M.S. Netrawali, D. S. Pradhan. Regulation of transcription in eukaryotic cells. Proc. Symp. on 'Regulation and growth of differentiated function in eukaryotic cells, (ed. G. P. Talwar, Raven Press) pp. 129-138, 1975.

R. K. Prakash, M. S. Netrawali, D. S. Pradhan and A. Sreenivasan. Ineffectiveness of rifampicin in inhibiting RNA synthesis in Escherichia coli and T_4-infected Escherichia coli cells after exposure to ultraviolet radiation. Biochim. Biophys. Acta, 383, 435-440, 1975.

M.S. Netrawali, K. Pasupathy and D. S. Pradhan. Repairs of nuclear and mitochondrial DNAs in Tetrahymena by nucleocytoplasmic relationships. Elsevier Scientific Publishing Co., The Netherlands, pp. 139-143, 1975.

K. Pasupahty, M. S. Netrawali and D. S. Pradhan. Oxidative phosphorylation by Tetrahymena pyriformis mitochonria exposed to gamma radiation in vivo and in vitro. Int. J. Radiat. Biol., 28, 593-597, 1975.

M.N.S. Rao, M.S. Netrawali, and D. S. Pradhan. Changes at transcriptional level in the rat liver following whole-body x-irradiation. FEBS Letts. 48, 160-163, 1974.

S.G. Satav, S. S. Kafyare, M.S. Netrawali, P. Fatterpaker. The significance of promitochondrial structures in rat liver for mitochondrial biogenesis. Biochem. J., 134, 687-695, 1973.

M. S. Netrawali. Chemical radioprotection in the absence of the nucleus in Acebatularia mediterranea. Radiation Botany. 10, 365-370. 1970.

M. S. Netrawali. On the presence of histones in Euglena gracilis var. bacillaris. Exptl. Cell. Res., 63, 422-426, 1970.

1039118

Biochem. J., 96, 552-556, 1965.

M. S. Netrawali, Radiolysi and radioprotective effect of glutathione. Int. J. Radiat. Biol., 15, 275-277, 1970.

1039119

B. Support received from other Federal Agenices:

Support is obtained from the National Institute of General Medical Sciences-Grant No. 2R01-GM-18617-07 at a level of $55,931 (direct costs for current year).

Our NIH program concentrates on:

(1) Molecular biology of the repair of γ-ray-, near-violet- and benzo(a)pyrene induced DNA damage in Fanconi's Anemia.

(2) Distinction of different excision repair pathways in human alveolar tumor cells: excisability of different guanine lesions.

(3) Modulation of DNA repair: effects of growth conditions and viral infection on DNA repair in monkey and human cells.

C. 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, CO_2 incubators, ^{137}Cs-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 and Molecular Biology 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.

1039120

D. BUDGET FOR PERIOD OF DECEMBER 1, 1977 to NOVEMBER 30, 1978.

1. Salaries

Name	Title/ % of Time		Amount
M. Netrawali	Ph. D., Post-doctoral Associate	60%	$ 5,862.
E. Walker	Laboratory Technician I	75%	6,669.
A. Lusby	Assistant-In Biochemistry	75%	7,850.
Fringe Benefits	(M. Netrawali).0065 U.C.		38.
	(E. Walker) 15.5% & Health Ins.		1,196.
	(A. Lusby) 15.5% & Health Ins.		1,379.
Total Salary, Wages & Fringe Benefits			$ 22,994.

2. Permanent Equipment

Unspecified	$ 2,000.

3. Supplies

Chemicals	$ 4,252.
Radioisotopes	3,000.
Biochemicals	2,000.
Tissue Culture Media	6,000.
Glass and Plastic Ware	4,000.
Total Supplies	$ 19,252.

4. Travel

Domestic	$ 2,000.

5. Other Expenses

Instrument Service Contracts	$ 3,000.
Publication Costs	2,000.
Personnel Assessment $8. per FTE per Quarter til 6/30/77	27.
$9. per FTE per Quarter as of 7/1/77	
Total Other Expenses	$ 5,027.

6. Indirect Costs (51% of Salaries, Wages and Fringe Benefits) $ 11,727.

TOTAL $ 63,000.

*The State University System is pursuing the development of an overhead rate for a statewide cost allocation agreement. We will apply this additional amount when the rate is available and the final plan is approved by DHEW auditors.

1039121

Progress Report for the Period of September 1, 1976 to November 30, 1977 of Contract EY-76-S-05-4155 of the United States Energy Research and Development Administration

Title: Formation and Repair of Physically and Chemically Induced DNA Damage in Human Cells.

P. A. Cerutti, Principal Investigator
Department of Biochemistry and Molecular Biology
University of Florida
Gainesville, Florida 32610

Prepared for the United States Energy Research and Development Administration under Contract EY-76-S-05-4155.

1039122

SUMMARY

During the period of Sept. 1, 1976 to Nov. 30, 1977 of our E.R.D.A. contract, we have continued our studies of the formation and repair of chromosomal damage which is induced in mammalian cells by energy-related carcinogens. We have focused our attention on the physical carcinogens γ-rays and ultraviolet light and the chemical carcinogen benzo(a)pyrene.

We have investigated the formation of monomeric thymine damage of the 5,6-dihydroxy-dihydrothymine type in the DNA of human carcinoma HeLa cells by the action of aerobic γ-rays and ultraviolet light. Our studies with specific radical scavengers demonstrate that aerobic γ-rays induce such lesions predominantly by indirect action via the intermediacy of hydroxyl radicals. The formation of products of the 5,6-dihydroxy-dihydrothymine type in addition to pyrimidine photodimers is also observed upon irradiation of HeLa cells with ultraviolet light. While photodimers represent the major DNA lesions in the far ultraviolet, comparable amounts of photodimers and monomeric, ring-saturated thymine lesions are formed in the near-ultraviolet (313nm).

Excision repair of γ-ray products of the 5,6-dihydroxy-dihydrothymine type was studied in cells from patients with the genetic disease Ataxia telangiectasia (AT). AT-patients possess increased susceptibility for the development of malignancies. Cells obtained from AT patients show increased frequencies of chromosomal aberrations and are highly sensitive to ionizing radiation in culture. We have studied the capacity of sonicates of AT skin fibroblasts for the excision of γ-ray products from irradiated DNA and chromatin. For preparations from five different AT cell lines and under a variety of experimental conditions, the excision capacity was comparable to preparations from normal human skin fibroblasts.

We studied the formation and repair of covalent benzo(a)pyrene-purine adducts in actively metabolizing rodent and human tissues. Adduct formation was observed in baby hamster kidney cells (BHK 21/C13), secondary mouse embryo fibroblasts, human alveolar tumor cells A549 and cultured explants of peripheral human lung. The benzo(a)pyrene-purine adducts were characterized chromatographically. Adduct removal during posttreatment incubation was studied in baby hamster kidney cells and mouse embryo fibroblasts.

1039123

OUTLINE

I. Formation and repair of γ-ray and ultraviolet induced DNA damage in human cells.

(1) Hydroxyl radicals are intermediates in the formation of products of the 5,6-dihydroxy-dihydrothymine type by aerobic γ-rays in HeLa S3 cells.

(2) Formation of products of the 5,6-dihydroxy-dihydrothymine type by ultraviolet light in HeLa S3 cells.

(3) In vitro excision of γ-ray induced thymine lesions by preparations from Ataxia telangiectasia skin fibroblasts is normal.

II. Formation and repair of benzo(a)pyrene induced DNA damage in rodent and human cells.

(1) Excision repair of benzo(a)pyrene-purine adducts in baby hamster kidney cells and secondary mouse embryo fibroblasts.

(2) Formation of benzo(a)pyrene-purine adducts in human alveolar tumor cells and in explants of peripheral human lung.

III. References

IV. Publications resulting from work supported by E.R.D.A. Contract AT-(40-1)-4155 from September 1, 1976 to November 30, 1977.

V. Financial statement of expended funds from December 1, 1976 to November 30, 1977

(note: - financial statement for the period of September 1, 1976 to November 30, 1976 was submitted and approved in the Spring of 1977).

1039124

I. Formation and repair of γ-ray and ultraviolet induced DNA damage in human cells

(1) Hydroxyl radicals are intermediates in the formation of products of the 5,6-dihydroxy-dihydrothymine type by aerobic γ-rays in HeLa S3 cells.

The formation in DNA of ring-saturated products of the 5,6-dihydroxy-dihydrothymine type (t') after gamma-ray irradiation of intact cells has been established in a number of mammalian cell-lines (Mattern, Hariharan, Dunlap and Cerutti, 1973, Mattern, Hariharan and Cerutti, 1975). The mechanism by which these products are formed in DNA can be studied by the use of specific radical scavengers, a method introduced by Johansen and Howard-Flanders (1965) for E. coli. The hydroxyl radical has been shown to be responsible for the formation of $[^3H]H_2O$ by hydrogen abstraction from the methyl group of thymine in pre-labeled CHO cells (RotiRoti and Cerutti, 1974) as well as for the production of single-strand breaks in DNA (Roots and Okada, 1972; Achey and Duryea, 1974). The purpose of this study was to determine whether t' in purified DNA and intact cells results from direct or indirect action and if indirect, which radical species is primarily responsible. Our data clearly indicates that products of the 5,6-dihydroxy-dihydrothymine type are primarily formed by indirect radiation action via the intermediacy of hydroxyl radicals (Remsen and RotiRoti, 1977).

(2) Formation of products of the 5,6-dihydroxy-dihydrothymine type by ultraviolet light in HeLa S3 cells

We have undertaken a study of the formation of thymine photodimers ($\hat{TT}$) and monomeric, ring-saturated thymine lesions of the 5,6-dihydroxy-dihydrothymine type (t^{uv}) in human carcinoma HeLa S3 cells upon irradiation with monochromatic light at 240, 265, 280, and 313 nm under aerobic conditions. We have also measured the formation of tritiated water from [methyl-^{3}H] thymidine-labeled cells which gives a measure of the photochemical reactivity of the methyl group of thymine. The efficiency of the formation of all three products was maximal at 280 nm and dropped towards longer and shorter wavelengths. The efficiency of $\hat{TT}$ formation dropped more strongly towards longer and shorter wavelengths than the efficiency of t^{uv} formation (comparison at a dose of 5×10^3 J m^{-2}). Total monomeric thymine ring saturation (t_{sat}) was estimated from the t^{uv} data. It was calculated that 0.06 t_{sat} was formed for each $\hat{TT}$ at 280 nm, but 0.73 t_{sat} per $\hat{TT}$ at 313 nm. It follows that monomeric thymine ring saturation represents a minor photochemical reaction relative to pyrimidine dimerization in the far-ultraviolet but a major reaction in the near-ultraviolet. The formation of ^3H_2O by ultraviolet light from [methyl-^{3}H] thymidine-labeled HeLa cells most likely indicates the attack of hydroxyl radicals on the cellular DNA; 5-methylenouracil radicals formed as a consequence of the reaction may be important intermediates in ultraviolet-induced DNA-DNA and DNA-protein crosslinking.

1039125

Epidemiological data indicates that sunlight plays a major role in the etiology of skin cancer in man (see, e.g., Epstein, 1970). No experimental data is available which allows the definition of an action spectrum for skin cancer. However, from the sun spectrum at the surface of the earth and an action spectrum for ultraviolet-induced DNA damage and its lethal and mutagenic effects in bacterial systems, Setlow (1974) speculated that light in the 300-304 nm region should possess maximal effectiveness for the induction of skin cancer. It is interesting to note in this context that the ratio of t^{UV} over $\hat{TT}$ increased dramatically in this region of the spectrum. (Hariharan and Cerutti, 1977).

(3) In vitro excision of γ-ray induced thymine lesions by preparations from Ataxia telangiectasia skin fibroblasts is normal.

Ataxia telangiectasia (AT) is an autosomal recessive disease of man with increased frequencies of spontaneous and X-ray induced chromsomal aberrations and cancer. (Taylor *et al*, 1975).

Cultured skin fibroblasts from AT patients exhibit increased sensitivity to killing by gamma-rays delivered under aerobic conditions and to the chemical mutagen actinomycin D and, possibly, to methyl methanesulphonate and mitomycin C (Hoar and Sargent, 1976; Taylor *et al*, 1975). While the resealing of radiation induced DNA single- and double-strand breaks was normal in AT cells (Paterson *et al*, 1976; Vincent *et al*, 1975), Paterson *et al*, 1976, observed a decreased ability of some AT strains to undergo repair replication following exposure to gamma-rays delivered under anoxic conditions. The same investigators also observed a decreased rate of removal from DNA of M. luteus gamma-endonuclease sensitive sites in line AT3BI which had been irradiated under anoxic conditions. The damage recognized by M. *luteus* gamma-endonuclease most likely involves the heterocyclic DNA bases.

The capacity of whole cell sonicates of skin fibroblasts of normal individuals and patients with the autosomal recessive disease Ataxia telangiectasia (AT) to remove aerobic gamma-ray products of the 5,6-dihydroxy-dihydrothymine type ($t^{\gamma}_{O_2}$) from exogenous DNA substrates was investigated. All five AT strains (AT CRL 1312, AT CRL 1343, AT GM 367, AT 4BI, AT 3BI) possessed normal capabilities to excise $t^{\gamma}_{O_2}$ from irradiated bacteriophage DNA and irradiated chromatin isolated from normal and AT-skin fibroblasts (Remsen and Cerutti, 1977).

II. Formation and repair of benzo(a)pyrene-purine adducts in baby hamster kidney cells and secondary mouse embryo fibroblasts.

It is estimated that approximately 1300 tons of B(a)P are emitted into the air of the United States per year by combustion engines, heating systems, electrical power plants, etc. B(a)P is also one of the major procarcinogens contained in tobacco smoke. Work by several groups including our own has identified the disasteromeric metabolies 7,8-dihydroxy-9,10-epoxy-tetrahydrobenzo(a)pyrenes (B(a)P-diol-epoxides) as potent proximate mutagens in rodent cells and as intermediates in the covalent binding of B(a)P to DNA in intact mammalian cells. The major DNA adducts are substitution products involving the exocyclic amino-group of guanine, N^2-(10-{7β,8α,9α or 9β-trihydroxy-7,8,9,10-tetrahydrobenzo(a)pyrene}yl) deoxyguanosine (dGua-B(a)P I and II). Minor adducts are also formed to adenine and possibly cytosine. *In vitro* reaction of highly purified bacteriophage DNA with chemically synthesized, radioactive B(a)P-diol-epoxide corroborates these structural assignments (Remsen *et al*, 1977; Shinohara and Cerutti, 1977a and refs. therein). We have studied the formation and excision repair of these covalent benzo(a)pyrene adducts in a number of rodent and human tissues.

(1) Excision repair of benzo(a)pyrene-purine adducts in baby hamster kidney cells (BHK) and secondary mouse embryo fibroblasts (MEF)

The formation and excision of benzo(a)pyrene-deoxyguanosine adducts in metabolizing baby hamster kidney cells (21/C13) and secondary mouse embryo fibroblasts (C57BL/6J) was investigated. Both diastereomeric adducts, N^2-(10-{7β,8α,9α-trihydroxy-7,8,9,10-tetrahydrobenzo(a)pyrene}yl)deoxyguanosine and N^2-(10-{7β,8α,9β-trihydroxy-7,8,9,10-tetrahydrobenzo(a)pyrene}yl)deoxyguanosine, were detected in both cell lines and both cell lines were capable of excising these lesions, albeit with low efficiency. Approximately 80% of the benzo(a)pyrene guanine adducts had been removed from the DNA in BHK cells in 72 hours posttreatment incubation at an approximate initial damage level 1.1 adducts per 10^6 undamaged nucleotides. The corresponding data for MEF was 39% adduct removal in 72 hours at an initial adduct level of 1.2 per 10^5 undamaged nucleotides. This difference in the initial level of damage may explain the difference in the degree of completion of the excision process during the same time period for the two cell types. (Shinohara and Cerutti, 1977a).

(2) Formation of benzo(a)pyrene-purine adducts in human alveolar tumor cells and in explants of peripheral human lung

Evidence for formation of DNA-B(a)P adducts in cultured human bronchus (Yang et al, 1977) and peripheral lung has been obtained (Harris et al, 1976). Considerable interindividual variation in the level of B(a)P binding to bronchial explants was observed (Yang et al, 1977) and may represent a factor determining susceptibility to lung cancer in the general population. Most primary malignancies of the human lung originate from the bronchial epithelium. In addition to the bronchogenic carcinomas a small fraction of the primary neoplasms of the lung derives from alveolar cells (Adamson and Senior, 1976; Coalson et al, 1970; Dobbins et al, 1973). We have studied the formation of covalent benzo(a)pyrene-purine adducts, in the DNA of alveolar tumor cells A549 (Lieber et al,) and cultured explants of peripheral human lung obtained from surgery. The lung explants were cultured according to Barret et al (1976) From the chromatographic analysis of digests of DNA extracted from these tissues, it was concluded that both the lung specimens and A549 cells metabolized benzo(a)pyrene to diastereomeric 7,8-dihydroxy-9,10-epoxy-tetrahydrobenzo(a)pyrene intermediates which mostly reacted with the exocyclic amino-groups of deoxyguanosine to N^2-(10-{7β,8α,9α- and 9β-trihydroxy-7,8,9,10-tetrahydrobenzo(a)-pyrene}yl)deoxyguanosine (dGua-B(a)P I and II). While comparable amounts of dGua-B(a)P I and II were formed in A549 cells, dGua-B(a)P I represents the predominant adducts in the DNA of lung specimens from six different donors (Shinohara and Cerutti, 1977b).

III. REFERENCES

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Paterson, M., Smith, B., Lohman, P., Andersen, A., and Fishman, L., Nature 260, 444 (1976).

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Taylor, M., Harnden, D., Arlett, C., Harcourt, S., Lehmann, A., Stevens, S., and Bridges, B., Nature, 258, 427 (1975).

Vincent, J., Sheridan, R., and Huang, P., Mutat. Res. 33, 357 (1975).

Yang, S., Gelboin, Trump, B., Autrap, H., and Harris, C., Cancer Res. 37, 1210 (1977).

1039130

IV. PUBLICATIONS RESULTING FROM WORK SUPPORTED BY E.R.D.A. CONTRACT AT-(40-1)-4155 FROM SEPTEMBER 1, 1976 to NOVEMBER 30, 1977.

A. Papers published or in press in refereed journals sponsored by E.R.D.A.:

J. Remsen and P. Cerutti - Excision of gamma-ray induced thymine lesions by preparations from Ataxia telangiectasia fibroblasts. Mutation Research, 43, 139 (1977).

P. V. Hariharan and P. A. Cerutti - Formation of Products of the 5,6-dihydroxy-dihydrothymine type by ultraviolet light in HeLa cells. Biochemistry, 16, 2791 (1977).

P. Cerutti and K. Shinohara - Formation of Benzo(a)pyrene-DNA adducts in peripheral human lung tissue. Cancer Letters, in press.

J. Remsen and J. RotiRoti, Formation of 5,6-dihydroxydihydrothymine type products in DNA by hydroxyl radicals. Int. J. Rad. Res. 32, 191 (1977).

B. Papers published or in press co-sponsored by E.R.D.A. and N.I.H.

K. Shinohara and P. Cerutti - Excision of Benzo(a)pyrene-deoxyguanosine Adducts in Baby Hamster Kidney 21/C13 Cells and in Secondary Mouse Embryo Fibroblasts C57 B1/6J. Proc. Natl. Acad. Sci. USA, 74, 979 (1977).

P. Cerutti, K. Shinohara, and J. Remsen - Repair of DNA damage induced by ionizing radiation and benzo(a)pyrene in mammalian cells. J. of Toxicology and Environmental Health, 2, 1375 (1977).

P. Cerutti, K. Shinohara, M. Ide and J. Remsen, Formation and repair of benzo(a)pyrene induced DNA damage in mammalian cells in Polycyclic Hydrocarbons and Cancer: Chemistry, Molecular Biology and Environment (eds. P. O. P. Ts'o and H. Gelboin) Academic Press, New York, (in press).

P. Cerutti and J. Remsen, Repair of DNA damage induced by Oxygen radical species, in Cellular Senescence and Somatic Cell Genetics:DNA Repair Processes (Eds. W. Nichols and D. Murphy, Symposia Specialists, Miami) 1977, in press.

1039131

V. FINANCIAL STATEMENT OF EXPENDED FUNDS FROM DEC. 1,1976 to Nov. 30, 1977

ERDA FUNDS EXPENDED

December 1, 1976 through November 30, 1977

	Allocation	Costs through 9/20/77	Anticipated Costs	Total Costs	Monies Transferred to Expense *	Balanc
Salary & Wages	$ 20,784.	$7,831.76	$7,214.41	$15,046.17	-$5,508.46	$ 229
Fringe Benefits	1,603.	801.80	732.	1,533.80	-0-	69
Equipment	4,484.	4,422.84	-0-	4,422.84	-0-	61
Travel	2,000.	819.20	-0-	819.25	- 1,180.75	-0
Other Direct Costs	22,695	27,000.60	-0-	27,000.60	-0-	5,192
		32,193.44*				
Indirect Costs	11,434.	4,405.11	4,052.66	8,457.77	- 2,809.23	167
Total	63,000.	45,281.36	11,999.07	57,280.43	--------	5,719

*Includes $9,498.44 transferred from Salary & Wages, Travel and Indirect Costs.

**Note: All balances appearing at this time will be spent out by the end of the grant period, November 30, 1977.

The financial report for the period of September 1, 1976 through November 30, 1976 has already been submitted in the Spring of 1977.

1039132